

Synthesis, Characterization, antimicrobial, anticancer and anti-hemolytic activity of Co (II), Ni (II) and Cu (II) complexes with 2-methoxy-6-(3-iminoquinoliny methyl) phenol

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Abstract: A Schiff base ligand was synthesized by condensation of o-vanillin with 3-aminoquinoline in 1:1 molar ratio. The Schiff base metal complexes of Co (II), Ni (II) and Cu (II) were prepared using the metal salt and ligand in 1:2 ratio. The metal complexes were characterized by using elemental analysis, molar conductance, magnetic susceptibility, UV-Visible, and IR spectra. They were also characterized by their thermogravimetric analysis. The compositions of the Schiff base ligand and the metal complexes were established by elemental analysis which indicated a metal: ligand ratio of 1:2. Magnetic, thermal and spectral studies indicated that ligand behaved as bidentate coordinating through phenolic oxygen and azomethine nitrogen and formed octahedral metal complexes. The metal complexes revealed their general formula as $[ML_2(H_2O)_2]$ where M= Co(II), Ni(II) or Cu(II) and L=deprotonated Schiff base ligand. The in-vitro antibacterial activities of the metal complexes were tested using bacterial species *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Escherichia coli*. These compounds were also tested for antifungal activities against *Candida albicans* and *Aspergillus niger*. All the Schiff bases were also screened for their anticancer activities against breast cancer MCF-7 cell line and colon cancer HT-29 cell line by sulforhodamine-B (SRB) assay. Interestingly, the Schiff base and its Co(II), Ni(II) and Cu(II) metal complexes showed superactive anticancer activity against breast cancer MCF-7 cell line while Schiff base metal complex of Co(II) showed superactive anticancer activity against colon cancer HT-29 cell line. The Schiff base ligand and its metal complexes were found to be anti-hemolytic in nature.

Keywords: Synthesis, Schiff base ligand, transition metal complexes, spectral characterization, antimicrobial, anticancer and anti-hemolytic activity.

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I. Introduction

Schiff bases were first reported by scientist Hugo Schiff in 1864. These compounds contain an azomethine group ($-HC=N-$). These are also known as imines or anils. They are prepared by the condensation of primary amines with aldehydes or ketones, resulting into the formation of a new C=N bond [1]. Schiff bases derived from aromatic aldehydes and amines have a wide variety of applications in various fields like biological, inorganic and analytical chemistry. Schiff bases can be synthesized by relatively simple procedures enabling us to design compounds which are structurally similar to some substances of biological origin [2-3].

It is well known that N and O atoms play a key role in the co-ordination of metals at the active sites of many metallic-biomolecules [4]. Schiff base metal complexes have been widely studied because they have various properties like antibacterial, antifungal, anticancer, herbicidal, analytical etc. They find wide applications as catalysts in many synthetic and biological reactions. Besides, they can undergo bonding with metal ions in a variety of ways and the resulting compounds show varied activities. 3-aminoquinoline and o-vanillin are especially known for their antibacterial and antifungal activities and are thus used as synthetic precursors in pharmaceuticals. Considering the distinct biological activities of these compounds, we herein put forth an account of the synthesis and characterization of ligand complexes of Co(II), Ni(II) and Cu(II) derived from 3-aminoquinoline and o-vanillin along with the biological screening of Schiff base ligand and its complexes against different bacteria, fungi and cancers [5].

II. Experimental

2.1 Apparatus

An UV-VIS-NIR-3600 recording spectrophotometer (Shimadzu, Japan) was used for the UV spectrum scanning and determination experiments. Bruker, Germany Model 3000 Hyperion Microscope with Vertex 80FTIR system range $400-4000\text{ cm}^{-1}$ (KBr discs) at SAIF, IIT, Bombay was used for IR spectral

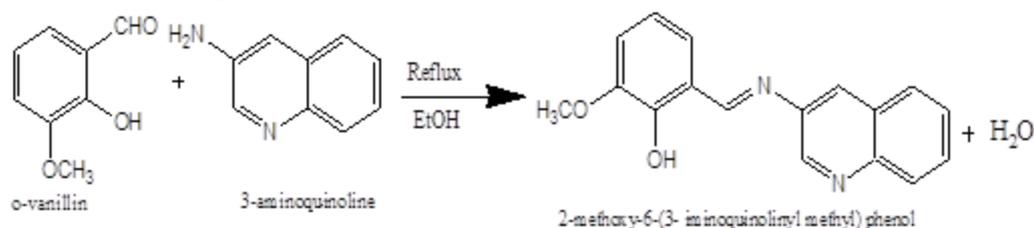
characterization of the Schiff base ligand and metal complexes. The thermogravimetric analysis of ligand and metal complexes was performed on Perkin-Elmer Diamond TG at SAIF, IIT, Bombay. The X-ray powder diffraction of ligand and metal complexes was scanned on X-ray diffraction system, Ultima IV Rigaku Corporation, Japan at Instrumentation centre, Solapur University, Solapur.

2.2 Reagents

All the chemicals / reagents used were of AR grade or higher grades. Sigma- Aldrich o-vanillin, 3-aminoquinoline, while Standard Qualigens (SQ)AR grade hydrated Cobalt (II) chloride, Nickel (II) chloride and Copper (II) chloride were used for synthesis. The solvents ethanol, DMSO etc. were used.

2.3 Synthesis of Schiff base ligand (SB-1)

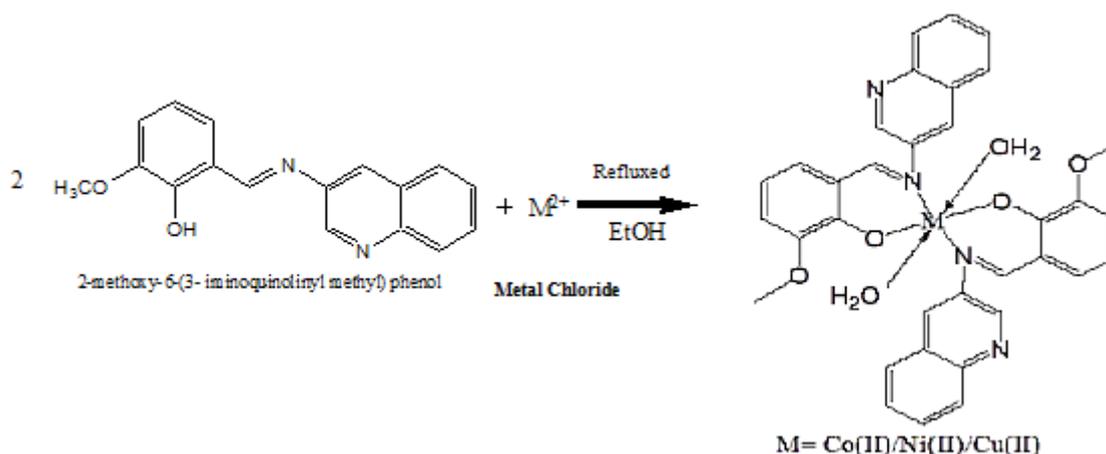
20 mmol (3.04g) of o-vanillin is dissolved in absolute ethanol. Then it was added dropwise into 20 mmol (2.88g) of ethanolic solution of 3-aminoquinoline. Then the mixture was refluxed for 2 hours then cooled and filtered [6-7]. Intense red orange colored crystals of Schiff base SB-1 were formed (yield 93%, 5.17g). The crystals were washed with cold ethanol and anhydrous diethylether and dried over anhydrous CaCl_2 . The synthesis of Schiff base ligand (SB-1) is shown in Scheme-1



Scheme-1: Synthesis of Schiff Base ligand (SB-1)

2.4 Synthesis of Schiff base transition metal complexes

20 mmol of Schiff base (SB-1) was dissolved in ethanol (30ml) and added to 10 mmol ethanolic solution (20ml) of metal (II) chloride salt. The metal-ligand molar ratio taken was 1:2. The mixture was refluxed for 3 hours. On cooling, a crystalline metal complex was obtained. It was filtered, recrystallized from ethanol and dried in desiccator over anhydrous CaCl_2 . The Co(II), Ni(II) and Cu(II) metal complexes of Schiff base (SB-1) were prepared by above general method using salts $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ and $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$. The preparation of metal complexes is shown in Scheme -2



Scheme -2: Synthesis of metal complexes

Schiff base (SB-1) and its Co (II), Ni (II) and Cu (II) complexes synthesized were stable at room temperature and soluble in common solvents like DMSO, methanol etc. The synthesized ligand and the metal complexes were characterized by elemental analysis. Biological activity of the ligand and its metal complexes were also studied. The geometry of the synthesized compounds has been elucidated on the basis of their elemental and spectral studies.

III. Results And Discussion

3.1 Analytical and Physicochemical data

The stoichiometry of ligand and their metal complexes is confirmed by the elemental analysis. The analytical and physicochemical data of Schiff base (SB-1) and its metal complexes is found in good agreement with the proposed structure of ligand and the metal complexes. The data being listed in Table-1

Table-1: Analytical and Physicochemical data of Schiff base (SB-1) and its metal complexes

Sr. No	Comp.	Molecular formula	m. p. (°C)	Mol. Weight	Color	% Observed (Theoretical)			
						C%	H%	N%	M%
1	SB-1	C ₁₇ H ₁₄ O ₂ N ₂	210	278	Red Orange	72.79 (73.7)	4.81 (5.03)	9.99 (10.07)	-
2	SB-1-Co	C ₃₄ H ₂₆ O ₄ N ₄ Co.(H ₂ O) ₂	>300	648.93	Dark Pink	62.50 (62.8)	4.90 (4.62)	8.42 (8.62)	8.94 (9.08)
3	SB-1-Ni	C ₃₄ H ₂₆ O ₄ N ₄ Ni.(H ₂ O) ₂	>300	648.69	Dark Green	62.64 (62.8)	4.89 (4.62)	8.92 (8.63)	8.98 (9.04)
4	SB-1-Cu	C ₃₄ H ₂₆ O ₄ N ₄ Cu.(H ₂ O) ₂	>300	653.55	Grey	62.13 (62.4)	4.77 (4.59)	8.33 (8.65)	9.52 (9.72)

3.2 UV Spectral Analysis

The UV spectrum of Schiff base ligand SB-1 is exhibiting two intense bands at 243 nm and 348 nm which can be assigned respectively to $\pi \rightarrow \pi^*$ transition within aromatic moiety and $n \rightarrow \pi^*$ transition of azomethine group of ligand. In the spectra of complexes, the bands due to azomethine group are shifted to higher frequency indicating that azomethine nitrogen atom is involved in coordination to the metal ion.

In the present investigation, the electronic spectra of Co(II) complex showed two absorptions bands associated with d-d transitions at 630 nm and 539 nm due to ${}^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ (ν_2) and ${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(P)$ (ν_3) transitions respectively. The electronic spectra of Ni(II) complex exhibited three bands associated with d-d transitions at 1050 nm, 670 nm and 430 nm assignable to transitions ${}^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ (ν_1), ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ (ν_2) and ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ (ν_3) respectively. The electronic spectrum of Cu(II) complex showed one low intensity broad absorption band due to d-d transition at 610 nm assigned to ${}^2E_g \rightarrow {}^2T_{2g}$ (ν_2). These observed d-d transitions for metal complexes are consistent with octahedral geometry of Co(II), Ni(II) and Cu(II) metal complexes of Schiff base ligand. The UV spectra of Schiff base (SB-1) and its metal complexes are represented in Figure-1

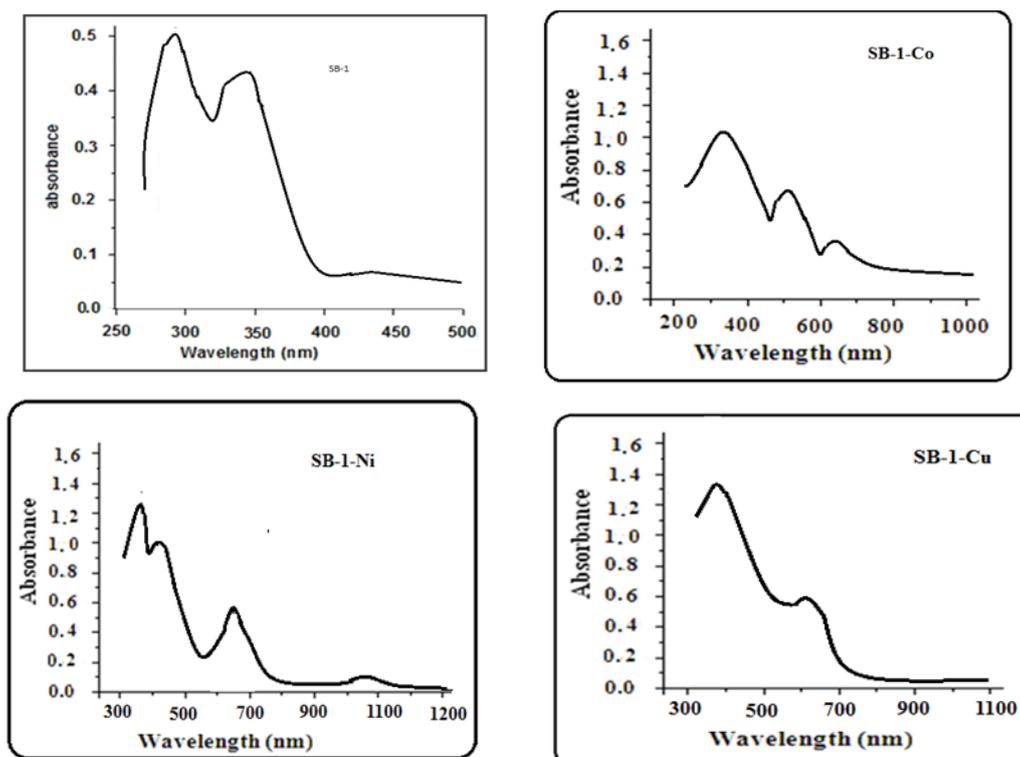


Figure-1: The UV spectra Schiff base and metal complexes

3.3 Magnetic Susceptibility studies of complexes

The magnetic moments obtained at room temperature for the metal complexes indicate paramagnetism. The Co(II) complex showed the magnetic moment of 4.81 BM. The spin free octahedral complexes of Co(II) are reported to exhibit magnetic moment in the range of 4.70 to 5.20 BM. Hence the observed moment of Co(II) complex under study indicates that, it has an octahedral configuration. The Ni(II) complex showed magnetic moment of 2.92 BM. This value of magnetic moment is within the range of 2.7 to 3.3 BM, reported for octahedral Ni(II) complexes thereby suggesting octahedral geometry for Ni(II) complex under study. For octahedral Cu(II) complexes, the magnetic moment values are expected to be in the range 1.80 to 2.20 BM. The Cu(II) complex under study exhibited magnetic moment of 1.87 BM which indicated its octahedral configuration.

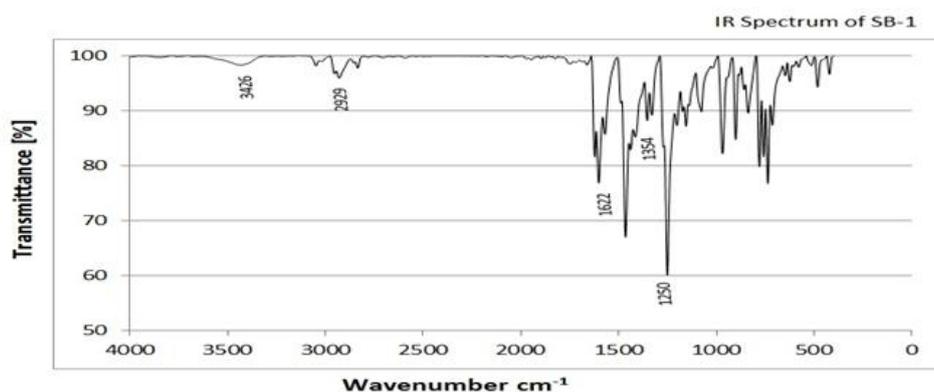
3.4 FTIR Spectral Analysis

The IR spectra of complexes are compared with those of ligand in order to determine the coordination sites that may be involved in chelation. The prominent FTIR data with the assignments of bond frequencies of Schiff base ligand (SB-1) and its Co(II), Ni(II) and Cu(II) metal complexes are presented in Table-2 and FTIR spectra of these compounds are reported in Figure-2.

Table-2: FTIR Spectral data (cm⁻¹) of Schiff base ligand and its metal complexes

Assignments	SB-1	SB-1-Co	SB-1-Ni	SB-1-Cu
v(Hydrogen bonded O-H)	3426	-	-	-
v(Coordinated water)	-	3300	3320	3316
v(C=N)	1622	1602	1604	1592
v(Ar-O)	1250	1271	1275	1277
v(C-N)	1354	1389	1391	1388
v(O-CH ₃)	2929	2930	2929	2931
v(H ₂ O rocking)	-	820	857	861
v(M-O)	-	554	554	555
v(M-N)	-	437	435	438

The broad peak observed at 3426 cm⁻¹ in the IR spectra of Schiff base ligand assigned to v(O-H), was found to be disappeared in all the complexes, thereby indicating deprotonation and formation of metal-oxygen bond. This is further supported by the shifting of phenolic v(Ar-O) towards higher frequency, indicating the coordination of phenolate oxygen to metal ion. The v(C=N) vibration of the Schiff base ligand (SB-1) occurs at 1622 cm⁻¹, which is shifted to a lower frequency of the complexes, indicating the involvement of azomethine nitrogen in chelation with the metal ion. The observed increase in magnitude of v(C-N) on complexation may be attributed to increase in double bond character of aryl azomethine C-N bond. It is due to π electron delocalization from the metal ion to the nitrogen atom and resonance interaction with the benzene ring. However the stretching frequencies of Methoxy O-CH₃ bonds for ligand and complexes are almost same, indicating the nonparticipation of methoxy oxygen in coordination. The new bands in IR spectra of complexes in the regions 554-555 cm⁻¹ and 435-438 cm⁻¹ are ascribed to v(M-O) and v(M-N) vibrations respectively. In the IR spectra of complexes appearance of a new broad band in the region 3300-3320 cm⁻¹ corresponding to the stretching frequency of v(O-H) of H₂O indicated the presence of coordinated water. It is further confirmed by the appearance of non-ligand band in complexes in the region 820-861 cm⁻¹ assignable to rocking mode of water. Presence of two molecules of coordinated water in molecules of metal complexes is further confirmed by their thermogravimetric analysis. From the above observations, it can be concluded that the ligand behaves as bidentate coordinating through deprotonated phenolic oxygen and azomethine nitrogen.



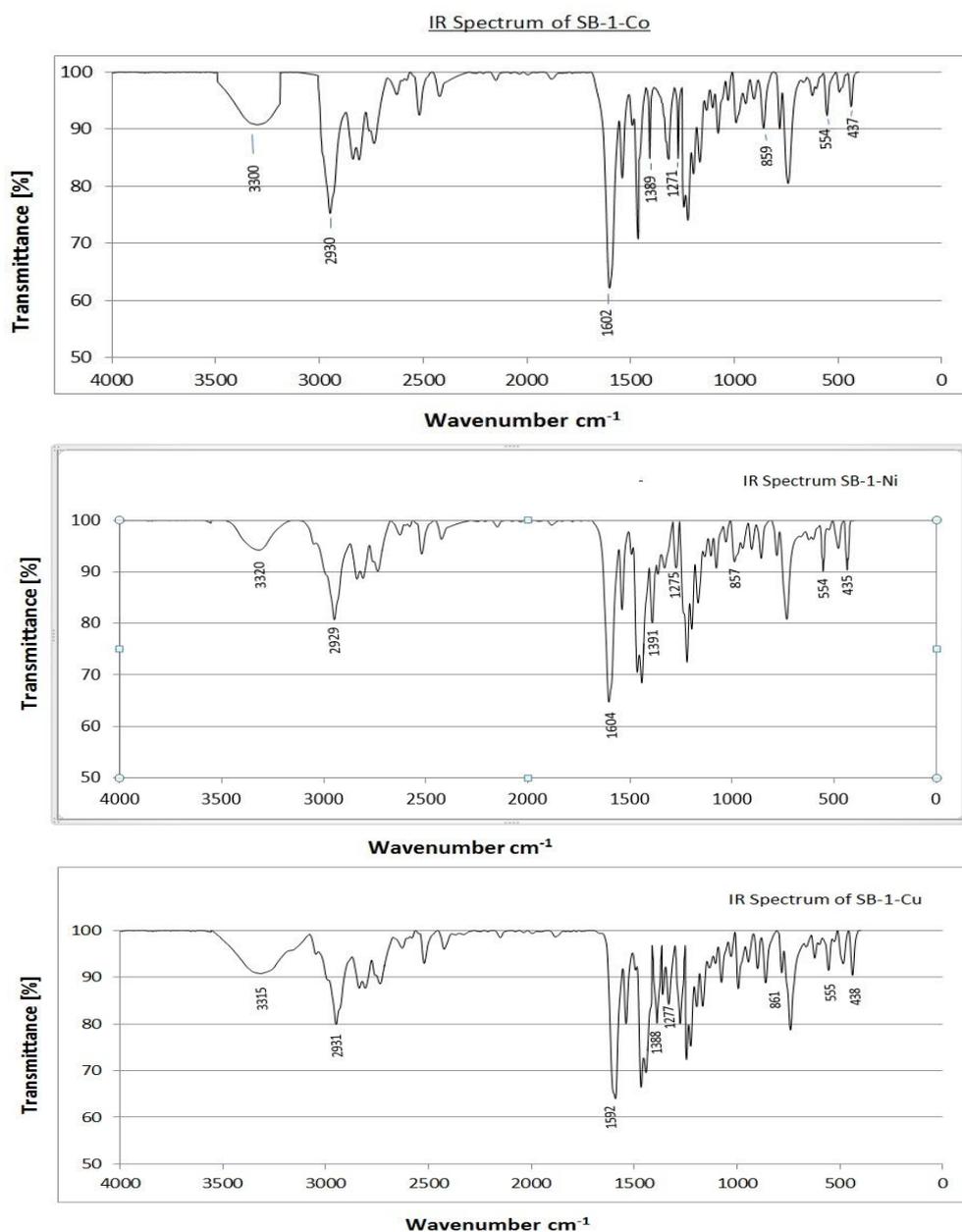


Figure-2: FTIR spectra of Schiff base ligand and its metal complexes

3.5 Thermal decomposition studies of metal complexes

Thermal decomposition studies of metal complexes have been undertaken to know the presence or absence of coordinated water molecules. Thermal decomposition data of Schiff base and its metal complexes is reported in Table-3. The thermograms of complexes are shown in Figure-3.

Table-3: Thermal data of metal complexes

Sr.No.	Complex	Temperature (°C)	Loss of molecule	Percentage weight loss	
				Calculated	Observed
1.	SB-1 Co	150°C-250°C	2H ₂ O	5.54	5.33
		250°C-610°C	Org. moiety	85.37	83.23
		>610°C	CoO	11.54	11.43
2	SB-1 Ni	150°C-260°C	2H ₂ O	5.54	5.10
		260°C-600°C	Org. moiety	85.40	80.20
		>600°C	NiO	11.51	11.30
3	SB-1 Cu	150°C-240°C	2H ₂ O	5.50	5.20
		240°C-570°C	Org. moiety	84.76	79.80
		>570°C	CuO	12.17	11.80

In case of Co(II) complex a weight loss of 5.33% between temperature range 150°C-250°C indicated the presence of two molecules of coordinated water (calculated 5.54%). The anhydrous compound further decomposed rapidly between 250°C-610°C with 83.23% weight loss corresponding to decomposition of organic moiety of the complex (calculated 85.37%) leading to the formation of 11.43% stable residue of cobalt oxide (calculated 11.54%) above 610°C. All the complexes are found to follow two step decomposition pattern. In the first step there is loss of two molecules of coordinated water and in the second step there is oxidative decomposition of organic part of the ligand leading to the formation of stable residue of metal oxide. Thermal studies supported the octahedral structure of all the metal complexes indicating presence of two coordinated water molecules.

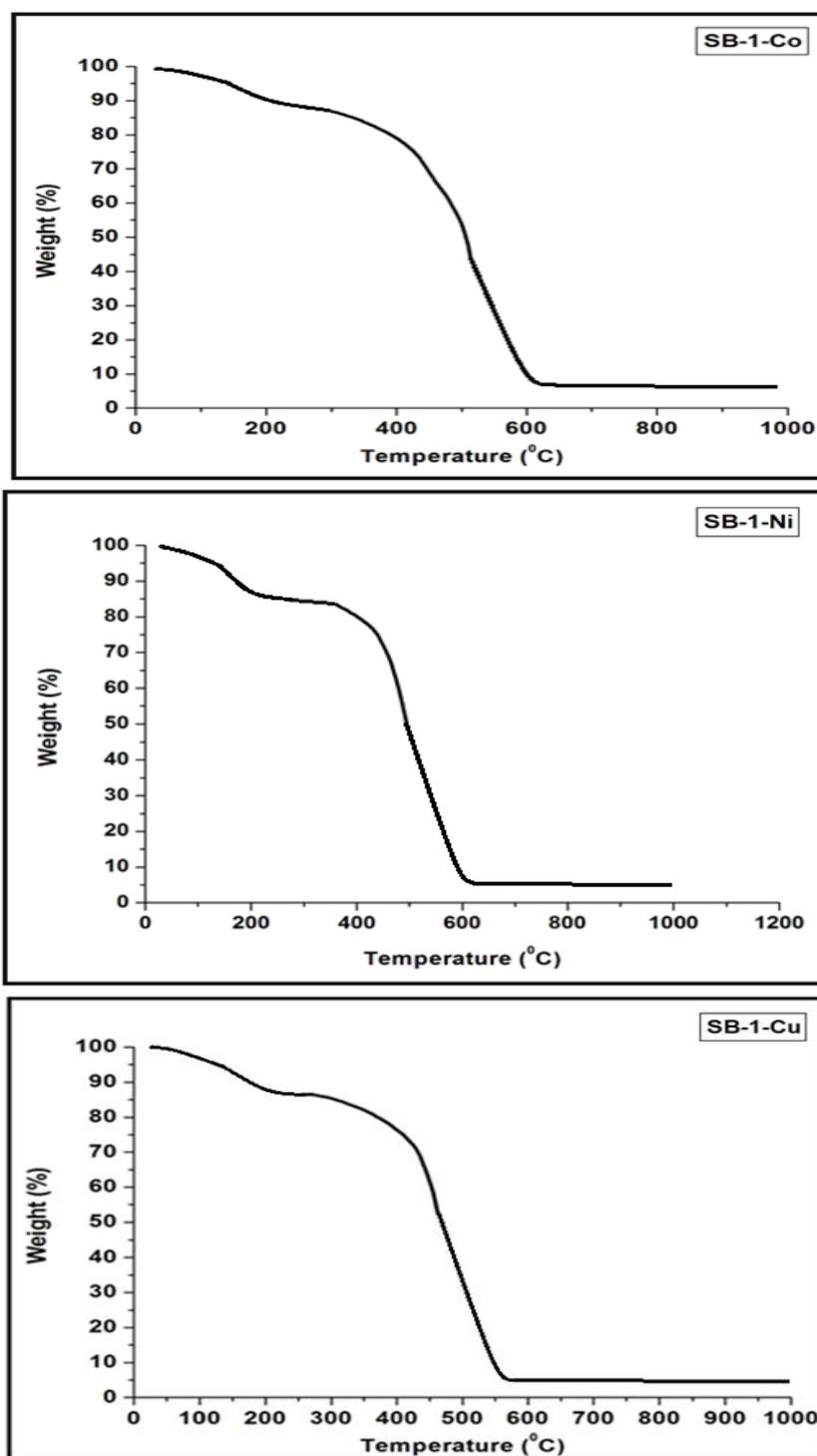


Figure-3: Thermograms of metal complexes

3.6 H^1 NMR Spectroscopic Studies

The H^1 NMR Spectrum of the Schiff base (SB-1) Shows D_2O exchangeable broad singlet at 13.258ppm integrating for one proton is assigned to $-OH$. The H^1 NMR spectrum of Schiff base shows multiplet in the range 6.947-8.172ppm due to aromatic protons. The $-N=CH-$ proton of Schiff base exhibits singlet in the region 8.966ppm. The H^1 NMR spectrum of Schiff base is shown in Figure-4.

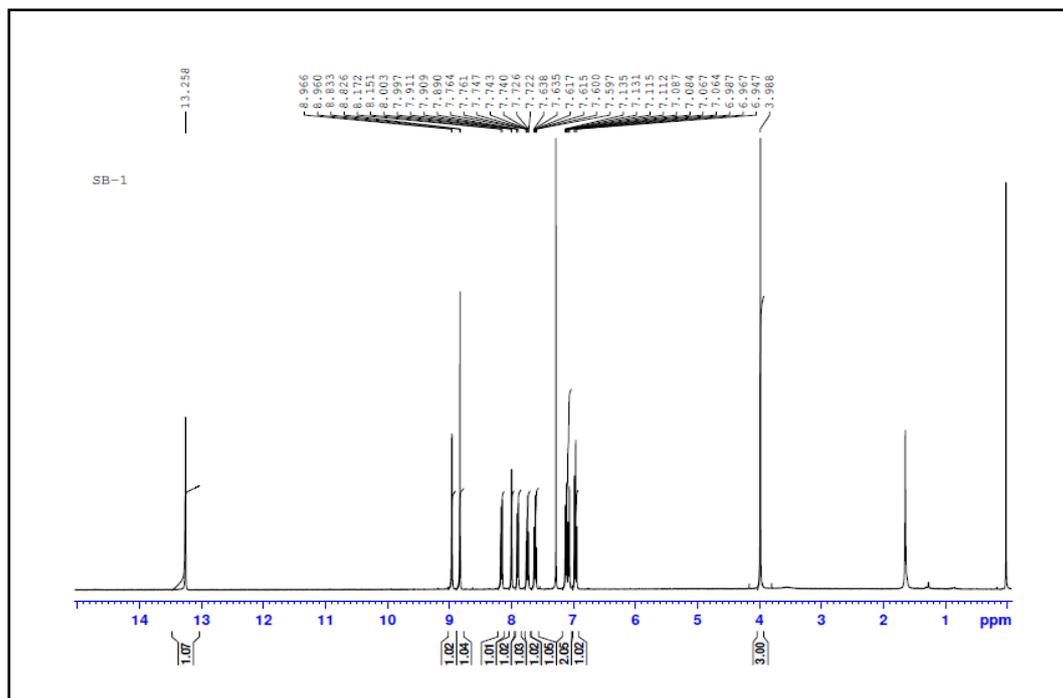


Figure- 4 : H^1 NMR spectra of Schiff Base (SB-1)

3.7 Mass spectroscopic Studies

The mass spectrum of Schiff base (SB-1) was recorded. It shows parent peak due to molecular ion (M^+). The proposed molecular formula of Schiff base was confirmed by comparing its molecular formula weight with m/z value. The molecular ion peak with $m/z = 279.3$, corresponds with the proposed molecular formula of Schiff base. In addition, the mass spectrum exhibited peaks assignable to various fragments arising from the thermal cleavage of the compound have been observed. The mass spectrum of Schiff base is shown in Figure-5.

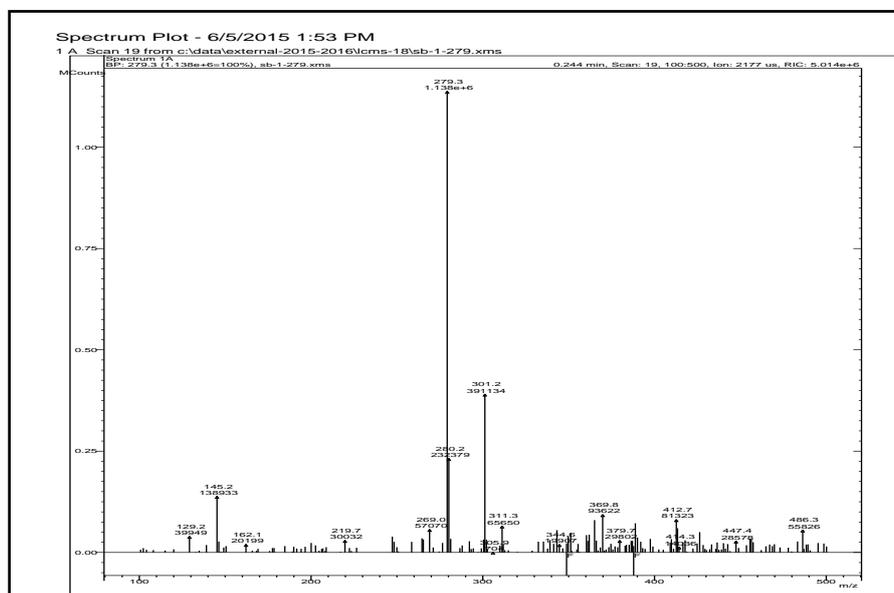


Figure-5 : Mass spectrum of Schiff base (SB-1)

3.8. Antibacterial and Antifungal activity

The antibacterial and antifungal studies of the Schiff base (SB-1) and its metal complexes SB-1-Co, SB-1-Ni and SB-1-Cu were tested on Gram positive bacteria such as S.aureus and B. cereus and Gram negative bacteria such as P.aeruginosa and E.coli while antifungal activities of this Schiff base and its metal complexes were tested on fungi C.albicans and A.niger. Well-known agar-well diffusion method was used for studies on antibacterial activity [1, 2] and Agar-Ditch method for studies on antifungal activity [3, 4]. The stock solutions of Schiff base and metal complexes of concentration 1000 µg/ml were prepared and used to prepare their various concentrations of 100,200,300,400 and 500 µg/ml. The bacteria and fungi were incubated on the surface of Nutrient agar and Sabouraud's agar respectively, the various concentrations of the compounds were incubated in the wells and ditches prepared on the agar plates. The plates were incubated at room temperature for 24 hours for bacteria and 48 hours for fungi. In order to clarify the effect of DMSO for its antimicrobial activity by agar plate assay, separate studies were carried out with DMSO and showed no activity against any bacteria and fungi, the results are as summarized in the Table-4. Metal complexes in general exhibited better antibacterial and antifungal activity than ligand. Co(II) complex exhibited highest antimicrobial activity among the compounds tested.

Table-4: Antibacterial and Antifungal activities of ligand (SB-1) and its Co (II), Ni (II) and Cu (II) Complexes

Compound	Concentration µg/ml	S.aureus	B.cereus	P.aeruginosa	E.coli	C.albicans	A.niger
SB-1	100	3-	2-	10+	6+	1-	1-
	200	5+	3-	14++	10+	2-	1-
	300	8+	4-	17++	12++	5+	2-
	400	10+	6+	15++	14++	7+	3-
	500	14++	7+	20++	16++	8+	4-
SB-1-Co	100	10+	4-	16++	14++	2-	2-
	200	12++	5+	17++	16++	4-	2-
	300	14++	7+	19++	17++	6+	3-
	400	16++	9+	21+++	18++	9+	5+
	500	18++	10+	24+++	21+++	11+	7+
SB-1-Ni	100	4-	5+	12+	6+	2-	1-
	200	6+	6+	13++	8+	3-	1-
	300	8+	6+	14++	10++	6+	2-
	400	11++	7+	15++	13++	7+	3-
	500	13++	8+	16++	17++	9+	5+
SB-1-Cu	100	5+	3-	9+	5+	2-	1-
	200	7+	5+	10+	8+	3-	2-
	300	8+	6+	12++	9+	5+	4-
	400	9+	7+	13++	11++	8+	5+
	500	11++	9+	14++	13++	10+	6+
Activity Scale	- ve= Inactive (Zone of inhibition <5mm)						
	+ve= weakly active (5 ≤ Zone of inhibition < 10mm)						
	++ve= moderately active (10 ≤ Zone of inhibition < 20mm)						
	+++ve= Highly active (Zone of inhibition ≥20mm)						

3.9 Anticancer activity studies

The anticancer activity of the ligand (SB-1) and its Co(II), Ni (II) and Cu(II) Complexes was determined by sulforhodamine -B assay on human breast cancer cell line MCF-7 and human colon cancer cell line HT-29 at ACTREC, Tata Memorial Centre, Kharghar, Navi Mumbai. The cell lines were cultured in RPMI 1640 medium, supplemented with 10% fetal bovine serum (FBS) and 2 millimolar L- glutamine at 37°C in a humidified atmosphere of 5% CO₂. About 5X10³ cells/well were seeded in 96-well micro titer plate using a culture medium. After 24 hours, Schiff base (SB-1) and its Co(II), Ni(II) and Cu(II) metal complexes at the concentrations of 10,20,40 and 80 µg/ml were added to respective wells at a single concentration and incubated for 48 hours. After incubation the sulforhodamine-B assay was performed [1,2].

Ligand (SB-1) and its Co(II), Ni (II) and Cu(II) complexes are super active on human breast cancer cell line MCF-7 in the assay system used with GI50 near or less than 10 µg/ml which is comparable to that of Adriamycin, a standard positive control drug with GI50 value less than 10 µg/ml. Therefore ligand and complexes may prove as lead compounds for in vivo screening of anticancer activity against malignant breast cancer.

Ligand (SB-1) is resistant to human colon cancer cell line HT-29 with GI50, 64.3 µg/ml. However its Co (II) complex exhibits super in vitro anti-cancer activity against colon cancer cell line HT-29 which is comparable to Adriamycin with GI50 value less than 10 µg/ml. Hence Co(II) complex can be considered as lead compound for in vivo screening of anticancer activity against malignant colon cancer.

The results of cytotoxicity of ligand (SB-1) and its complexes on human breast cancer cell lines MCF-7 and colon cancer cell lines HT-29 are shown in Tables-5 and 6.

Table -5: Cytotoxicity of Schiff base (SB-1) and its Co (II), Ni (II) and Cu (II) metal complexes on human breast cancer cell line (MCF-7)

Compound	Drug Concentration (µg/ml)		
	LC 50	TGI	GI 50
SB-1	>80	>76.5	<10
SB-1-Co	>80	8.2	<10
SB-1-Ni	>80	70.6	<10
Sb-1-Cu	>80	54.1	10.2
ADR	>80	30.11	<10

Table 6: Cytotoxicity of Schiff base (SB-1) and its Co (II), Ni (II) and Cu (II) metal complexes on human colon cancer cell line (HT-29)

Compound	Drug Concentration(µg/ml)		
	LC 50	TGI	GI 50
SB-1	>80	>80	64.3
SB-1-Co	59.6	34.7	9.7
SB-1-Ni	>80	>80	80
SB-1-Cu	>80	84.4	56.6
ADR	NE	34.7	<10

Value GI50* of <10 µg/ml- Super active 10-15 µg/ml- Moderately active 15-30 µg/ml – Weakly active 30-80 µg/ml – Resistant > 80 µg/ml – Inactive	GI50= Concentration of drug causing 50% inhibition of cell growth TGI= Concentration of drug causing total inhibition of cell growth. LC 50=Concentration of drug that 50% of the cells NE-Non evaluable data. ADR= Adriamycin (Doxorubicin, Positive control drug).
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In -vitro testing for anticancer activity in cell lines based on GI50 values shows that ligand and its Co(II) , Ni(II) and Cu(II) metal complexes are more active against human breast cancer cell line MCF-7 than human colon cancer cell lines HT-29. Cytotoxicity of ligand and its complexes on human breast and colon cancer cell lines is shown in Figure-6 and 7.

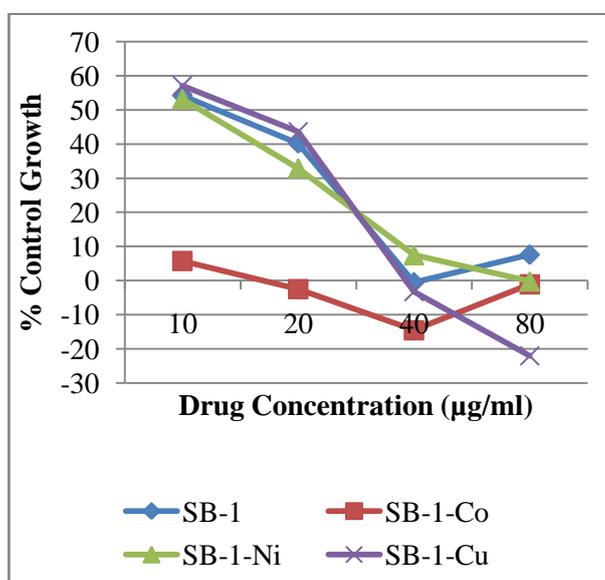


Figure- 6: Cytotoxicity of Schiff bases (SB-1) and its Co(II) , Ni(II) and Cu(II) metal complexes on human breast cancer cell line (MCF-7).

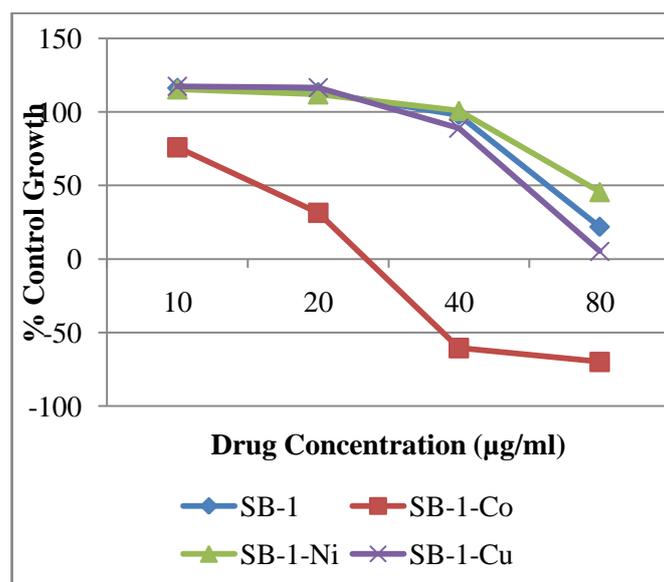


Figure- 7: Cytotoxicity of Schiff bases (SB-1) and its Co (II), Ni (II) and Cu (II) metal complexes on human colon cancer cell line (HT-2).

3.10: Antihemolytic activity

Antihemolytic activity was assessed by spectrophotometric method in a pathology laboratory of Shri Markandeya Sahakari Rughalaya and Research Centre, Solapur, From a well nourished healthy adult blood sample was collected and centrifuged at 1500 rpm for 3 minutes. Pellet of blood so obtained was washed three times in sterile saline solution (0.5% NaCl). The pellet was re-suspended in normal 0.5% saline solution. Schiff base (SB-1) and its Co (II), Ni (II) and Cu (II) metal complexes of concentrations 100, 200, 300, 400 and 500 µg/ml were prepared. A volume of 0.5 ml of the sample Schiff base (SB-1) was added in 0.5 ml of the cell suspension. After incubation of the mixture at 37°C for 30 minutes, it was centrifuged at 1500 rpm for 10 minutes. Anti-hemolytic activity was assessed by measuring the absorbance at 540 nm spectrophotometrically. For positive and negative control Triton-X-400 and DMSO were used respectively. The above procedure is repeated for various concentrations of Schiff base, Co (II), Ni (II) and Cu (II) complexes. The optical density values and percentage hemolysis (H%) obtained are presented in Table-7. The observed values of percentage hemolysis are below 5% indicating that Schiff base and its Co(II), Ni(II) and Cu(II) complexes are not showing hemolysis even at their highest concentration of 500 µg/ml.

Table-7: Percentage hemolysis (H%) of Schiff base SB-1 and its Co(II), Ni(II) and Cu(II) metal complexes

Compound		Concentration (µg/mL)				
		100	200	300	400	500
SB-1	OD	0.025	0.026	0.00275	0.028	0.031
	H%	1.26	1.476	1.793	1.898	2.531
SB-1-Co	OD	0.029	0.0295	0.0300	0.0312	0.0320
	H%	2.109	2.215	2.320	2.573	2.742
SB-1-Ni	OD	0.0292	0.0299	0.0320	0.0329	0.0333
	H%	2.151	2.299	2.742	2.932	3.016
SB-1-Cu	OD	0.029	0.0297	0.0312	0.0331	0.0334
	H%	2.109	2.257	2.573	2.974	3.037
Positive Control	OD	1.915	1.915	1.915	2.915	1.915
	H%	400	400	400	400	400
Negative Control	OD	0.019	0.019	0.019	0.019	0.019
	H%	0	0	0	0	0

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