ISSN: 2230-7850 IMPACT FACTOR : 5.1651 (UIF) VOLUME - 7 | ISSUE - 4 | MAY - 2017

INDIAN STREAMS RESEARCH JOURNAL

STUDIES ON ANTIMICROBIAL, ANTICANCER AND ANTI-HEMOLYTIC ACTIVITY OF CO (II), NI (II) AND CU (II) COMPLEXES WITH 2–METHOXY-6-(5-IMINOQUINOLINYL METHYL) PHENOL

S. N. Battin
Department of Chemistry, Walchand College of Arts and Science, Solapur, Maharashtra, India.

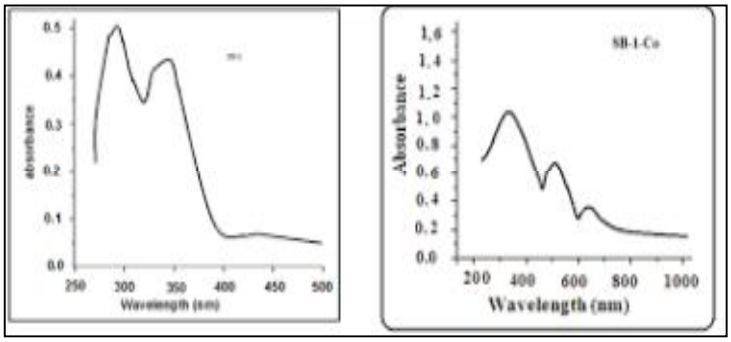
ABSTRACT:
A Schiff base ligand was synthesized by condensation of o-vanillin with 5-aminoquinoline in 1:1 molar ratio. The Schiff base metal complexes of Co (II), Ni (II) and Cu (II) were prepared using the metal salt and ligand in 1:2 ratio. The metal complexes were characterized by usingelemental thermal and spectral 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 and 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, antimicrobial, anticancer and anti-hemolytic activity.

INTRODUCTION:

Schiff bases 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 applications in various fields like biological, inorganic and analytical chemistry. Schiff bases can be synthesized byrelativelyby 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. 5-aminoquinoline and o-vanillin are especially known for theirantibacterial and antifungal activities and are thus used as synthetic precursors in pharmaceuticals. O-vanillin is flavor and aroma compound in vanilla which is used in food flavoring and pharmaceuticals. Schiff bases containing 5-aminoquinoline and o-vanillin Moiety form stable complexes with significant bioactivities.Considering the distinct biological activities of these compounds, we hereinput forth an account of the synthesis and characterization of ligand complexes of Co(II), Ni(II) and Cu(II) derived from 5-aminoquinolline and o-vanillin and the biological screening of Schiff base ligand and its complexes against different bacteria, fungi and cancer cell lines [5].

2. EXPERIMENTAL:

2.1 Apparatus:

The antibacterial and antifungal activity of Schiff base-2 and its metal complexes was carried out by standard agar well diffusion method and agar ditch method respectively for which pure R and D purpose strains of bacteria and fungi were obtained from National Collection of Industrial Micro-organisms, NCL, Pune. The anticancer activity was determined at ACTREC, Tata Memorial Centre, Navi Mumbai by standard SRB assay. The anti-haemolytic activity was determined by spectrophotometric method.

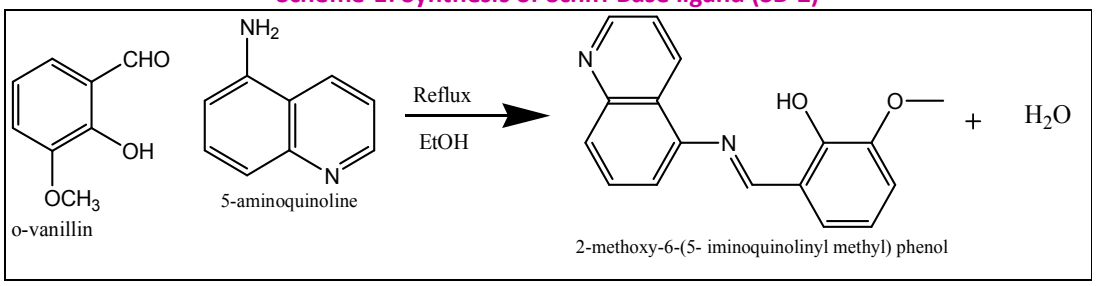
2.2 Reagents:

Analytical grade chemicals - Sigma- Aldrich o-vanillin, 5-aminoquinoline, andStandard Qualigens (SQ) hydrated Cobalt (II) chloride, Nickel (II) chloride and Copper (II) chloride were used for synthesis. The solvents AR grade absolute ethanol,DMSO etc. were used.

2.3 Synthesis of Schiff base ligand (SB-2)

20 mmol (3.04g) of o-vanillin is dissolved in absolute ethanol and added dropwise into 20 mmol (2.88g) of ethanolic solution of 5-aminoquinoline. The mixture was refluxed for 2hours then cooled and filtered [6-7]. Intense yelloworange colored crystals of Schiff base SB-2 were formed (yield 90%, 5 g). The crystals were washed with anhydrous diethylether and dried over anhydrous$CaCl_2$[6-10]The synthesis of Schiff base ligand (SB-2) is shown in Scheme-1

Scheme-1: Synthesis of Schiff Base ligand (SB-2)

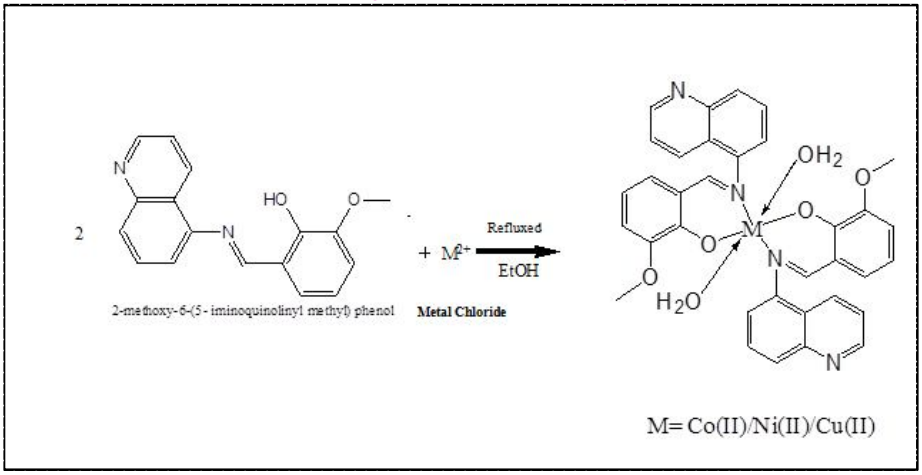


2.4 Synthesis of Schiff base transition metal complexes:

10 mmol ethanolic solution (20ml) of metal (II) chloride salt was added to 20 mmol of Schiff base (SB-2) dissolved in ethanol (30ml). The metal-ligand molar ratio taken was 1:2. The mixture was refluxed for 3 hours when a crystalline metal complex was obtained on cooling. 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-2) were prepared by above general method using salts $CoCl_2.6H_2O$, $NiCl_2.6H_2O$ and $CuCl_2.2H_2O$[11-19]. The preparation of metal complexes is shown in Scheme -2.

Scheme -2: Synthesis of metal complexes



Schiff base (SB-2) 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 Schiff base and the metal complexes were characterized by elemental, thermal and spectral analysis and their molecular formulae were determined. Biological activity namely antibacterial, antifungal and anticancer activity of the ligand and its metal complexes were studied.

3. RESULTS AND DISCUSSION:

3.1 Analytical and Physicochemical data:

The stoichiometry of ligand and their metal complexes is confirmed by the elemental and spectral analysis. The analytical and physicochemical data of Schiff base (SB-2) and its metal complexes is found in good agreement with the proposed structure of ligand and the metal complexes.[20-26] The data being listed in Table-1

Comp.	Molecular formula	m. p. (°C)	Mol. Weight	Color	% Observed (Theoretical)			
					C%	H%	N%	M%
SB-2	$C_{17}H_{14}O_2N_2$	162	278	Yellw Orange	72.46 (73.3)	4.83 (5.03)	9.94 (10.07)	-
SB-2-Co	$C_{34}H_{26}O_4N_4Co.(H_2O)_2$	>350	648.93	Dark Pink	61.95 (62.8)	4.81 (4.62)	8.45 (8.62)	8.92 (9.08)
SB-2-Ni	$C_{34}H_{26}O_4N_4Ni.(H_2O)_2$	>350	648.69	Dark Green	62.41 (62.8)	4.91 (4.62)	8.81 (8.63)	8.89 (9.04)
SB-2-Cu	$C_{34}H_{26}O_4N_4Cu.(H_2O)_2$	>350	653.55	Grey	62.19 (62.4)	4.89 (4.59)	8.51 (8.65)	9.43 (9.72)

Table-1: Analytical and Physicochemical data of Schiff base (SB-2) and its metal complexes

3.2. Antibacterial and Antifungal activity:

The antibacterial and antifungalstudies of the Schiff base (SB-2) and its metal complexes SB-2-Co, SB-2-Ni and SB-2-Cu were tested on Gram positive bacteria such as *S.aureus* and *B.cereus* and Gram negative bacteria such as *P.auruginosa*and *E.coli* while their antifungal activities were tested on fungi *C.albicans* and *A.niger*. Well-knownagar-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 standards Gentamycin and Fluconazole are used for antibacterial and antifungal studies respectively. The results are as summarized in the Table-2. Metal complexes in general exhibited better antibacterial and antifungal activity than ligand. Co(II) complex exhibited highest antimicrobial activity among the compounds tested.

Table-2: Antibacterial and Antifungal activities of ligand (SB-2) and itsCo (II), Ni (II) and Cu (II)Complexes

Compound	Concentration µg/ml	S.aureus	B.cereus	P.auruginosa	E.coli	C.albicans	A.niger
SB-2	100	2-	2-	3-	3-	1-	1-
	200	4-	4-	5+	5+	2-	2-
	300	5+	5+	8+	7+	5+	3-
	400	7+	7+	10+	10+	7+	4-
	500	10++	10++	12++	12++	8+	4-
SB-2-Co	100	4-	4-	6+	7+	2-	1-
	200	7+	6+	10++	9+	4-	3-
	300	10++	8+	15++	12++	8+	4-
	400	13++	10++	18++	15++	10++	5+
	500	16++	15++	22+++	20+++	12++	7+
SB-2-Ni	100	4-	4-	6+	4-	1-	2-
	200	6+	6+	9+	6+	3-	2-
	300	8+	8+	11++	8+	5+	4-
	400	11++	9+	13++	12++	8+	5+
	500	13++	11++	16++	15++	9+	5+
SB-2-Cu	100	5+	9+	15++	8+	3-	2-
	200	8+	12++	18++	10++	4-	3-
	300	12++	14++	20+++	12++	7+	4-
	400	16++	20+++	27+++	19++	8+	5+
	500	11++	9+	14++	13++	10++	6+
Standard	100	20+++	25+++	21+++	20+++	21+++	22+++
	200	23+++	27+++	28+++	23+++	25+++	25+++
	300	25+++	30+++	30+++	28+++	30+++	29+++
	400	25+++	35+++	35+++	32+++	35+++	34+++
	500	38+++	37+++	40+++	35+++	42+++	40+++

DMSO	100	1-	1-	2-	1-	1-	1-
	200	2-	2-	2-	2-	1-	1-
	300	2-	2-	3-	3-	2-	1-
	400	3-	3-	3-	3-	2-	2-
	500	3-	3-	3-	3-	2-	2-

Activity Scale	- ve= Inactive (Zone of inhibition <5mm)
	+ve= weakly active (5 ≤Zone of inhibition < 10mm)
	++ve= moderatively active (10 ≤ Zone of inhibition < 20mm)
	+++ve= Highly active (Zone of inhibition ≥20mm)
Standard	Gentamycin for study of antibacterial activity.
	Fluconazole for study of antifungal activity.

3.3 Anticancer activity studies:

The anticancer activity of the ligand (SB-2) 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 2millimolar L- glutamine at 37^0C in a humidified atmosphere of 5% CO_2. About $5X10^3$ cells/well were seeded in 96-well micro titer plate using a culture medium. After 24 hours, Schiff base (SB-2) 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-2) and its Co(II), Ni (II) and Cu(II) complexes are showing anticancer activity 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.The metal complex SB-2-Cu with GI50<10 is superactive in anticancer activity on human malignant breast cancer MCF-7 cell line. Therefore ligand and complexes may prove as lead compounds for *in vivo* screening of anticancer activity against malignant breast cancer.

Ligand (SB-2) is resistant to human colon cancer cell line HT-29 with GI50, >80 µg/ml. Howeverits Cu (II) complex exhibits weak in vitro anti-cancer activity against colon cancer cell line HT-29 which is comparable to Adriamycin with GI50 value 38.4 µg/ml. Hence Cu(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-2) and its complexes on human breast cancer cell lines MCF-7 and colon cancer cell lines HT-29 are shown in Table3 and Table 4.

Table -3: Cytotoxicity of Schiff base (SB-2) and its Co (II), Ni (II) and Cu (II) metal complexes on human breast cancer cell line (MCF-7)

Sr.No	Compound	Drug Concentrations (μg/ml)		
		LC 50	TGI	GI-50
1	SB-2	>80	>80	<19
2	SB-2-Co	>80	>80	15.65
3	SB-2-Ni	>80	79.4	30.4
4	SB-2-Cu	>80	<10	<10
6	ADR	>80	30.11	<10

Table 4: Cytotoxicity of Schiff base (SB-2) and its Co (II), Ni (II) and Cu (II) metal complexes on human colon cancer cell line (HT-29)

Sr.No	Compound	Drug Concentrations (μg/ml)		
		LC 50	TGI	GI-50
1	SB-2	>80	>80	>80
2	SB-2-Co	>80	>80	>80
3	SB-2-Ni	>80	>80	>80
4	SB-2-Cu	75.6	57.0	38.4
6	ADR	NE	<10	<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. LC50= Drug Concentration that kills 50% of the cells NE = Non evaluable data ADR= Adriamycin (Doxorubicin, Positive control drug).

In -vitro testing for anticancer activity in cell lines based on GI50 values shows that ligand SB-2 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.[27-31] Cytotoxicity of ligand SB-2 and its complexes on human breast and colon cancer cell lines is shown in Figure-1 and Figure-2.

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

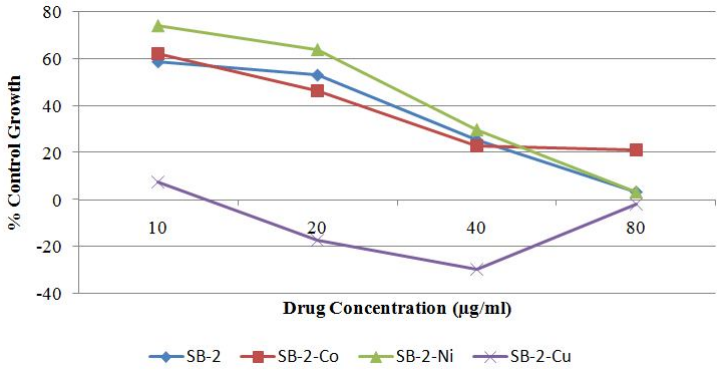
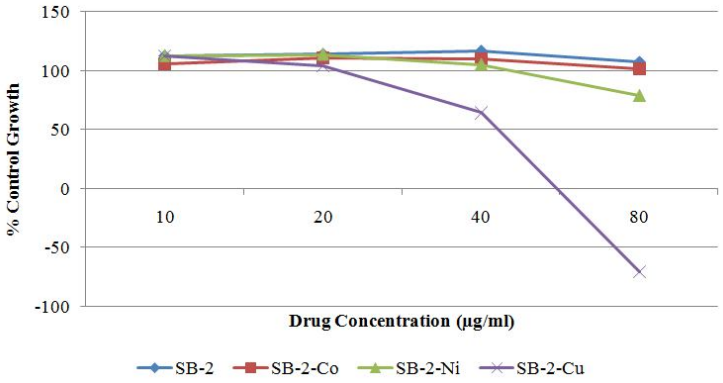


Figure-2 : Cytotoxicity of Schiff bases (SB-2) and its Co (II), Ni (II) and Cu (II) metal complexes on human colon cancer cell line (HT-29).



3.4: Antihaemolytic activity:

Antihaemolytic activity was assessed by spectrophotometric method in a pathology laboratory of Shri Markandeya Sahakari Rugnalaya and Research Centre, Solapur, from a well-nourished healthy adult blood sample was collected and centrifuged at 1500 rpm for 3minutes. 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-2) and itsCo (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-2) was added in 0.5 ml of the cell suspension. After incubation of the mixture at 37^0C 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 [31]. The optical density values and percentage hemolysis (H%) obtained are presented in Table-5.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 500µg/ml.

Table-5: Percentage hemolysis (H%) of Schiff base SB-2 and its Co(II), Ni(II) and Cu(II) metal complexes

Compound		Concentration (µg/mL)				
		100	200	300	400	500
SB-2	OD	0.026	0.028	0.00282	0.0292	0.0312
	H%	1.476	1.645	1.940	2.151	2.573
SB-2-Co	OD	0.0291	0.0300	0.0321	0.0334	0.0340
	H%	2.13	2.320	2.763	3.037	3.164
SB-2-Ni	OD	0.0292	0.0309	0.0324	0.0336	0.0346
	H%	2.151	2.510	2.820	3.080	3.291
SB-2-Cu	OD	0.0292	0.0299	0.0312	0.0333	0.0342
	H%	2.151	2.299	2.573	3.016	3.206
Triton-X-400 Control	OD	1.915	1.915	1.915	2.915	1.915
	H%	400	400	400	400	400
DMSO Control	OD	0.019	0.019	0.019	0.019	0.019
	H%	0	0	0	0	0

4. ACKNOWLEDGEMENT:

I acknowledge, Department of Microbiology, Walchand Centre of Biotechnology, Solapur for providing facility of microbial studies. I am also thankful to ACTREC, Tata memorial Centre, Kharghar, Navi Mumbai for assistance in the study of anticancer activity of sample compounds. I also wish to acknowledge, Department of Pathology, Shri Markandeya Sahakari Rugnalaya and Research Centre, Solapur.

REFERENCES:

[1] M. Gulcan, M. Sonmez, *Phosphorus, Sulfur, Silicon Relat. Elem.* 186,(2011),1962.
[2] P. Rajavel, M.S.S.C. Anitha, *European J. of Chemistry*,5(3) (2008) 620.
[3] E.A. Keskioglu, B.G. Hamuraw , *Spectrochimica Acta Part A*,70(3) (2008) 634
[4] C. Aladin, Ibrahim Yilmaj, Alabibe Ozman and Misir Ahmedizade, *Trans. Met.Chem.*27 (2002) 171.
[5] M. B. Fugu, N.P. Ndahi, B.B. Paul and A.N. Mustapha, *J. Chemical and Pharmaceutical Research,* 5(4) (2013) 2 .
[6] A.H. Manikshete, M.M. Awatade, S.K. Sarsamkar, M.R. Asabe, *International J. of Engg. Sci. Invention* Vol. 4, Issue 1 (2015) 22.
[7] Bo Tang, Taixing Yue, Junsen Wu, Yuming Dong, Yi Ding, Hongjian Wang, *Talanta* 64, (2004) 955.
[8] Sanjit Nayak, Patrick Gamez, Bojan Kozlevcar, Andrej Pevec, Olivier Roubeau, Stefanie Dehnen, Jan Reedjik,*Polyhedron* 29 (2010) 2291.
[9] E.V. Shekhar, K.N. Jayaveera and S. Srihari *J. of Chemical and Pharmaceutical Research,* 4(12) (2012) 5121.
[10] J. Bassett, R. C. Denny, G. H. Jeffery and J. Mendham, *'Vogel's Text Book of Quantitative Inorganic Analysis,* ELBS Longman, England P 849-868 (1985).
[11] M. C. Day (Jr) and Selbin J., *'Theoretical Inorganic Chemistry'*, 2nd Edition. Affil. East West Press, New Delhi P 483-486 (1971).
[12] D. A. Skoog,*"Principles of Instrumental Analysis"* 3rd International Edition, Holt Saunders College Publisher, New York P 344-346, 484, 713-726 (1968).
[13] Earnshaw,*' Introduction to Magneto chemistry'* U. S. Ed. Academic press Inc. New York (1968).
[14] K. Burger,*" Coordination Chemistry Experimental Methods"*, First Edition, East West Press New Delhi, P 215 (1972).
[15] R.M. Silverstein, G.C. Bassler and T.C. Norrid "Spectrometric Identification of Compounds" John Wiley and Sons, 4th edition. (1981).
[16] K. Nakamoto *Infrared and Raman Spectra of Inorganic coordination compounds,* 5th ed., John Wiley and Sons. New York, (1998).
[17] D.T. Sakhare, T.K. Chondhekar, S.G. Shankarwar and A.G. Shankarwar, *Pelagia Research Library, Advances in Applied Science Research,* 6(6) (2015) 10.
[18] Masoumeh Orojloo, Fereshtech Nourian Raziyen Arabahmadi, Saeid Amani *Quimica Nova*, Vol. 38, No.9, Sao Paulo Nov. (2015).
[19] K. Siddappa and Nabiya Sultana Mayana, Hindawi Publishing Corporation *Bioinorganic Chemistry and Applications,* Volume 2014, Article ID 483282.
[20] W.W. Wendladt, *'Thermal methods of analysis'* Heyden Philadelphia (1977).
[21] K. Miyokawa and H. Wakida, *Thermo Chimica Acta* 21 (1991) 182.
[22] R. Selwin Joseyphus; C. Shiju; J. Joseph; C. Justin; Dhanaraj and K.C. Bright *Der Pharma Chimica* 7(6) (2015) 265.
[23] W. Kemp, *'Organic Spectroscopy'* McMillan Press Ltd. 2nd Edition (1991).
[24] E.F. Bowrsand, L.R. Jeffries, *J. Clin. Patho. 8* (1995) 58.
[25] Srinivasan Durairaj, Sangeetha, Srinivasan, P. Lakshmana Perumalasamy, *Elect. J. of Biol.,*5 (1) (2009) 5.
[26] B. Rizwana, Santha Lakshmi S. *International J. ChemTech Research,* Vol.4, No.1, (2012) 464.
[27] A.A. Osowale and S.A. Balogun, *Eur. J. Applied Sciences* 4(1) (2012) 06.

[28] Bao-dui Wang Zheng-Yin Yang et al., *J. Photochem. Photobiol. A*, 194 (2008) 49.
[29] Houghton Peter et al., *Methods*, 42 (2007) 377.
[30] M. M. Lubran Ann *Clin. Lab.Sci.* 19 (1989) 114.
[31] Devajani Chakraborty, Barakha Shah*International Journal of Pharmacy and Pharmaceutical Sciences* Vol.3 (2011) 192.