

**SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL STUDIES ON MIXED LIGAND COMPLEXES OF CO (II), NI (II) AND CU (II) WITH ISOXAZOLE SCHIFF BASE AND 1, 10-PHENANTHROLINE/ 2, 2' -BIPYRIDINE LIGANDS**

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**Abstract**

Synthesis, Characterization and antimicrobial studies of Cobalt (II), Nickel (II) and Copper (II) ternary complexes of mixed ligands with Schiff base derived from 3-amino 5-methyl isoxazole with 2-hydroxy 1-naphthaldehyde and 1, 10-phenanthroline/ 2, 2' bipyridine. The micro analytical, magnetic moment, IR and electronic spectral data analysis have been used to confirm the structure of these complexes, their lower electrical conductance values indicates that all the complexes are non- electrolytes. The magnetic moment values and electronics spectral data of the Co (II) and Ni (II) complexes further indicates the octahedral geometry and Cu (II) complexes are tetragonal geometry. The synthesized compounds have been tested against microorganisms such as (*bacillus* and *pseudomonas*) bacteria and (*R.Saloni* and *A. niger*) fungi. A comparative study of the MIC (minimum inhibitory concentration) values of the ligands and their complexes indicates that the complexes exhibit higher antimicrobial activity than the free ligand and control.

**Key words** Isoxazole Schiff base and 1, 10-phenanthroline/ 2, 2'-bipyridine ligands; antimicrobial activity.

**Introduction**

The coordination chemistry of Schiff bases as multi dentate ligands gained much importance for more than two decades because of their use as models in biological system (1-4). Transition metal complexes of 1, 10-phenanthroline (phen), 2,2'-bipyridine (bpy) or their modified variants are widely employed in several research areas including bioinorganic and biomedical chemistry (5-12). Ternary complexes with 1,10-phen as the ligand moiety presented in this work is of considerable interest because of biological or pharmacological properties (anti fungal, anti mycoplasma and antiviral) some of its metal complexes (13). The metal complexes of Schiff bases derived from condensation of 3-amino 5-methyl isoxazole with substituted salicylaldehyde and heterocyclic aldehyde were prepared with metal ions Co (II), Ni (II), Cu (II) and Zn (II).

These complexes characterized by elemental analysis magnetic moment and spectral analysis (IR, UV,  $^1\text{H-NMR}$  and mass) antimicrobial activities of the Schiff bases and their metal complexes were also screened (14). In review of this, we report here in the synthesis, structural characterization and antimicrobial studies of the Co (II), Ni (II) and Cu (II) complexes derived from mixed ligands of MIIMN Schiff base and 1,10-phenanthroline/ 2,2' bipyridine. The ligand MIIMN reported earlier, synthesized in our laboratory with known method and the spectral data and elemental analysis are coinciding with literature data (15).

## Experimental

### *Synthesis of ternary metal complexes*

The appropriate quantity of MIIMN was dissolved in methanol and a solution of  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ /  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ /  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  in methanol was added. The mixture was stirred for 30 minutes at  $70^\circ\text{C}$ , and then a solution of 2, 2' Bipyridine or 1, 10-Phenanthroline in methanol was added drop-wise. The reaction mixture was refluxed with stirring further for 3 hours at  $80^\circ\text{C}$ . The precipitated coloured solid was then filtered, washed with cold methanol and dried in vacuum desiccators.

### *Physical measurements*

$^1\text{H-NMR}$  spectra of the ligands were recorded at 200 MHz and 300 MHz on Varian Gemini Unity Spectrometer using TMS as internal standard. The EI mass spectra were recorded on a VG micro mass 7070-H Instrument, ESIMS spectra were recorded on VG AUTOSPEC mass spectrometer. IR spectra of the ligand and complexes were recorded using KBr pellets in the range ( $4000\text{-}400\text{cm}^{-1}$ ) on Perkin-Elmer Infrared model 337. Electronic spectra of metal complexes in DMSO were recorded on Shimadzu UV-VIS by using micro analytical techniques on Perkin Elmer 240C (USA) elemental analyzer. TGA of complexes were carried on Mettler Toledo Star system in the temperature range of  $0\text{-}1000^\circ\text{C}$ . Melting points of the ligands and decomposition temperature of complexes were determined on Polmon instrument (model No. MP-96). The conductivity measurements were measured in DMSO solutions (0.001 M) using Digisun Electronic Digital conductivity meter of model: DI-909 having a dip-type cell calibrated with KCl solution.

### *Antibacterial activity*

The invitro biological screening effects of the investigated compounds were tested against bacteria *Bacillus* and *Pseudomonas* by the well diffusion method using agar nutrient as the medium. The stock solutions were prepared by dissolving the compounds (0.001-0.007 M) in ml of DMSO. In a typical procedure, a well (50  $\mu\text{g}$ ) was made on the agar medium inoculated with microorganisms. The well was filled with the test solution using a micropipette and the plate was incubated at  $35^\circ\text{C}$  for 48hr. During this period, the test solution diffused and the growth of the inoculated microorganisms was affected. (Inhibitory) activity. The lowest concentration which completely inhibited visible microbial growth was recorded as the minimum inhibitory concentration (MIC, g/ml)

### Antifungal screening

After solidification of media. Petri plates were inoculated with actively growing culture of *A. niger* and *R. solani* separately. Filter paper discs of 4mm diameter were dipped in the test solution (DMSO). After drying the disc, it was kept on potato dextrose agar in Petri plates seeded with *A. niger* and *R. solani* and incubated at 35°C for 48hrs the Petri dishes were checked for growth inhibition zone. The presence of clear zone of growth inhibition around the paper disc indicates the compound was considered to be active. The diameter of zone of inhibition was calculated in millimeters.

### Results and Discussion

All the complexes are colored, stable and unaffected by air and moisture. They melt at higher temperatures. They are insoluble in water and alcohols but all ternary complexes are completely soluble in DMSO. The analytical data of all ternary complexes are presented in Table-1. The data in the table reveal that the metal to ligand ratio is 1:1:1.

**Table-1. Analytical data of all the Ternary complexes are presented.**

Complexes	Formula	M.Wt	C	H	N	M
[Co (II)-(MIIMN)-(1,10phen)(H <sub>2</sub> O) <sub>2</sub> ] <sup>+1</sup>	[CoC <sub>27</sub> H <sub>24</sub> N <sub>4</sub> O <sub>4</sub> ]	521.00	62.05 (62.16)	4.35 (4.61)	10.45 (10.75)	10.06 (10.17)
[Ni (II)-(MIIMN)-(1,10phen)(H <sub>2</sub> O) <sub>2</sub> ] <sup>+1</sup>	[NiC <sub>27</sub> H <sub>24</sub> N <sub>4</sub> O <sub>4</sub> ]	524.00	61.52 (61.83)	4.45 (4.58)	10.56 (10.69)	10.52 (10.72)
[Cu (II)-(MIIMN)-(1,10phen)(H <sub>2</sub> O) <sub>2</sub> ] <sup>+1</sup>	[CuC <sub>27</sub> H <sub>24</sub> N <sub>4</sub> O <sub>4</sub> ]	526.00	61.23 (61.60)	4.13 (4.56)	10.35 (10.65)	10.95 (11.03)
[Co (II)-(MIIMN)-(2,2' bipy)(H <sub>2</sub> O) <sub>2</sub> ] <sup>+1</sup>	[CoC <sub>23</sub> H <sub>22</sub> N <sub>4</sub> O <sub>4</sub> ]	471.00	58.32 (58.60)	4.53 (4.67)	11.68 (11.89)	11.13 (11.25)
[Ni (II)-(MIIMN)-(2,2' bipy)(H <sub>2</sub> O) <sub>2</sub> ] <sup>+1</sup>	[NiC <sub>23</sub> H <sub>22</sub> N <sub>4</sub> O <sub>4</sub> ]	474.00	58.12 (58.23)	4.38 (4.64)	11.45 (11.81)	11.64 (11.83)
[Cu(II)-(MIIMN)-(2,2' bipy)(H <sub>2</sub> O) <sub>2</sub> ] <sup>+1</sup>	[CuC <sub>23</sub> H <sub>22</sub> N <sub>4</sub> O <sub>4</sub> ]	476.00	57.65 (57.98)	4.32 (4.62)	11.65 (11.76)	11.56 (11.78)

(The values mentioned in bracket are calculated values)

### Infrared spectra

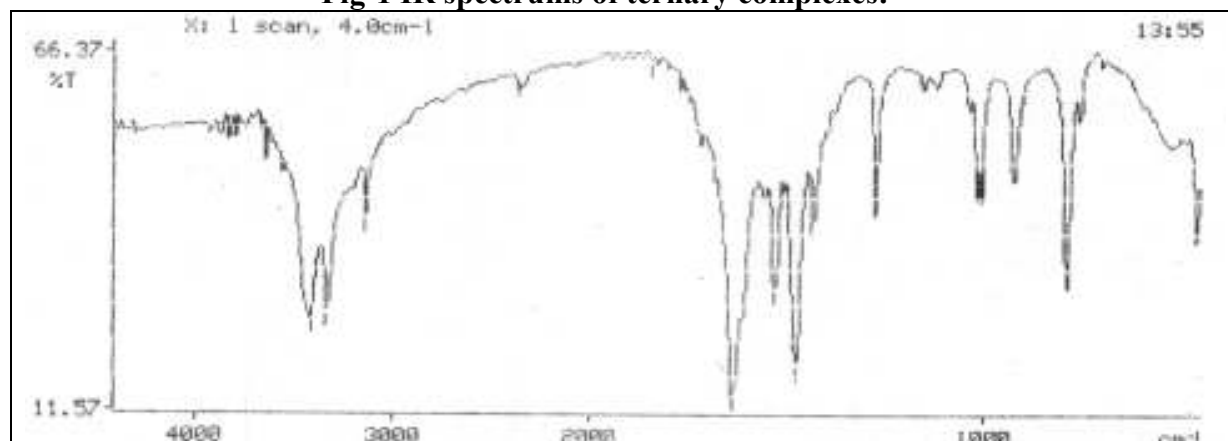
In order to study the binding mode of the ligands to metal ion, the IR spectra of the free ligands were compared with the spectra of the metal complexes. The IR spectra of Co (II), Ni (II) and Cu (II) ternary complexes are shown in Fig-1. The important absorption frequencies of these complexes with all the ligands and their assignments are presented in Table -2. IR spectrum of MIIMN ligand showed a medium intensity band 1648 cm<sup>-1</sup> due to  $\nu$  (C=N) shifted to lower frequency region to the extent 20-35 cm<sup>-1</sup> in complexes indicating the nitrogen of azomethine is coordinated to the metal ion (16). A broad band 3448 cm<sup>-1</sup> in MIIMN due to the phenolic  $\nu$ (OH) group disappeared in their complexes indicating coordination through phenolic hydroxyl group (17). A medium intensity band 1378 cm<sup>-1</sup> due to phenolic  $\nu$ (C-O) group of the ligand shifted to negative side by 20-40 cm<sup>-1</sup> in their complexes suggesting the participation of the oxygen of the hydroxyl group in bonding with the metal ion (18). These facts suggest that the shifts are due to co-ordination of ligand to the metal ion by the azomethine nitrogen and phenolic oxygen. The bands corresponding to the ring stretching frequencies  $\nu$  (C=C) and  $\nu$  (C=N) at 1535-1563 cm<sup>-1</sup> and 1450-1454 cm<sup>-1</sup> shifted to higher frequencies and appeared at 1546-1568 and 1459-1467

cm<sup>-1</sup> indicating the co-ordination of the 1,10-phen and 2,2' bipy nitrogen atoms to the metal ion (19-20). All the ternary complexes showed a broad diffuse band at 3400-3436 cm<sup>-1</sup> and another band at 772-801 cm<sup>-1</sup> at lower frequency region indicating the presence of coordinated water molecules (21-22). Similarly, in the far-infrared region the new bands at 535-563 cm<sup>-1</sup> and 450-474 cm<sup>-1</sup> are assigned to the  $\nu$  (M-O) and  $\nu$  (M-N) vibrations respectively (23).

**Table-2. IR absorption frequencies (cm<sup>-1</sup>) of Ternary complexes**

Complexes	$\nu$ (OH)	$\nu$ (C=N)	$\nu$ (C-O)	Coordinated water	$\nu$ (M-O)	$\nu$ (M-N)
MIIMN	3448	1648	1378	-	-	-
[Co (II)-(MIIMN)-(1,10phen)(H <sub>2</sub> O) <sub>2</sub> ] <sup>+1</sup>	-	1607	1353	782	560	466
[Ni (II)-(MIIMN)-(1,10phen)(H <sub>2</sub> O) <sub>2</sub> ] <sup>+1</sup>	-	1631	1342	801	549	450
[Cu (II)-(MIIMN)-(1,10phen)(H <sub>2</sub> O) <sub>2</sub> ] <sup>+1</sup>	-	1624	1341	777	549	450
[Co (II)-(MIIMN)-(2,2' bipy)(H <sub>2</sub> O) <sub>2</sub> ] <sup>+1</sup>	-	1633	1347	791	563	460
[Ni (II)-(MIIMN)-(2,2' bipy)(H <sub>2</sub> O) <sub>2</sub> ] <sup>+1</sup>	-	1613	1337	768	542	452
[Cu (II)-(MIIMN)-(2,2' bipy)(H <sub>2</sub> O) <sub>2</sub> ] <sup>+1</sup>	-	1619	1351	772	535	474

**Fig-1 IR spectra of ternary complexes:**



**Fig – 1.1 IR spectrum of Co (II) –MIIMN-bpy**

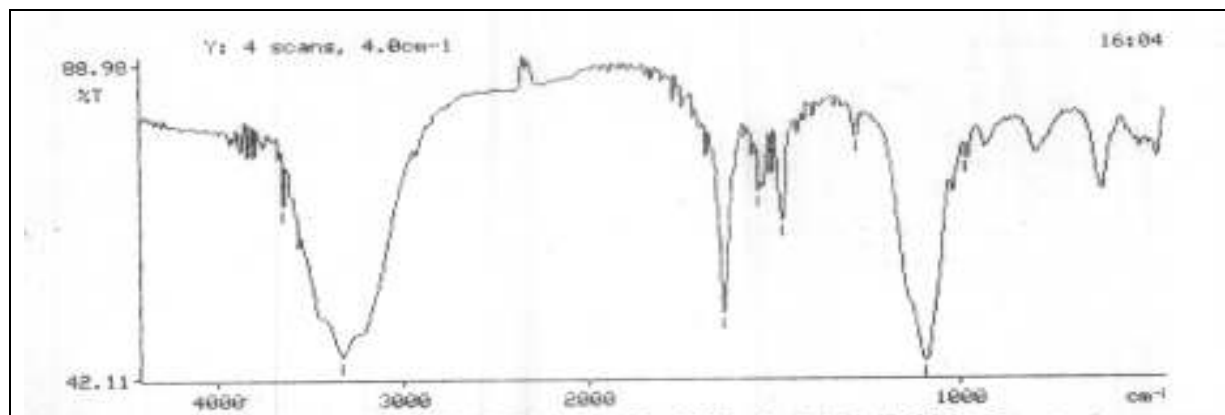


Fig – 1.2 IR spectrum of Cu (II) –MIIMN-Phen

### Thermo gravimetric analysis

It has been observed that the complex of Cu(II)-(MIIMN)-1,10-Phen, it reveal show that a loss in weight between 100-170 °C, indicating that crystal water molecules and coordinated water molecules are present in the complex. The t.g. curve in the above 200°C temperatures the loss in weight corresponds to the remaining organic ligands molecule. The ligands gradually decompose to the corresponding metal oxide at higher temperature. This result in accordance with the composition of the complex. The suggested structure for the complex is shown in Fig-2.

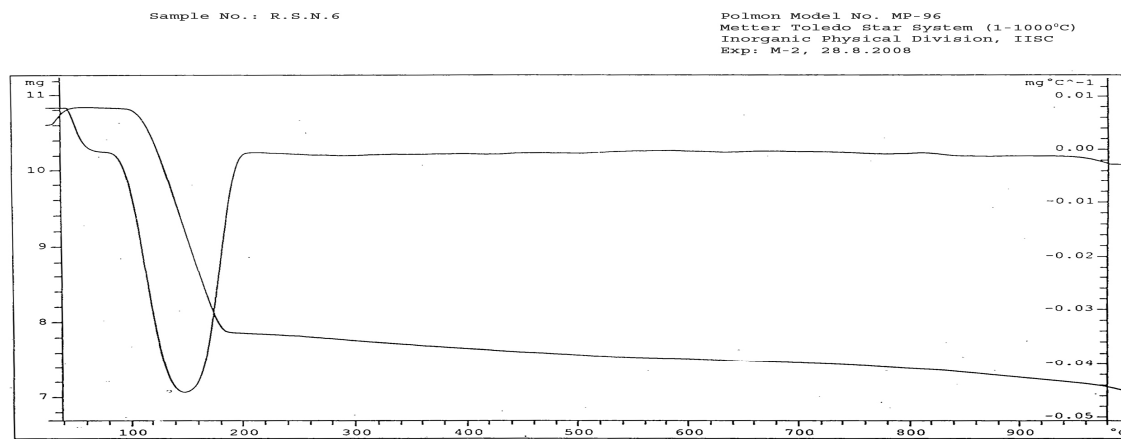


Fig – 1.3 TG & GTA curves of Cu (II) –MIIMN-Phen

### The magnetic moments and electronic spectra

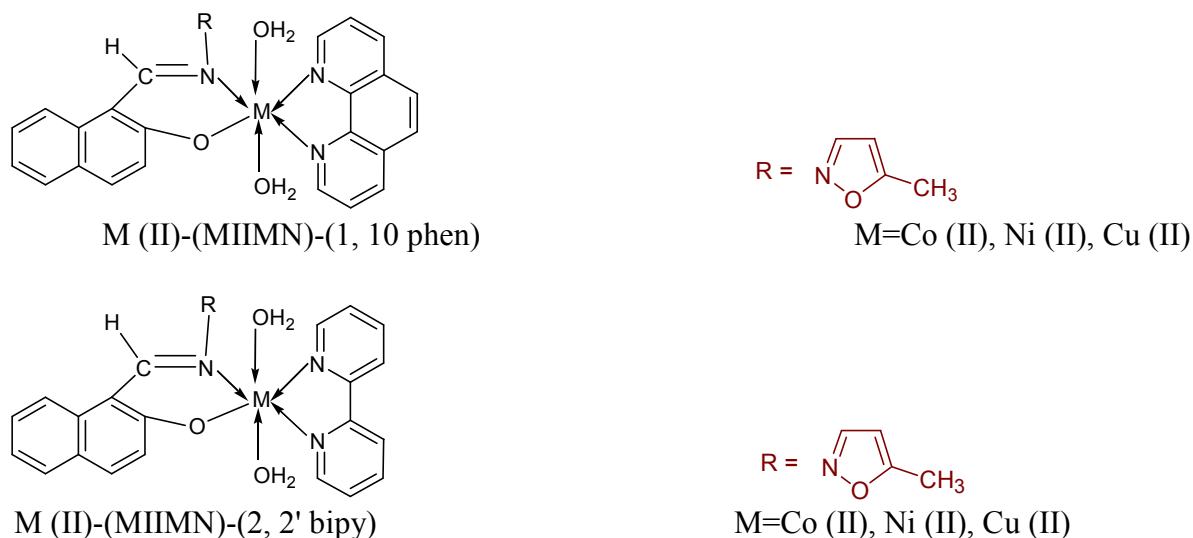
The electronic spectra of metal complexes were recorded in DMSO in the range of 200-1100nm. The electronic spectra of ternary complexes are represented in Fig-2. The electronic spectral data above complexes are presented in Table-3. The electronic spectra of ternary Co(II) complexes showed three characteristic bands in the range 11,606-14,201; 14,561-18,692 and 23,402-24,269  $\text{cm}^{-1}$  which are assigned to the three spin-allowed transitions  ${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ ,  ${}^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$  and  ${}^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$  respectively. The magnetic moments values for these complexes ranging between 4.12-5.01 B.M. which are in fair agreement with the predicted values of octahedral geometry (24). The electronic spectra of ternary Ni (II) complexes showed three

bands in the range 12,463-12,658; 16,327-19,262 and 26,394-28,321 $\text{cm}^{-1}$  assigned to the three spin-allowed transitions  ${}^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ ,  ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$  and  ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$  respectively. The magnetic moments values for these complexes ranging between 3.13-3.62 B.M. which are in fair agreement with the predicted values of octahedral geometry (25). The electronic spectra of ternary Cu (II) complexes each showed, a single broad band around 12,469-21,651  $\text{cm}^{-1}$  due to transition between  ${}^2E_g \rightarrow {}^2T_{2g}$  and the magnetic moments values for these complexes ranging between 1.72-1.81 B.M. which are in fair agreement with the predicted values of tetragonal geometry. Tetragonal or square planar Cu (II) complexes are expected to give three bands. However, these three bands usually overlap in tetragonal complexes, to give one broad absorption band (26-27).

**Table-3. Magnetic moments and Electronic spectral of ternary complexes**

Complexes	$\mu_{\text{eff}}$ (B.M)	Frequencies ( $\text{cm}^{-1}$ )
$[\text{Co}(\text{II})-(\text{MIIMN})-(1,10\text{phen})(\text{H}_2\text{O})_2]^{+1}$	4.12	11,606; 14,561; 24,269
$[\text{Ni}(\text{II})-(\text{MIIMN})-(1,10\text{phen})(\text{H}_2\text{O})_2]^{+1}$	3.13	12,658; 16,327; 28,321
$[\text{Cu}(\text{II})-(\text{MIIMN})-(1,10\text{phen})(\text{H}_2\text{O})_2]^{+1}$	1.81	12,571-20,463
$[\text{Co}(\text{II})-(\text{MIIMN})-(2,2'\text{ bipy})(\text{H}_2\text{O})_2]^{+1}$	5.01	14,201; 18,692; 23,402
$[\text{Ni}(\text{II})-(\text{MIIMN})-(2,2'\text{ bipy})(\text{H}_2\text{O})_2]^{+1}$	3.62	12,463; 19,262; 26,394
$[\text{Cu}(\text{II})-(\text{MIIMN})-(2,2'\text{ bipy})(\text{H}_2\text{O})_2]^{+1}$	1.72	12,469-21,651

**Fig-2. Tentative structures of ternary complexes**



### Antimicrobial activity

Ternary Co (II), Ni (II) and Cu (II) complexes with 1, 10-phenanthroline and 2,2' bipyridine were screened against bacteria and fungi and the results obtained are presented in Table-4 and Fig-3.

### Antibacterial activity

Antibacterial activity of the ligand and its complexes has been carried out against the bacteria, *Bacillus* and *Pseudomonas* using Muller –Hiton agar by well diffusion method (28). A comparative study of MIC values of the Schiff base and its complexes indicate that the metal complexes exhibit higher antibacterial activity than the free ligands. It has been observed from the results that the metal complexes have higher activity than that of the free ligands. This is probably due to the greater lipophilic nature of the complexes. Such increased activity of the metal chelates can be explained on the basis of Overtones concept and chelation theory. According to Overtone's concept, the cell favors the passage of only the lipid-soluble materials due to which lip solubility are an important factor, which controls the antimicrobial activity. On Chelation the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further it increases the delocalization of  $\pi$  electrons over the whole chelate ring and enhances lipophilicity of the complexes. This increased lipophilicity enhances the penetration of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of the protein that restricts further growth of the organism. Furthermore, the mode of action of the compounds may involve formation of a hydrogen bond through the azomethine group with the active centers of cell constituents, resulting in an interference with the normal cell process.

**Table-4. Antimicrobial activity of Ternary complexes**

Complexes	<i>Bacillus</i>	<i>Pseudomonas</i>	<i>R.solani</i>	<i>A.ngier</i>
Co (II) -MIIMN-1,10-phen	+	+	+	+
Cu (II) -MIIMN-1,10-phen	+++	+++	++	++
Ni (II) -MIIMN-1,10-phen	+++	++	++	+
Co (II)-MIIMN-2, 2' Bipy	+	++	+	+
Cu (II)-MIIMN-2, 2' Bipy	+++	+++	++	++
Ni (II)-MIIMN-2,2' Bipy	+	++	+	+
<i>Gentamycine</i>	+++	++	++	++
<i>Contrimoxazole</i>	+++	++	+++	++
<i>Ampicillin</i>	+++	++	++	++

High active = +++ (inhibition zone > 20 mm); moderately active = ++ (inhibition zone > 15 mm); slightly active = + (inhibition zone > 10 mm);

### Antifungal activity

Ternary Co (II), Ni (II), Cu (II) complexes with 1, 10-phenanthroline and 2,2' bipyridine were screened against fungi. It is observed that the activity of ternary complexes is more compared to their corresponding binary complexes. This result is expected since the complexes possess a greater planar area and  $\pi$ -systems, which make stacking more strongly. The variation in the activity of different complexes against different organisms depend either on the impermeability of the cells of the microbes or difference in ribosome's of microbial cells (29-30). Comparison of the biological activity of the synthesized compounds with some known antibiotics (*gentamycin*, *contimoxazole*, *Ampicillin*) presented in Table-4 show generally the free Schiff base ligand and some of its complexes exhibit better activity than these antibiotics or comparable effect.

**Fig: 3 Biological activity pictures**

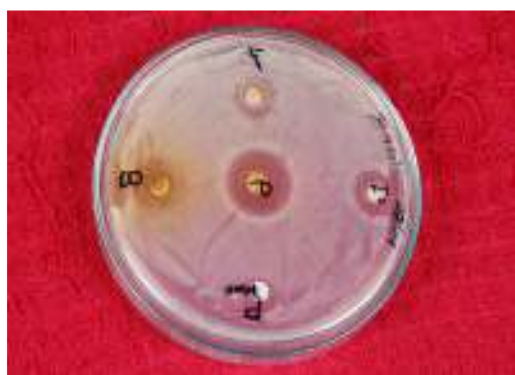


Fig: 3.1 *Bacillus* actions on Cu (II)-MIIMN-byp MIIMN-phen



Fig: 3.2 *Pseudomonas* actions on Cu (II)-MIIMN-byp MIIMN-phen



Fig: 3.3 *Bacillus* action on Ni (II)-MIIMN-phen



Fig: 3.4 *Bacillus* action on Ni (II) - byp

### Conclusion:

The mixed ligand ternary Co (II), Ni (II) and Cu (II) complexes containing MIIMN as primary ligand and 1, 10-phenanthroline or 2, 2' bipyridine as secondary ligands have been



structurally characterized. The analytical data of all ternary complexes are presented in the Table-1. The values reveal that the metal to ligands ratio is 1:1:1. Based on these IR spectral data it is observed that the metal ion coordinates through phenol ate oxygen, azomethine nitrogen and nitrogen atoms of phenanthroline or 2,2' bipyridine. Based on analytical, magnetic moment and electronic spectral data, octahedral geometry has been proposed for the Co (II) and Ni (II) complexes and tetragonal geometry for Cu (II) complexes. These ternary metal complexes have been screened against microorganisms such as (*Bacillus* and *Pseudomonas*) bacteria and (*R.Saloni* and *A. niger*) fungi. A comparative study of the MIC (minimum inhibitory concentration) values of the ligands and the complexes indicates that the complexes exhibit higher antimicrobial activity than the free ligands and control.

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