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Synthesis, Characterization And Biological Evaluation Of Schiff Base Of 5-Chloro Salicylaldehyde Hydrazine N-Methyl Carbo-Thioamide And Its Metal Complexes: Cytotoxicity, Dna Interaction & Incision, Antibacterial And Antifungal Studies

Pilli Jyothi[1], C. Gyana Kumari[2]

[1]*Department of Chemistry, Osmania University College of Science, Telangana, India, 500007*
[2]*Professor, Department of Chemistry, OUCS, Osmania University*

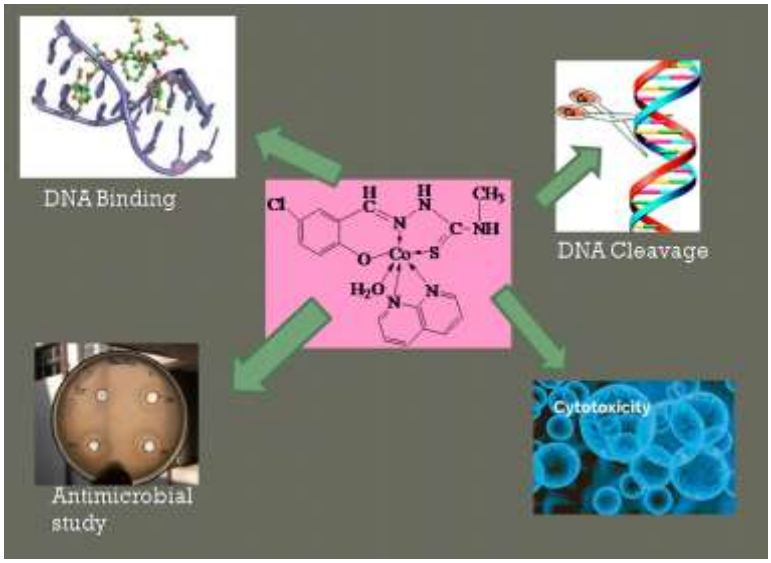
Email: [1]jyothi.p59@gmail.com

Abstract:
A Schiff base ligand of N-methyl Thiosemicarbazide and its complexes with Co metal ion of binary $[Co(L)_2]$ (Complex-A), and ternary, formulae $[Co(L)(L_1)(H_2O)]$ (Complex-B), $[Co(L)(L_2)(H_2O)]$ (Complex-C) where L is Schiff base ligand, L_1 is Bi-pyridine and L_2 is oxalic acid have been synthesized ***and the compounds had been characterized based on the several spectroscopic results.*** ***The interaction of these*** ***compounds with DNA was investigated by electronic absorption spectroscopy and fluorescence spectroscopy. Our experiments indicated that these complexes could strongly bind to CT- DNA via intercalation mode with K_b values of 1.2×10^4 M^{-1} to 5.7×10^4 M^{-1} and the nucleotide incision against super coiled pBR322 DNA had been investigated by both photolytic and oxidative techniques to reveal the efficient manner of cleavage by the metal complexes. Furthermore, these complexes exhibited significant in vitro Cytotoxicity against HeLa & MCF7 cell lines showing IC_{50} values around 46.03 to 62.05 μM. Finally, an antimicrobial assay of these complexes over one gram-positive bacterium of Bacillus Subtilis and one gram-negative bacterium of Escherichia Coli and also two fungal species had given a good result.***

Highlights:

- ***Schiff's base ligand and its metal complexes of Co (II) were synthesized and characterized.***
- ***The cytotoxicity of these metal complexes against MCF7 and HeLa cell lines was evaluated.***
- ***DNA binding studies of these metal complexes were studied by using Electron Absorption & Fluorescence spectra and DNA incision studies had been studied by both photolytic and oxidative methods.***
- ***Metal complexes were also screened for their Antibacterial &Antifungal studies.***
- ***All the complexes exhibited satisfied results in all biological activities.***

Graphical Abstract:



Keywords:

Schiff Base; Metal Complex; Cytotoxicity; DNA Binding; DNA incision; Antimicrobial

1. INTRODUCTION:

Schiff base ligands are one of the well known chelating ligands which play a vital role in the preparation of Metallo-organic hybrid species as they coordinate with different metal ions to form different metal complexes with various oxidation states and have applications for their metal complexes [1]. The Schiff base ligand of Thiosemicarbazide having Oxygen, Nitrogen, and Sulphur as donor sites are widely studied to their complexes has been notably developed during the past years due to their wide range of biological applications including antitumor, antibacterial, antiviral, and anti-malarial activities [2, 3]. Earlier studies had demonstrated that Thiosemicarbazide ligands are less potent than its metal complexes in all activities.

Evaluation of in vitro Cytotoxicity study is a useful tool in the investigation of chemotherapy agents that procures preliminary data for further studies [4]. Despite the occurrence of platinum drugs in cancer treatment, several identified disadvantages exist that remain a challenge for new chemotherapeutics to overcome and to prepare efficient anticancer drugs [5-7]. In general Cytotoxicity activity of compounds could be showed by interacting with DNA, followed by effecting its replication, which inhibits the growth of tumor cells. Hence DNA binding studies of metal complexes own significant research area in life sciences and play a vital role in the study of DNA molecular probes to create new therapeutic reagents, where the efficiency of a drug depends on mode and tendency of binding [8-10]. From our laboratory, we have been published several articles of the synthesis, characterization and biological activities of Thiosemicarbazide Schiff base metal complexes earlier, [11,12] but in this publication, our interest was to focus on other metal complexes that bind to DNA preferentially with extreme curative power and low side effects and also on cleavage ability. Concerning the facts mentioned, we are reporting the synthesis, characterization, cytotoxicity, and DNA interaction studies of Co (II) complexes. UV absorption spectra& fluorescence spectral studies were employed to evaluate the DNA binding studies, and MTT assay was used to assessing Cytotoxicity experiments against HeLa & MCF7 cell lines. Finally, the complexes were also subjected to antimicrobial studies against one gram-positive, gram-negative bacterium and few fungi species. Additionally, compounds were characterized by different spectral methods.

2. PROCEDURE:

Materials and instrumentation:

All the chemicals and solvents were of analytical grade and purchased from Merk, Sigma-Aldrich Chemicals and Hi-Media Ltd. India.

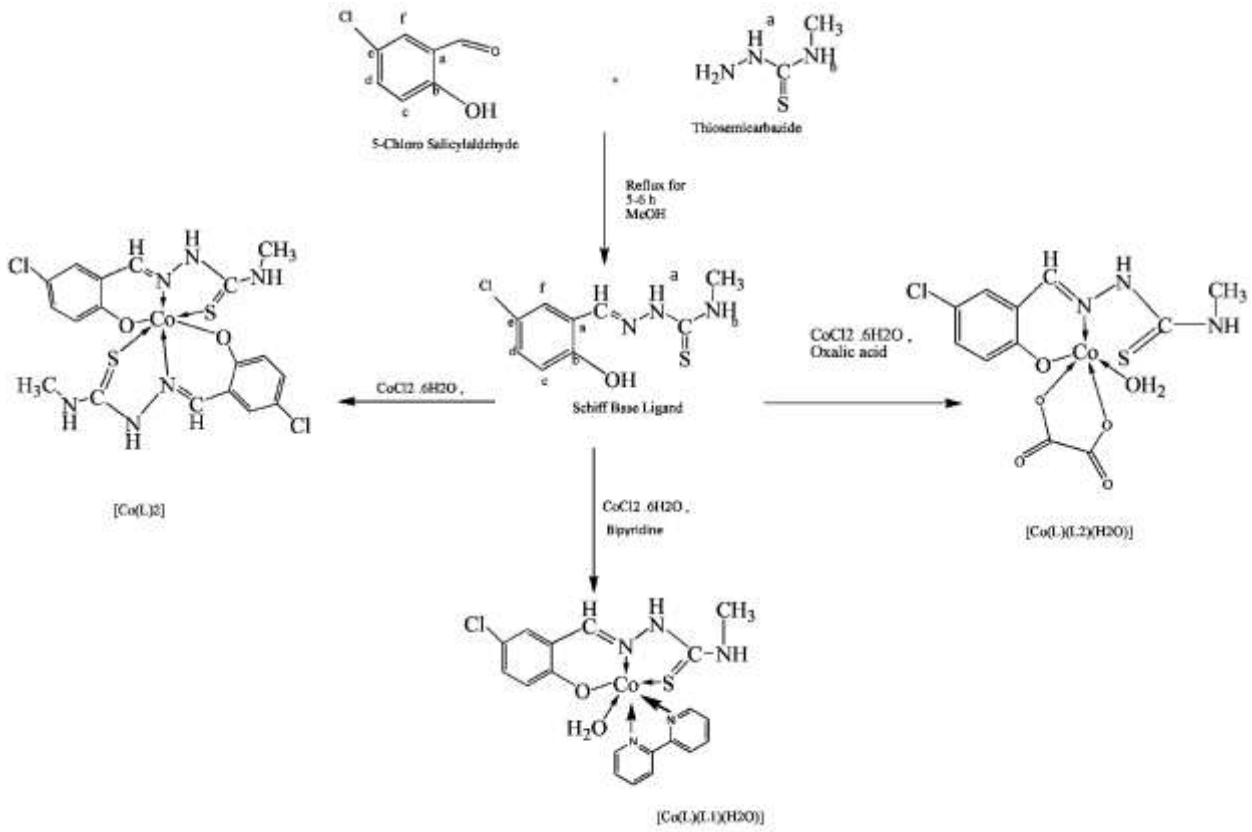
The FT-IR Spectra of metal complexes and Schiff's base ligand were recorded employing KBr disks with a Perkin- Elmer IR spectrometer model 337 between 4000-250 cm^{-1}. H^1-NMR spectrum was recorded in a Bruker 400 MHz spectrometer using TMS (Tri Methyl Silane) as an internal standard. The mass spectra were recorded by the ESI technique on VQ AUTO SPEC mass spectrometer. Fluorescence spectra were recorded on the JASKO Spectro fluorometer FP-8500. Electronic spectral data obtained in the 200-1000 nm range using a Shimadzu UV-3101 PC spectrometer and $BaSO_4$ is used as a reference. Magnetic susceptibility values of complexes were performed on the Gouy balance model 7550 with Hg [Co (NCS) $_4$] as the calibrant. Thermo Gravical analysis of compounds was carried out on a Mettler Toledo Star system about 30-1000 ^{0}C under Nitrogen atmosphere (20 ml / min) at the heating rate of 10 ^{0}C / min.

3. SYNTHESIS:

Schiff Base Ligand:

The Schiff base Ligand (L) has been synthesized by mixing the hot Methanolic solution of (40 ml) N-Methyl Thiosemicarbazide (2.10 g) to a Methanolic solution of 5-Chloro Salicylaldehyde (3.13 g). To this, a few drops of sulphuric acid were added, and the resultant mixture was refluxed for 3 h; the resulting solid product was isolated by filtration and recrystallized from methanol.

Yield 80%; ESI-MS (DMSO): m/z=245(calcd.244) (M+1);
Anal. Calc (%): C, 44.2; H, 4.5; N, 17.2; O, 6.5. found (%): C, 44.3; H, 4.4; N, 17.5; O, 6.4. IR (KBr): $\nu_{(O-H)}$ 3394, $\nu_{(CH=N)}$ 1600, $\nu_{(C=S)}$ 1267. H^1-NMR (400MHz,$CDCl_3$,ppm): 6.8-7.0 (d, H_c, 1H), 7.0-7.5 (d, H_d, 1H), 8.0 (s, H_f, 1H), 11.5 (s,-OH, 1H), 10-10.5(s, -NH_a, 1H), 8.0-8.5(s, -NH_b, 1H), 8.0(s, -CH, 1H), 2.5-3.5 (s, -CH_3, 3H) (Fig.1& Fig.2) (Scheme.1).



Scheme.1 synthesis of Schiff's base ligand and metal complexes

[Co (L) $_2$] complex (Complex-A):

This complex was prepared by mixing the 1: 2 ratio of $CoCl_2$. $6H_2O$ (1 mM) in MeOH (50 ml) and Schiff's base (L) (1mM) in 15 ml methanol and the mixed solutions refluxed for 2-5 h at 70-80 ^{0}C. The product was separated and washed with ethanol and dried in desiccators.

Yield 80%; ESI-MS (DMSO): m/z=545(calcd.544) (M+1). Anal. Calc (%): C, 39.7; H, 3.3; N, 15.4; O, 5.8. found (%): C, 39.8; H, 3.4; N, 15.5; O, 5.7. IR (KBr): ν $_{(O-H)}$ 3552, ν ($_{CH=N}$) 1610, ν($_{C=S}$) 1286. (Fig.3) (Complex-A)

[Co (L) (L_1) H_2O] (Complex-B) and [Co (L) (L_2) H_2O] (Complex-C) complexes:

These complexes were synthesized by mixing $CoCl_2$ .6 H_2O (1 mM) in MeOH (50 ml) and Schiff's base (1 mM) in 15 ml Methanol and refluxed for 2 h. To this, Bipyridine (L_1) (1 mM) and Oxalic acid (L_2) (1 mM) in 15 ml methanol were added to get Complex-B and Complex-C, respectively and refluxed at refluxing temperature for 3 h. The resulting products were filtered, washed with cold ethanol, and dried in Vacuum.

Yield 75%; ESI-MS (DMSO): m/z=474(calcd.474) (M). Anal. Calc (%): C, 48.1; H, 4.0; N, 14.7; O, 6.7. found: C, 48.2; H, 3.9; N, 14.8; O, 6.6. IR (KBr): ν $_{(O-H)}$ 3556, ν ($_{CH=N}$) 1608, ν($_{C=S}$) 1286. (Fig.4) (Complex-B)

Yield 76%; ESI-MS (DMSO): m/z=430(calcd.407) (M+Na). Anal. Calc (%): C, 32.4; H, 2.7; N, 10.3; O, 23.5. found: C, 32.3; H, 2.8; N, 10.4; O, 23.6. IR (KBr): ν $_{(O-H)}$ 3337, ν ($_{CH=N}$) 1610, ν($_{C=S}$) 1292. (Fig.5) (Complex-C)

Cytotoxicity studies:

The Cytotoxicity activity of synthesized compounds was examined on *HeLa* & *MCF7* cell lines adopting MTT dye reduction assay, which is a widely accepted method of evaluation, in the concentration range of 20-80 µM. This method measures mitochondrial activity based on the reductive cleavage of the yellow tetrazolium salt (MTT) to a purple

formazan compound by the dehydrogenase activity of mitochondria. Cells were seeded in 96 well plate culture medium, counted by hemocytometer with a density of 5.0 x 10^3 cells / well in 100 µl media followed by incubation at 37 ^{0}C for 12 h. Then take off the old media and add new media of 100 µl with different concentrations of a test compound. After two days, the cells were washed thrice with buffer and added MTT solution (0.5 mg / ml in phosphate buffer) to each well, followed by plates were allowed to incubate at 37 ^{0}C for three hours, the supernatant was removed and the formazan product obtained was dissolved in DMSO and measured for the absorbance was at 570 nm [13].

The IC_{50} value is used as a criterion for the cytotoxicity study, which defined as "the concentration of compound required to reduces cell growth by 50 %" was determined by using equation $Y = Mx + C$ where $Y = 50$, M and C values were calculated from the viability graph. The cytotoxicity efficiency of a drug relies on its ability to bind with DNA and inhibiting the replication and transcription process, which leads to cell death.

DNA Binding Studies:

Electron Interaction study is an effective method of determining the binding modes of prepared compounds with DNA, which results in drug-DNA interactions. The binding affinity of Schiff base and metal complexes with DNA by the absorption titrations were being done at room temperature by maintaining the concentration of compound constant (10 µM) and altering the concentration of CT-DNA about 0-10 µM [14]. The resulted DNA solutions were allowed to incubation for five minutes for each titration, and then recorded the spectrum. The binding constant (K_b) of the compound can be determined by a plot made between [DNA] / (E_a-E_f) Vs [DNA] from which, the binding ability of the complex can be predicted. [15, 16]

Fluorescence quenching study further supported the Absorption spectral results about the mode of binding with DNA by using the Ethidium Bromide (EB) displacement method to investigate the relative binding ability of complexes with DNA in comparison with EB. EB is one of the potent probes that bind to DNA through intercalation resulting in the enhancement of the intensity of fluorescent spectra of EB bound DNA.

The competitive fluorescence binding of Schiff base and metal complexes with CT-DNA were carried out in TAE buffer (40 mM Tris-acetate, 1 mM EDTA) at 25 ^{0}C on fluorescence spectrophotometer. The intensity of the EB- bound CT-DNA was measured in the range about 520-800 nm by maintaining the concentration of EB-bound DNA as constant and increasing the concentration of the compounds from 0-100 µM. The Stern-Volmer constant was calculated for each complex to evaluate the binding properties of metal complexes.

DNA Incision study:

Cleavage of Super coiled pBR322 DNA with the prepared compounds have been studied using the Oxidative (in presence of H_2O_2) and Photolytic (under UV light) methods using agarose gel electrophoresis technique. DNA was treated with metal complexes of different concentrations and diluted with Tris-HCl buffer having P^H of 7.2. Sample solutions were incubated at 37 ^{0}C for 3 h followed by the addition of Bromo Phenol Blue about 2 µl. Then sample solutions were placed on to the agarose gel and carried out to Electrophoresis for 45 min at 70 V and the resultant bands were being photographed.

The nuclease activity of the compounds can be expressed in the form of conversion of Form-I (ds circular DNA) to Form-II (nicked circular DNA). If incision takes place on the single strand of the ds circular DNA, it relaxes to form-II, which moves slightly slower than Form-I and if incision takes on both the strands, it relaxes to Form-III, which migrates between Form-I and Form-II.

Antimicrobial Study:

The Schiff base and its metal complexes were screened for their in-vitro antibacterial activity against one gram-positive bacterium of *Bacillus Subtilis* and one gram-negative bacterium of *Escherichia Coli* and antifungal activity against *Fusarium Oxysporium Lycopersicum* and *Fusarium recini* by employing the Agar disc diffusion method using Ampicillin and ketoconazole were used as standard drugs for antibacterial and antifungal activity respectively where the cultures are maintained on agar solution and incubated at 37 ^{0}C for 24 h. Wells of 5 mm size were cut and loaded with various concentrations of the complexes followed by incubation at 37 ^{0}C for 24-48 h. The activity was determined by measuring the diameters of the inhibition zone (mm) [17].

4. RESULT AND DISCUSSION:

Characterization:

Mass Spectral evidence has given the apparent confirmation of the formation of ligand and complexes (Fig.2 – Fig.5).

The IR spectrum of a free ligand showed bands at 3394 cm^{-1}, 1554 cm^{-1}, and 1267 cm^{-1}due to phenol OH, azomethine nitrogen and thioketo sulfur were found to be altered in the IR spectra of metal complexes, indicating coordination takes place by them. Moreover, the formation of Co metal complexes was also revealed by the presence of medium intensity υ (Co-O), υ (Co-N), and υ (Co-S) bands in the region of 576-582 cm^{-1}, 468-484 cm^{-1} and 356-420 cm^{-1} respectively. Additionally, the presence of a water molecule that coordinated to the Co(II) metal ion in the complexes indicated by the appearance of broadband around 3350-3480 cm^{-1} and weaker bands around 800-870 cm^{-1} range [18-20] (Fig.6 – Fig.9).

The UV-DRS spectrum of the Co (II) metal complexes exhibited bands in the region of 200 to 400 nm, which is indicative of metal to ligand charge transfer transitions (MLCT). Further, the spectrum also showed one broadband with an elegant structure in the range of 700-800 nm due to spin allowed d-d transition υ_1 [(T_{1g} (F) - T_{2g} (F)] is a characteristic of an octahedral geometry which also proven by the magnetic moment value of 4.8 BM. [21] (Fig.10 –Fig.13).

The elemental composition of ligand and metal complexes had shown in EDX spectra are depicted in *Table.1.*

Cytotoxicity studies:

The Cytotoxicity results were examined by cell viability curves and given with IC_{50} values (Fig.14). The IC_{50} values of Schiff base and metal complexes were presented in *Table.2*. The results proved that cell viability is decreased by increasing the concentration of the compound and Complex-B is found to more potent when compared to other complexes. Probably the higher cytotoxicity had shown by the compounds could be related to the interacting ability of the complexes with DNA. [22, 23]

s.no.	Sample name	IC_{50} (µM)	
		MCF 7	HeLa
1	Schiff base ligand	62 ± 0.5	61±0.5
2	Complex-A	59± 0.2	57±0.2
3	Complex-B	48±0.5	46±0.3
4	Complex-C	54±0.2	53±0.3

Table.2 IC_{50} values of Schiff base and metal complexes against HeLa and MCF7 cell lines

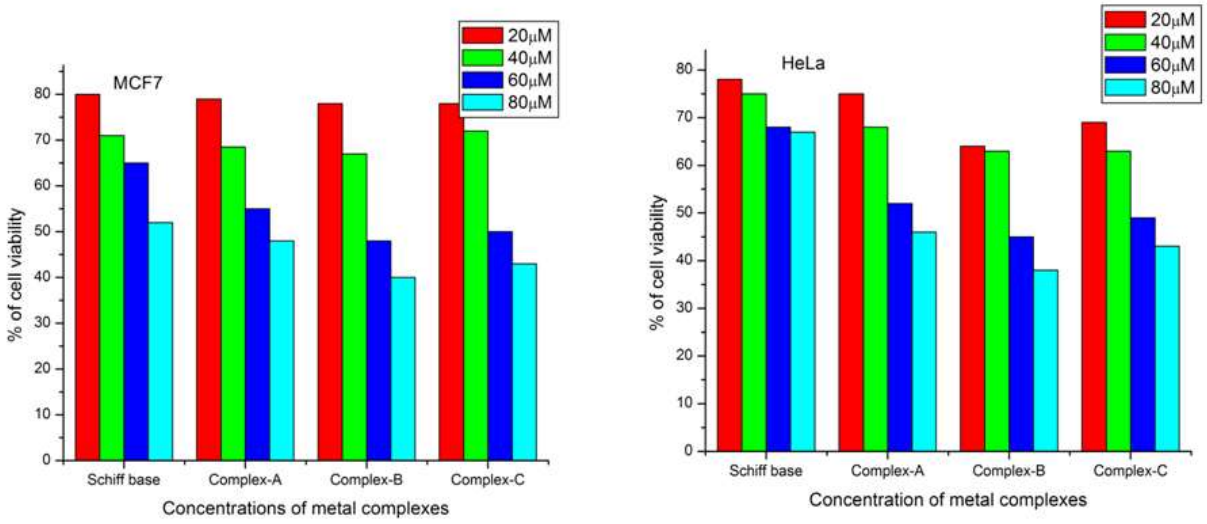


Fig.14 Percentage of cell viability versus compounds in the concentration range of 20-80µM exposed to HeLa & MCF7 cell lines

DNA Intercalation Studies:

In Electron absorption study, the binding of synthesized compounds to the DNA was found to be a reduction in intensities about 10-25 % (hypo chromic) and red shift in wavelength owing to abundance binding between aromatic chromophore and the DNA base pairs [24, 25] indicates intercalative mode of binding has existed *(Fig.15).* The k_b values for compounds are found to be 1.05 x 10^4 M^{-1}, 1.2 x 10^4 M^{-1}, 5.7 x 10^4 M^{-1}, 2.8 x 10^4 M^{-1} for Schiff base, complex-A, complex-B, complex-C respectively. However, the lighter group-Chlorine showed its mark towards increasing binding affinity of the complex slightly, in comparison with earlier reported articles.Though, the results were not as reliable as Ethidium bromide [EB], a potential intercalator [26, 27].

Fluorescence quenching results revealed that there is a reduction in the intensity of EB bound DNA was observed on the addition of compounds to EB-DNA due to the aggressive binding of complexes to the EB-DNA system by replacing the bounded EB from DNA [28, 29]. Intercalative mode of binding has been taken place as the emission intensity at 595 nm declined progressively by raising the concentration of the compound *(Fig.16).*The fluorescence quenching constants were found to be between 1.4 x 10^4 to 3.0 x

10^4 M^{-1}, consistent with the order Complex-B > Complex-C > Complex-A > Schiff base which denotes they are good in agreement with the results of absorption studies.

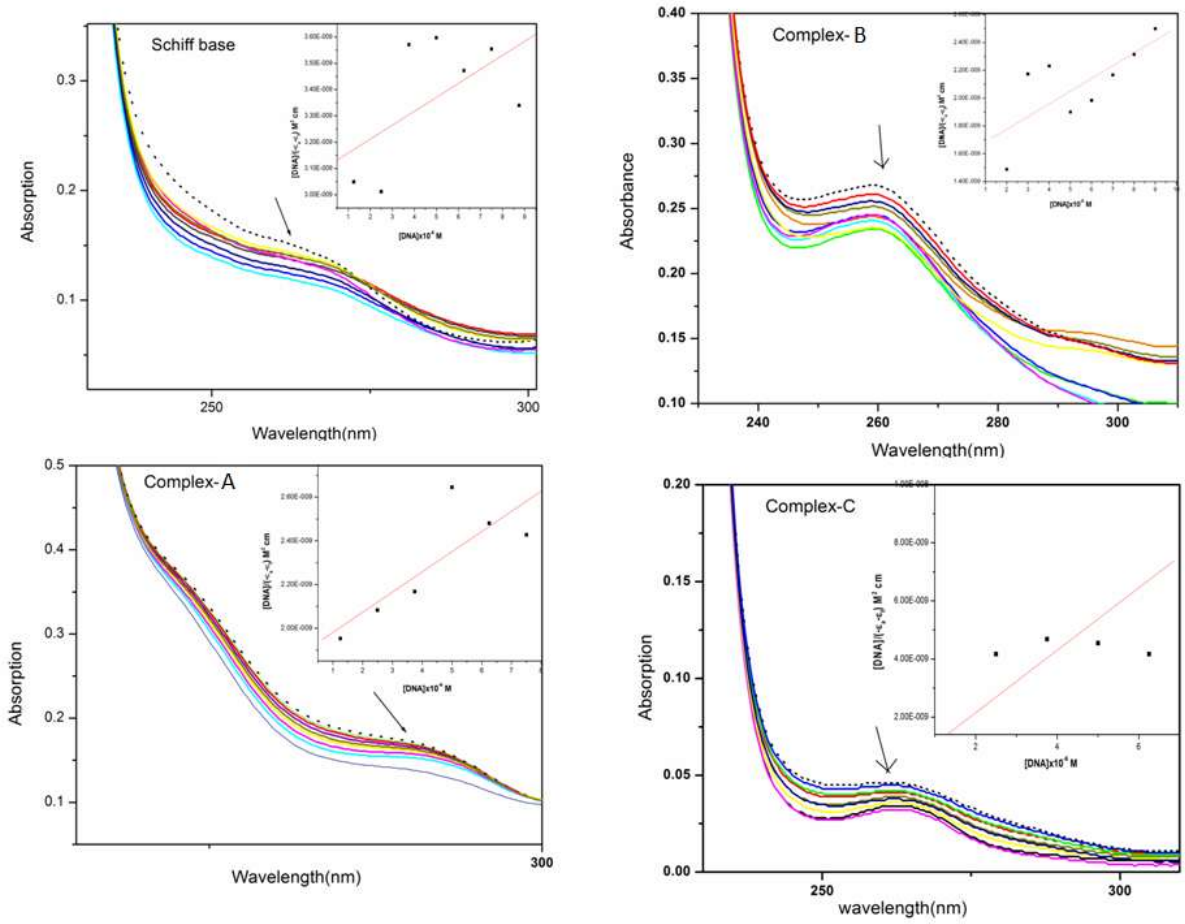


Fig.15 *Electron Absorption Studies of the Schiff base and metal complexes in the absence (dashed line) and presence (solid lines) of increasing amounts of CT-DNA ([DNA] = 0-10 µM), [complex] = 10 µM). Inset: Linear plots used for the calculation of K_b values. Arrow mark (↓) shows the hypochromism upon increasing DNA concentration*

DNA Incision Study:

In the DNA incision study, the DNA control (not treated with test compound) didn't show any incision in both methods (shown in of lane 1) and H_2O_2 alone (absence of compound) also didn't show any effect on the oxidative cleavage of DNA (shown in lane 2 of *Fig.17*). The complexes showed remarkable cleavage capacity at 50 µM concentration *i.e. Form-II* (lane 3-6 in oxidative cleavage and lane 2-3 in photolytic cleavage). Relatively all the complexes have shown greater incision capacity than Schiff base (*Fig.17 and Fig.18).*

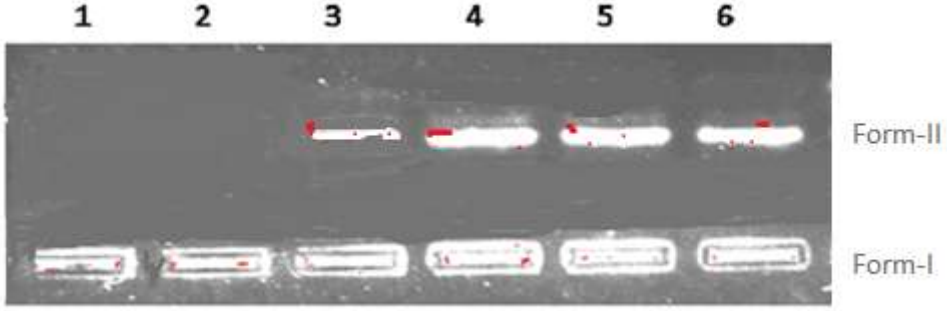


Fig.17 *Oxidative cleavage of pBR 322 DNA (0.2* µg / ml) *at 37 ^{0}C in TAE buffer by the Schiff base and metal complexes. Lane 1: DNA control, Lane2: DNA+ H_2O_2 (1µM), Lane 3-6: DNA+ Schiff base, DNA + complex-A, DNA + complex-B, DNA + complex-C in 50 µM respectively.*

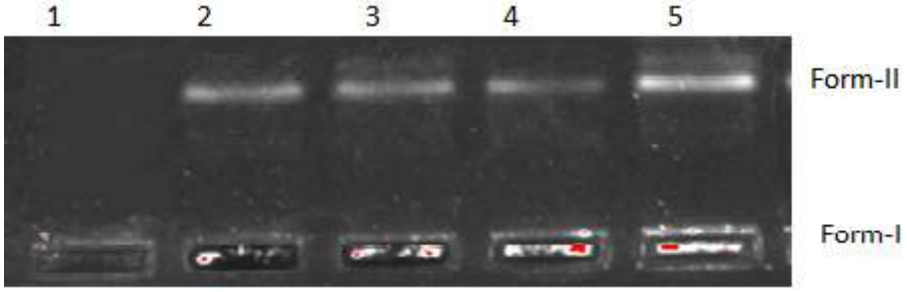


Fig.18 Photolytic cleavage of pBR 322 DNA (0.2 µg/ml) at 37 ^{0}C in TAE buffer by the Schiff base metal complexes using UV light at 345 nm. Lane 1: DNA control, Lane 2-4: DNA+ Schiff base, DNA + complex-A, DNA + complex-B, DNA + complex-C in 50 µM respectively.

Anti-Microbial activity:

Metal complexes showed the higher zone of inhibition than Schiff base *(Fig.19).* Interestingly the synthesized metal complexes showed more potent inhibition of fungal abilities than antibacterial with significant inhibition zone (diameter). The more vigorous activity may be attributed to the fact that the lipophilicity, which controls antimicrobial activity [30, 31].

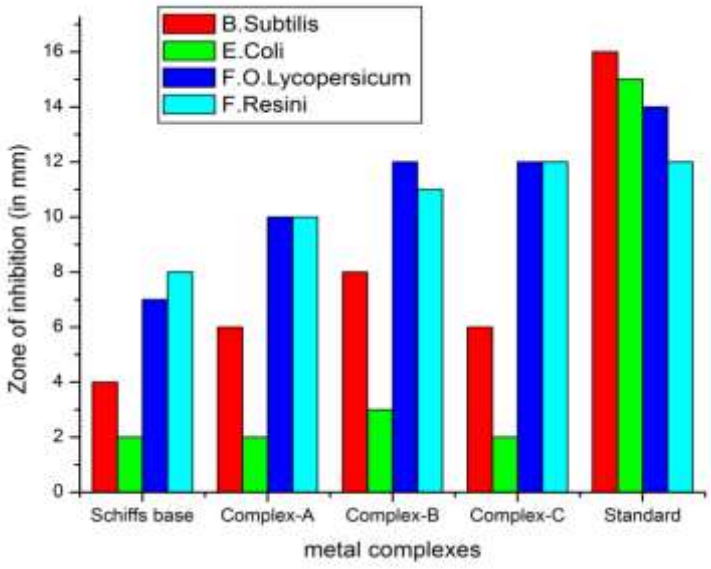


Fig.19 Antimicrobial activity of Schiff base and metal complexes comparing with standards

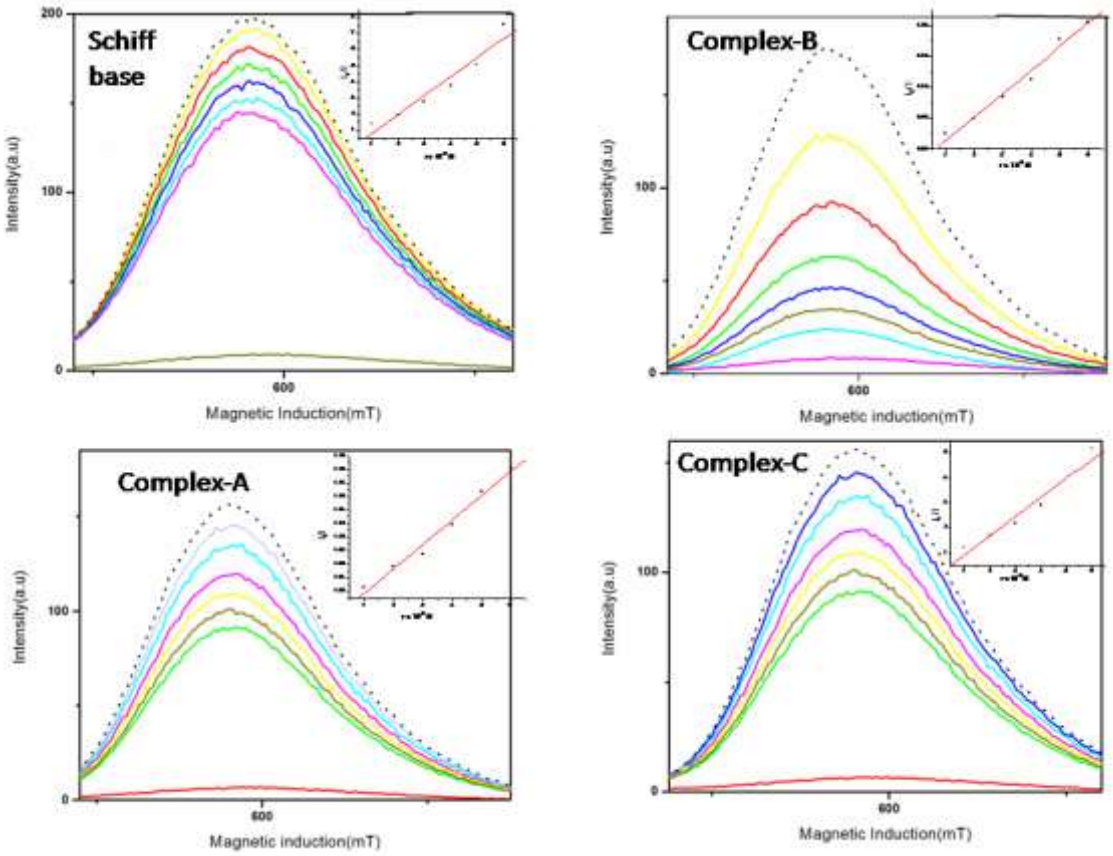


Fig.16 Fluorescence Emission spectra of EB bound DNA in the absence (dashed line) and presence of synthesized compounds (0-100 µM) [EB] = 12.5 µM, [DNA] = 125 µM Inset: the plot of emission intensity I_o / I Vs [complex].

Compound	% of C,H,N analysis found(calculated)							Molecular structure
	C	H	N	S	O	Co	Cl	
Schiff's base	44.3 (44.2)	4.4 (4.5)	17.5 (17.2)	13.2 (13.1)	6.4 (6.5)	-	14.4 (14.5)	$C_9H_{11}N_3OSCl$
Complex-A	39.8 (39.7)	3.4 (3.3)	15.5 (15.4)	11.5 (11.7)	5.7 (5.8)	10.6 (10.8)	13.1 (13.0)	$C_{18}H_{18}N_6O_2S_2Cl_2Co$
Complex-B	48.2 (48.1)	3.9 (4.0)	14.8 (14.7)	6.5 (6.7)	6.6 (6.7)	12.4 (12.4)	7.8 (7.7)	$C_{19}H_{19}N_5O_2SClCo$
Complex-C	32.3 (32.4)	2.8 (2.7)	10.4 (10.3)	7.8 (7.8)	23.6 (23.5)	14.3 (14.4)	8.8 (8.7)	$C_{11}H_{11}N_3O_6SClCo$

Table.1 Elemental analysis of ligand and metal complexes

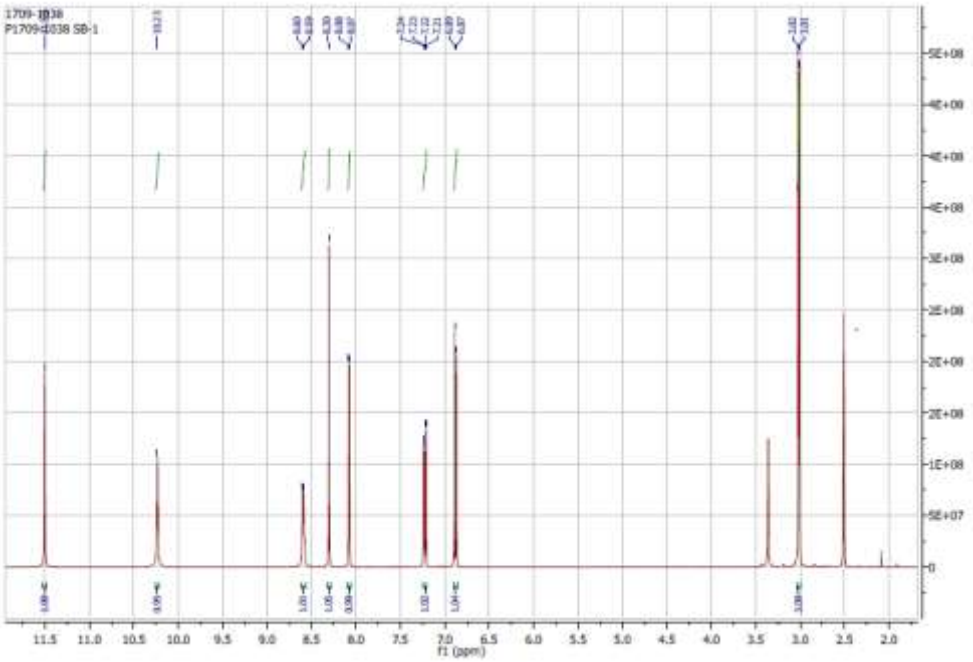


Fig.1 H^1- NMR spectra of schiffs base ligand

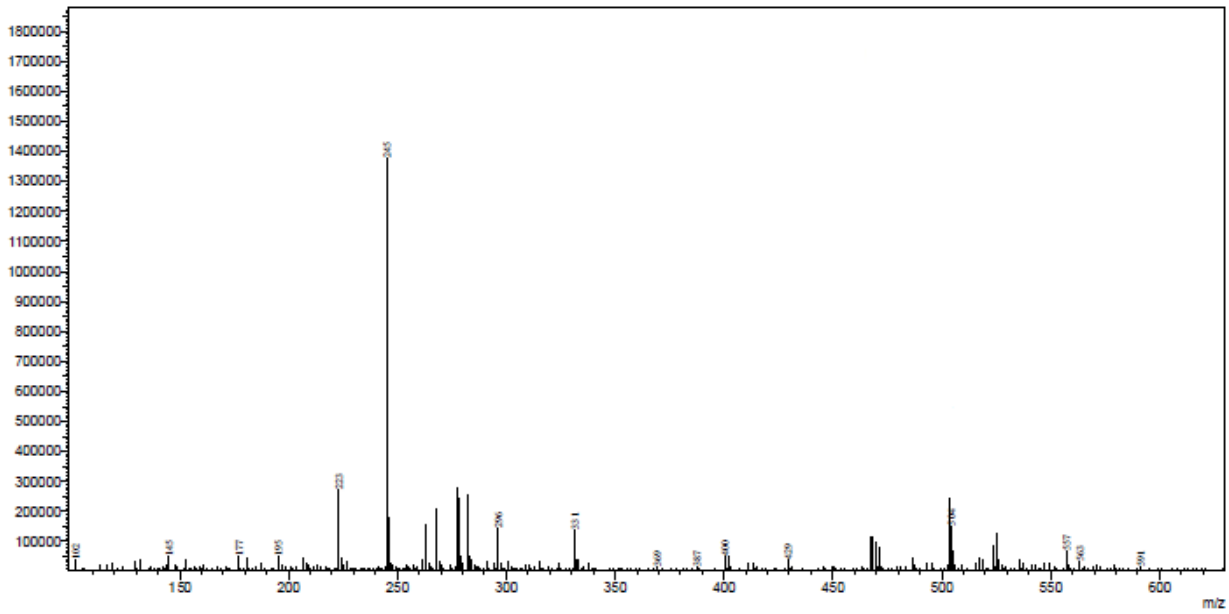


Fig.2 Mass spectrum of schiffs base ligand

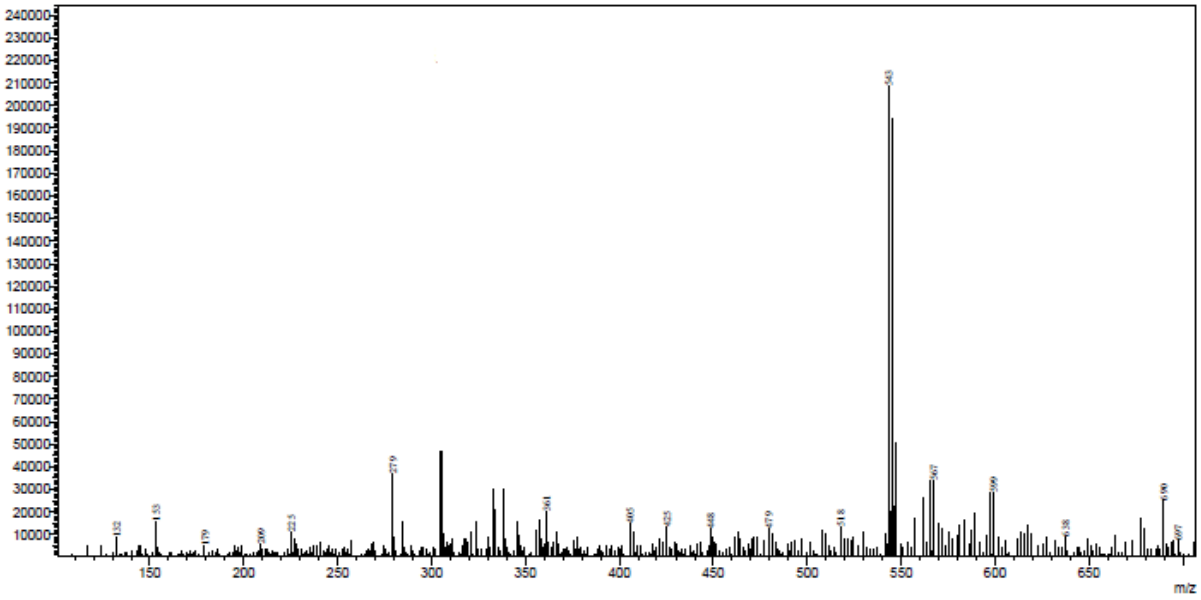


Fig.3 Mass spectrum of Complex-A

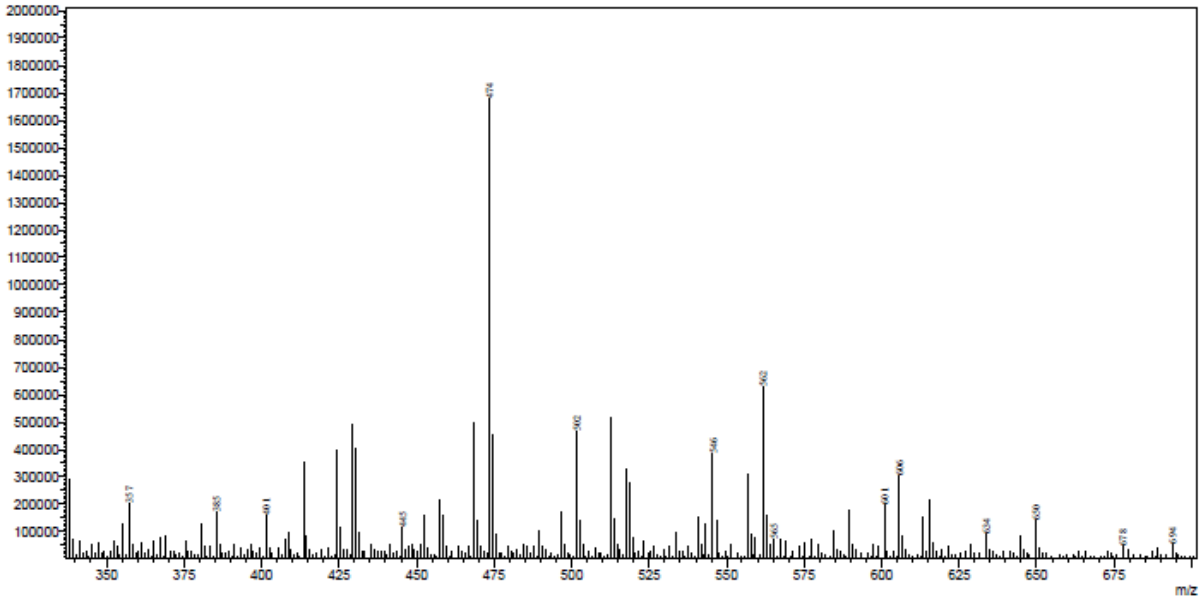


Fig.4 Mass spectrum of Complex-B

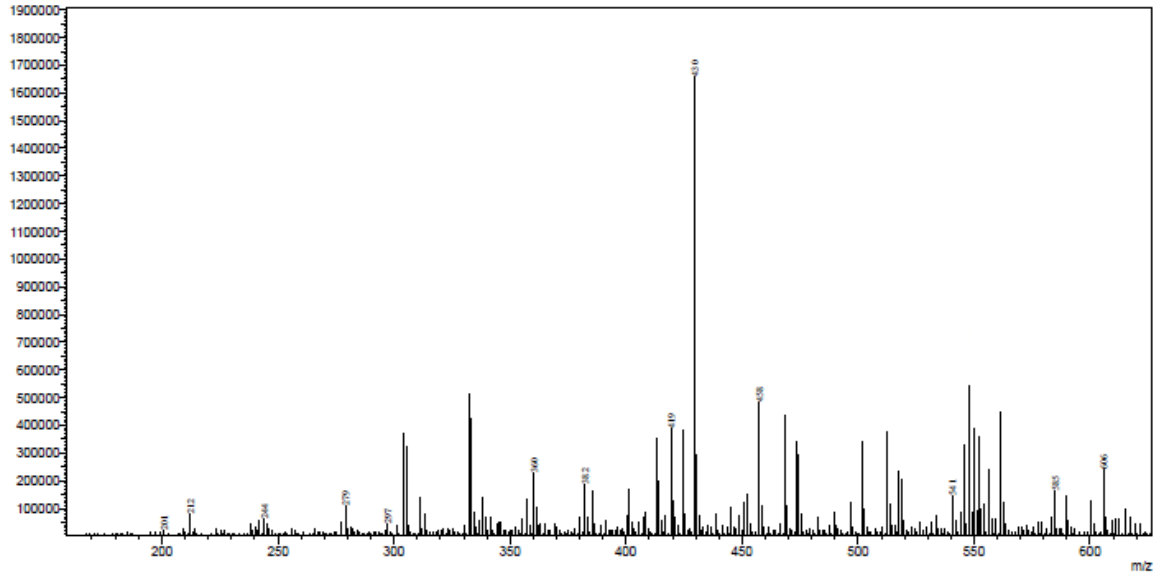


Fig.5 Mass spectrum of Complex-C

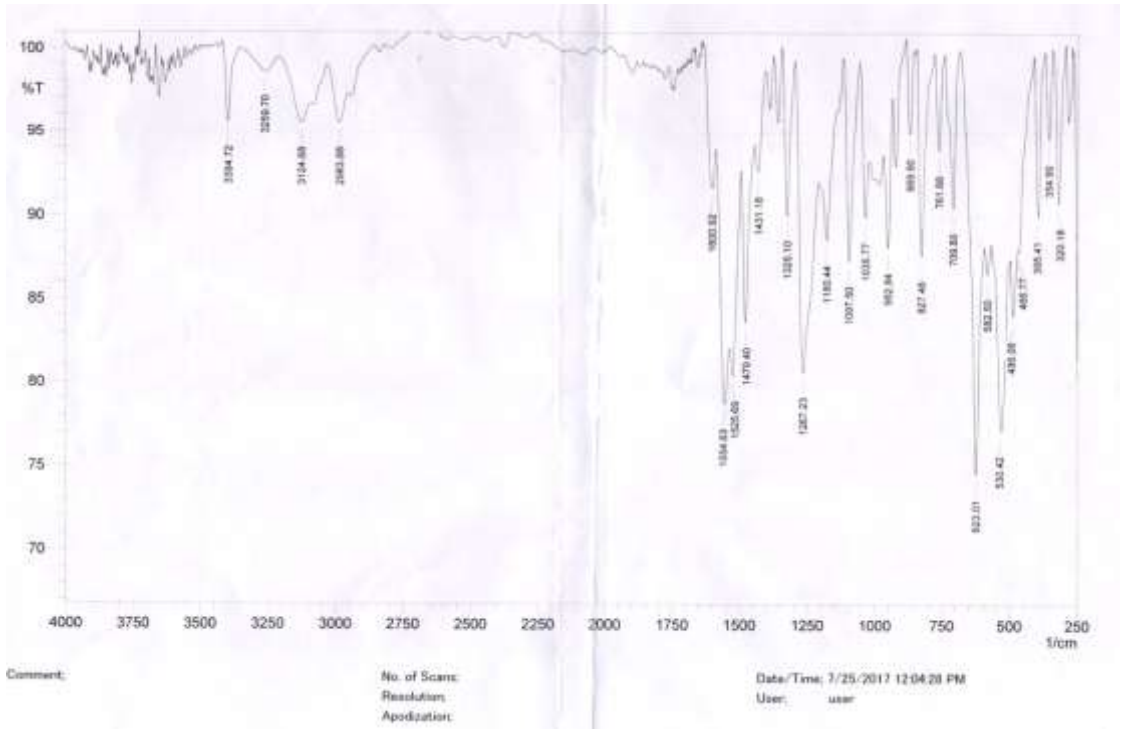


Fig.6 IR spectrum of schiffs base ligand

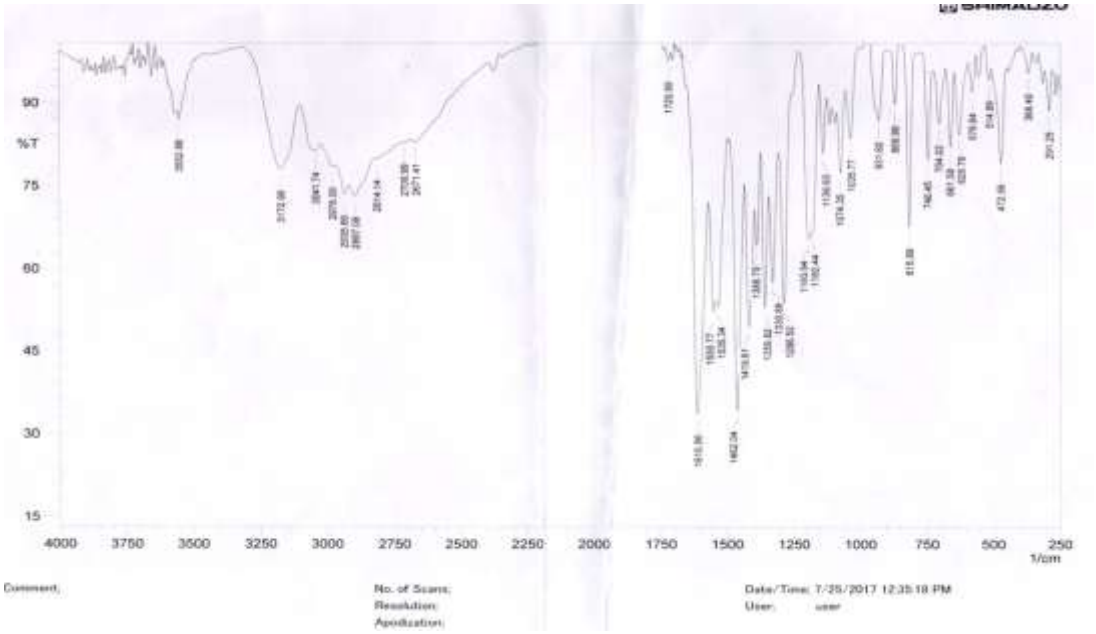


Fig.7 IR spectrum of Complex-A

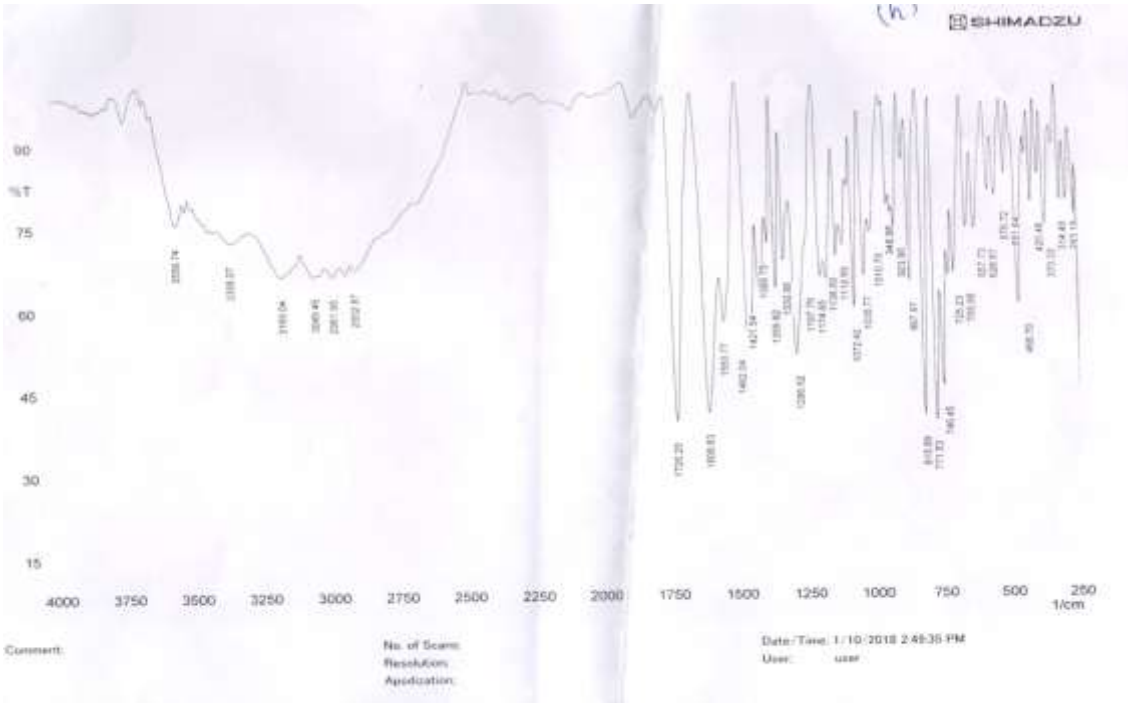


Fig.8 IR spectrum of Complex-B

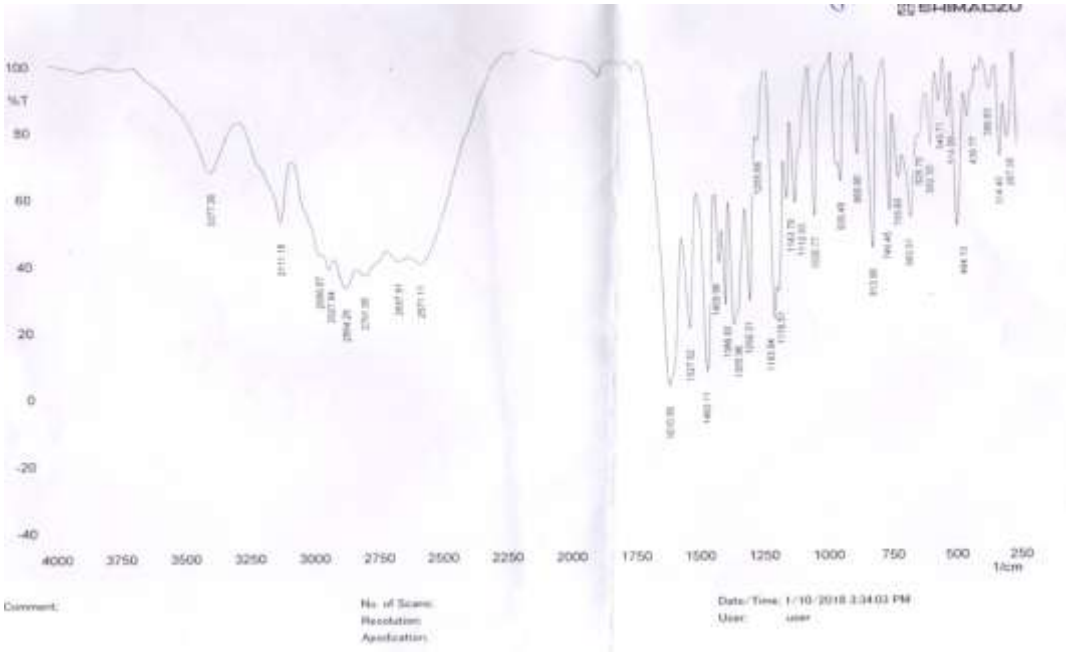


Fig.9 IR spectrum of Complex-C

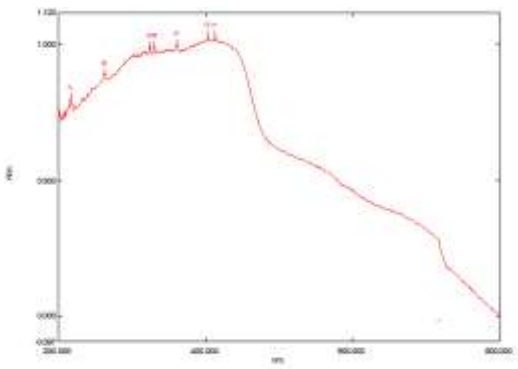


Fig.10 UV-DRS of Schiff's base ligand

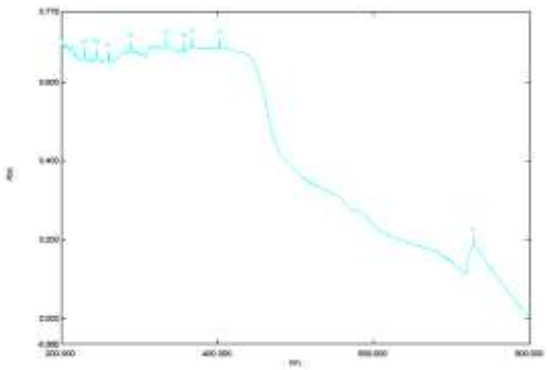


Fig.11 UV-DRS of Complex-A

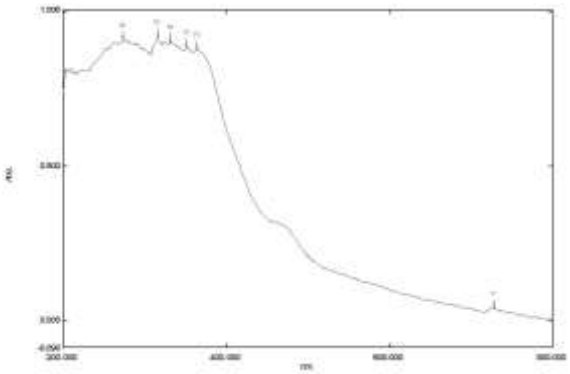


Fig.12 UV-DRS of Complex-B

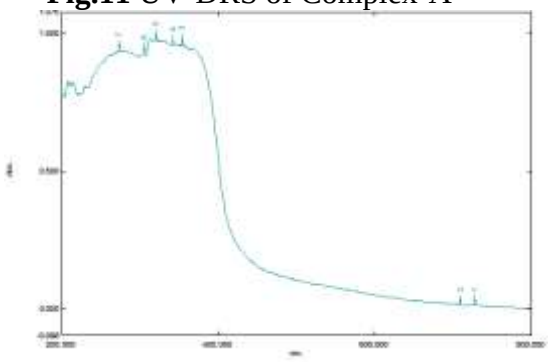


Fig.13 UV-DRS of Complex-C

5. CONCLUSION:

In this paper, we have illustrated that the synthesis of Schiff's base ligand & it's Co(II) complexes and characterization had been done by the elemental data and spectral analysis suggesting octahedral geometry to the compounds consisting with Schiff base ligand acts as a tridentate ligand with O, N, and S are donor sites.

The complexes were actively interacted with CT-DNA, and the binding mode of compounds with CT-DNA was determined as intercalation mode, evaluated by UV-Visible absorption spectroscopy & Fluorescence studies. Besides, complexes can also cleave the pBR322 super coiled DNA efficiently.

The compounds can also inhibit the growth of MCF7 and HeLa cancer cells effectively in MTT assay. Additionally the compounds were also showed moderate to maximum antibacterial and antifungal activities. Further, concluded that in all aspects synthesized metal complexes are more potent than Schiff base ligand.

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Declarations:

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Conflicts of Interest:

The authors declare that they have no conflicts of interest.