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SYNTHESIS OF BIOACTIVE MONONUCLEAR COPPER (II), NICKEL (II), COBALT (II) AND MANGANESE (II) SCHIFF BASE COMPLEXES DERIVED FROM ISATIN: ANTIOXIDANT, ANTI-BACTERIAL AND DNA CLEAVAGE ACTIVITIES

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ABSTRACT: Potentially bioactive tetradentate novel Schiff base ligand and its mononuclear metal (II) complexes have been synthesized by the condensation method of p - nitroaniline, isatin, and 2, 2' bipyridyl molecules in the ratio of 1:1:1. The ligand and its reported complexes exactly categorized by diverse physicochemical techniques that include elemental analysis, conductance measurements, IR, electronic spectra studies, ¹H NMR spectroscopy, and EPR studies. Derived complexes are exactly non-electrolyte in nature in Dimethylformamide solution because of their lower conductance values. The IR data tells us the association of C=N nitrogen atom in coordination to the metal ion. Electronical studies and magnetic measurements suggested the octahedral geometry around metal ion under investigation. The EPR spectral data shows additional proof for the octahedral geometry. The nucleolytic cleavage of the reported compounds was checked upon pUC18 DNA by gel - electrophoresis method. In this study, anti-oxidant and anti-microbial activities have also been evidently studied. The compounds show exact growth inhibitory activity over bacteria such as *S. aureus*, *E. coli*, *B. subtilis* and *K. pneumonia* than derived free ligands.

INTRODUCTION: The success of developing cisplatin as an effectual anticancer agent has drawn the notice of numerous bio-inorganic chemists en route to preparing the numerous transition metal complexes with enhanced anticancer efficacy¹. Even though there are several biological issues in anticancer cells, which includes Ribonucleic acid, protein or enzyme and is admitted universally that Deoxy Ribonucleic acid is the prime achievement for several anticancer drugs.

Therefore the connections between DNA and small molecules between the main action mechanism of designing molecules and anticancer activity that bind and cleave DNA have paying extensive attention. Transition metal complexes have been largely oppressed for metallohydrolases capable of mimicking endonucleases and extending cleavage and binding agents for Deoxyribonucleic acid².

Especially, metal complexes such as Copper (II), Cobalt (II), Nickel (II) and Manganese (II) has N-containing aromatic rings exposed to admirable binding as well as cleavage activities. Schiff base complexes containing ligand with N donors are well-known for revealing exciting electrochemical, stereochemical and electronic properties³. At this point, we account for the preparation, characterizing, Deoxy Ribonucleic acid cleavage, anti-oxidant and also anti-bacterial abilities of

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Copper (II), Cobalt (II), Nickel (II) and Manganese (II) complexes with N-containing ligands. Derived complexes were characterized structurally by a variety of physicochemical techniques. $[ML_1L_2]$ complex cleaves DNA capably compared to other Schiff base systems.

Physical and Materials Measurements: Diverse agents and chemicals utilized for current analysis purchased from sources like Loba, Merck, and Aldrich chemicals. Metal (II) salts, isatin, p-nitroaniline, and 2, 2' bipyridyl got from Loba chemicals and utilized as same. CH_3CH_2OH , Dimethyl Sulphoxide, and Dimethyl formamide were taken as solvents and are from Aldrich and Merck chemicals. The elemental analyzer herein case for elemental analysis was Carlo Erba Model 1106. Perkin-Elmer FT-IR-8300 spectrophotometer on KBr disks were for recorded IR spectra, range of 4000–400 wavenumber region. ELICO CM 185 Bridge measured the molar conductivity values using a freshly prepared DMF solution. Perkin-Elmer Lambda 40 spectrometer using DMF recorded electronic spectra of complexes at 300 K in the 200-800 nm range. EPR spectral analysis was recorded on an E-112 ESR spectrometer for powdered samples. BRUKER ADVANCED III 400 MHz spectrophotometer recorded Proton Nuclear magnetic Resonance spectra in Dimethyl Sulphoxide - d_6 by Tetramethylsilane here for interior reference. Magnetic moment dimensions run out at room temperature by balance named Gouy.

Synthesis of (1-Methyl-4-nitro-benzene(1, 2-dihydrogen – indol – 3 – yilidene – 2 - imino-)-(4-nitro-phenylene)-amine): (1-Methyl – 4 - nitro-benzene (1,2-dihydrogen-indol-3-yilidene-2-imino-)-(4-nitro-phenylene)-amine) was derived via adding 4- nitroaniline (2 mmole) in 10 ml of sol. ethanol, Isatin (1 mmole) in CH_3CH_2OH sol. (20 ml) and induce heating by relaxation for 2-3 hours as in **Fig. 1**. The product yellow colour precipitate was allowable to cooling process and dried in desiccator using silica gel⁴.

Product Yield: 85%. Melting point: 139 °C. *Analytical calculation For* $C_{20}H_{13}N_4O_4$: Carbon, 69.30; Hydrogen, 6.11; Nitrogen, 12.92. Found: Carbon, 68.37; Hydrogen, 8.90; Nitrogen, 12.82 %. UV-Visible $[\lambda(\text{nm}) (\text{dmf})]$: 340 (34, 000)($\pi \rightarrow \pi^*$),

364 (36,400) ($n \rightarrow \pi^*$). IR cm^{-1} (KBr): $\nu(\text{C}=\text{N})$ 1620.

Synthetic Methodology of Cu ($C_{30}H_{21}N_7O_4Cl_2$): The Schiff base (1-Methyl-4-nitro-benzene (1,2-dihydrogen – indol – 3 – yilidene 2 - imino-) - (4 - nitro-phenylene)-amine) (1 mmole) dissolved in ethanol 20 ml followed by adding absolute hot solution of Cu (II) Cl_2 salts (1mM) in ethanol then followed by the 2, 2' bipyridyl in ethanolic solution. The mixed sol. was heated with constant stirring ~20 min followed by refluxation stoutly for 3-5 hrs on a water bath. Then the resultant product was kept for full night; finally, a darkish green coloured precipitate was meticulously strained, cleaned with CH_3CH_2OH , and dried in vacuo.

Product Yield: 85%. Melting point: >200 °C. Elemental analyses, *Analytical calculation for* $Cu(C_{30}H_{21}N_7O_4Cl_2)$: Carbon, 56.90; Hydrogen, 5.54; Nitrogen, 10.52. Found: Carbon, 56.88; Hydrogen, 5.51; Nitrogen, 10.53%. UV-Visible $[\lambda(\text{nm}) (\text{dmf})]$: 270 (27,000) ($\pi \rightarrow \pi^*$), 330 (33,000) ($n \rightarrow \pi^*$), 345 (34,500) (L \rightarrow M), 510, 609 (51,000) (60,900) (d \rightarrow d). IR cm^{-1} (KBr): ν [C=N: 1595, M-N: 468, M-Cl: 310].

Synthetic Methodology of Ni ($C_{30}H_{21}N_7O_4Cl_2$): 20 ml ethanolic solution of (1-Methyl-4-nitro-benzene (1,2-dihydrogen-indol-3-yilidene -2-imino-)-(4-nitro-phenylene)-amine) (1 mmole) followed by adding an absolute hot solution of Ni(II) Cl_2 salts (1 mmole) in ethanol then followed by the 2, 2' bipyridyl in ethanolic solution. The mixed sol. was heated with constant shaking for 20 min then refluxation stoutly for 3 to 5 hrs on top of water bath. Then the resultant mixture was kept overnight; finally, a brownish black coloured precipitate was obtained was thoroughly strained out, completely cleaned with CH_3CH_2OH , and dried in vacuo.

Product Yield: 85%. Melting point: >200 °C. Elemental analyses, *Analytical calculation For* $Ni(C_{30}H_{21}N_7O_4Cl_2)$: Carbon, 57.69; Hydrogen, 5.60; Nitrogen, 10.63. Found: Carbon, 57.65; Hydrogen, 5.58; Nitrogen, 10.63%. UV-Visible $[\lambda(\text{nm}) (\text{dmf})]$: 285 (28,500) ($\pi \rightarrow \pi^*$), 357 (35,700) ($n \rightarrow \pi^*$), 370(37,000) (L \rightarrow M), [450(45,000), 582(58,200), 630(63,000)] (d \rightarrow d). IR wave number region (KBr): ν [C=N: 1609, M-N: 454, M-Cl: 317].

Synthetic Methodology of Co ($C_{30}H_{21}N_7O_4Cl_2$):

20 ml ethanolic solution of (1-Methyl-4-nitrobenzene (1,2-dihydrogen-indol-3-ylidene-2-imino)-(4-nitro-phenylene)-amine) (1 mmole) followed by adding absolute hot sol. of Co (II) Cl_2 (1 mmole) in ethanol then followed by the 2, 2' bipyridyl in ethanolic solution.

The mixed sol. was heated with constant shaking for 20 min then refluxation firmly for 3 to 5 hrs resting lying on a water bath. The resultant product allowed to be kept overnight.

Finally, a dark pink coloured precipitate was obtained was, thoroughly strained, rinsed CH_3CH_2OH , and dried in vacuo.

Product Yield: 80%. Melting point: >200 °C. Elemental analyses, *Analytical calculation For Co ($C_{30}H_{21}N_7O_4Cl_2$):* Carbon, 57.68; Hydrogen, 5.61; Nitrogen, 10.62. Found: Carbon, 57.64; Hydrogen, 5.57; Nitrogen, 10.63%. UV-Visible [λ (nm),dmf]: 285 (28,500) ($\pi \rightarrow \pi^*$), 360 (36,7000) ($n \rightarrow \pi^*$), 370 (37,000) ($L \rightarrow M$), [470(47,000), 560(56,000), 630(63,000)] ($d \rightarrow d$). IR wavenumber region (KBr): $\nu[C=N: 1595, M-N: 468, M-Cl: 310]$.

Synthetic Methodology of Mn ($C_{30}H_{21}N_7O_4Cl_2$):

20 ml ethanolic solution of (1-Methyl-4-nitrobenzene (1,2-dihydrogen-indol-3-ylidene-2-imino)-(4-nitro-phenylene)-amine) (1 mmole) followed by adding an absolute hot solution of Mn(II) Cl_2 salts (1 mmole) in ethanol then followed by the 2, 2' bipyridyl in ethanolic solution.

The mixed sol. was heated with constant shaking for 20 min then refluxation doughtily for 3 to 5 hrs lying on a water bath. The resultant product allowed to kept for overnight finally a dark brown coloured precipitate were obtained was, thoroughly strained, cleansed with CH_3CH_2OH , and dried in vacuo.

Product Yield: 80%. Melting point: >200 °C. Elemental analyses, *Analytical calculation For Mn($C_{30}H_{21}N_7O_4Cl_2$):* Carbon, 57.60; Hydrogen, 5.60; Nitrogen, 10.71. Found: Carbon, 57.58; Hydrogen, 4.58; Nitrogen, 10.62%. UV-Visible [λ (nm) (dmf)]: 285 (28,500) ($\pi \rightarrow \pi^*$), 295 (29,500) ($n \rightarrow \pi^*$), 370 (37,000) ($L \rightarrow M$), [525 (52,500), 560(56,000), 695(69,500)] ($d \rightarrow d$). IR wave number region (KBr.): $\nu[C=N: 1600, M-N: 444, M-Cl: 312]$.

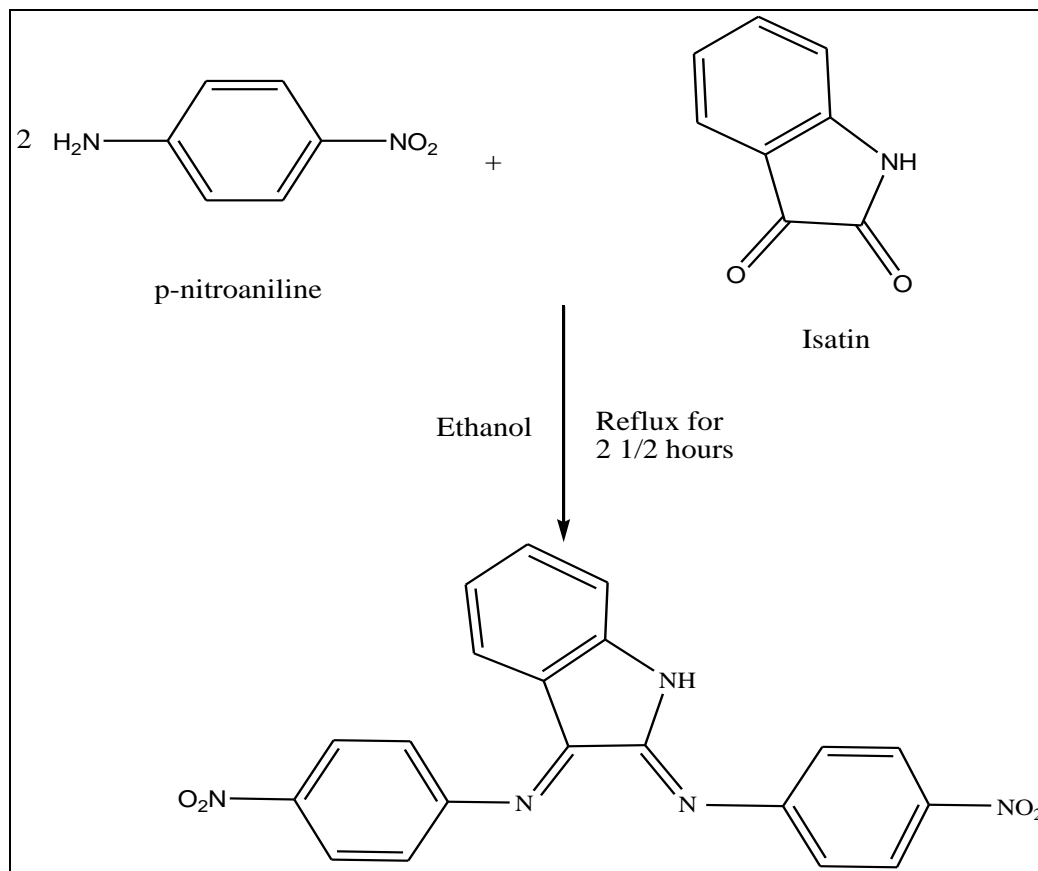


FIG. 1: SYNTHESIS OF SCHIFF BASE LIGAND ($C_{20}H_{13}N_4O_4$)

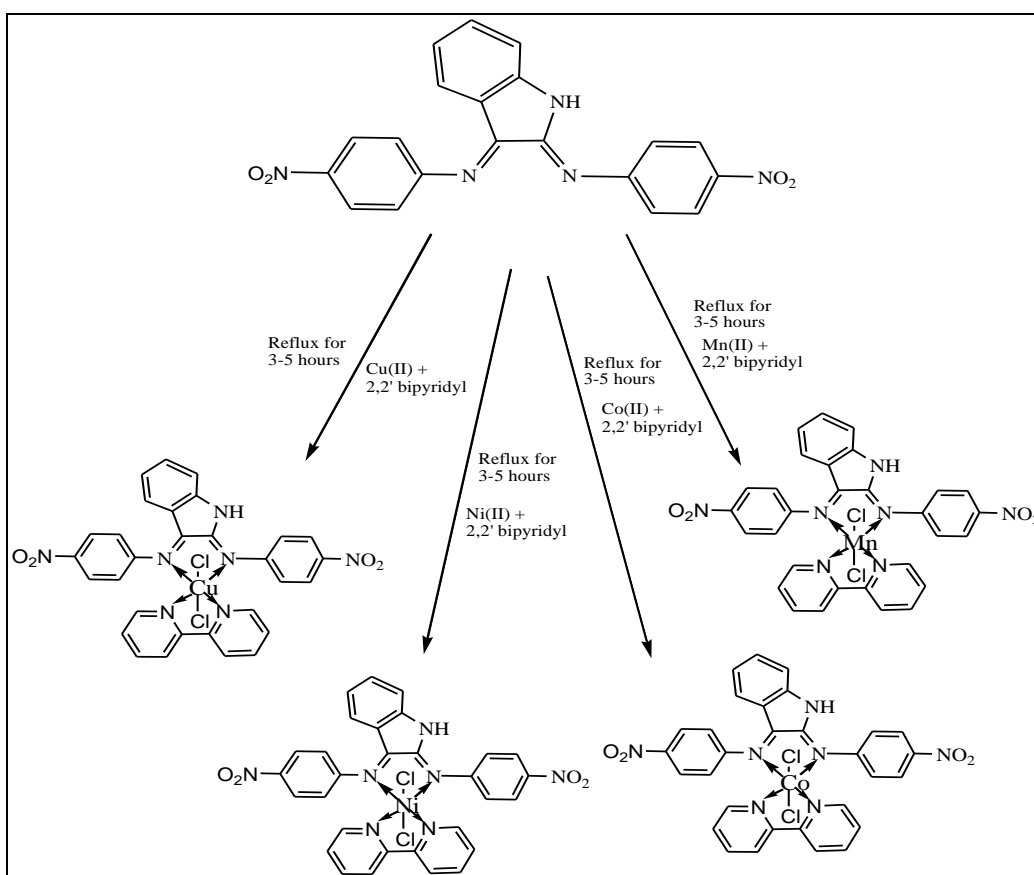


FIG. 2: SYNTHETIC ROUTE OF MONONUCLEAR SCHIFF BASE METAL (II) COMPLEXES

RESULTS AND DISCUSSION: Derived compounds are unsolvable in H_2O along with various organic solvents, which are common, but they all are instantly dissolvable in powerful coordinating agents like Dimethyl formamide, Acetonitrile as well as Dimethylsulphoxide. The physical properties and elemental analysis data of synthesized compounds are listed in **Table 1**.

The derived compounds can be signified as ML_1L_2 where: (M= Copper (II), Nickel (II), Cobalt (II), Manganese (II), and (L= ligand). Lower conductance values of derived complexes in DMF were precised at 10^{-3} Molar concentration, indicating that all reported complexes perform as non-electrolytes⁵.

TABLE 1: ANALYTICAL DATA OF THE SCHIFF BASE LIGAND AND ITS MONONUCLEAR METAL COMPLEXES

Molecular Formula	color	Yield %	Melting Point(°)	% of Nitrogen		% of Metal		Molar conductance Λ_m ($ohm^{-1}cm^2mol^{-1}$)
				Cal	Exp	Cal	Exp	
$C_{20}H_{13}N_4O_4$	Pale yellow	85	139	12.92	12.82	-	-	-
Cu(II)complex	Dark green	85	>200	10.52	10.53	9.60	9.55	6.7
Ni(II)complex	Dark green	85	>200	10.63	10.63	8.90	8.95	5.8
Co(II)complex	Dark pink	80	>200	10.62	10.63	8.96	8.95	7.9
Mn(II)complex	Dark brown	80	>200	10.71	10.62	8.42	8.45	8.4

* L1= Ligand 1, L2= 2, 2' bipyridyl and X = Chloride ion.

Infrared Spectra: Derived compounds were recorded fort IR- spectra to prove the binding form of electron donor atoms with central metal ions. Vibrational frequencies along with their timid assignments of reported metal (II) complexes were exactly listed in **Table 2**. The band shown at 1620

cm^{-1} represented for $\nu(C=N)$ that is shifted near low region approximately $1595-1609\ cm^{-1}$ in derived complexes and its clearly telling the confirmation of C=N molecule in the prepared formation of complexes⁶. The formation of non- ligand bands in complexes just about $444-468\ cm^{-1}$ are due to

(Metal-N) bond respectively ⁷. The absorption band are at 310-317 cm⁻¹ region that is assigned because of ν (Metal-Cl) Vibrations.

TABLE 2: IR DATA OF THE SCHIFF BASE LIGAND AND ITS MONONUCLEAR METAL COMPLEX

Compounds	(C=N) (cm ⁻¹)	(M-N) (cm ⁻¹)	(M-Cl) (cm ⁻¹)
Ligand	1620	--	--
Co(II)complex	1595	468	310
Mn(II)complex	1600	444	312
Cu(II)complex	1605	460	315
Ni(II)complex	1609	454	317

Magnetic Moment and Electronic Spectral: UV spectra of derived ligand in Dimethyl formamide exhibit band assigned to $\pi-\pi^*$ transitions of the aromatic ring in ligand and imino group at 340 nm and another one extra band is noted at 364 nm and is dependable for $n-\pi^*$ transitions ⁸. Cu (C₃₀H₂₁N₇O₄Cl₂) complex gave bands at 505 and 604 nm due to ${}^2B_{1g} \rightarrow {}^2E_{1g}$ and ${}^2B_{1g} \rightarrow {}^2A_{1g}$ transitions. This electronical data shows us

octahedral geometry for the reported Cu (C₃₀H₂₁N₇O₄Cl₂) complex. Cu (II) complex has magnetic moment value at 1.90 B.M. Octahedral geometry for darkish coloured Co (C₃₀H₂₁N₇O₄Cl₂) complex established by magnetic moment at 3.60 B.M. And the assignment of the three most intense bands are ${}^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$, ${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$, and ${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(P)$ at the range 464, 595 and 625 nm. The Mn(II) complex depicts transition bands by 694, 555, and 522 nm attributed to ${}^6A_{1g} \rightarrow {}^4A_{1g}$, ${}^6A_{1g} \rightarrow {}^4E_g$, ${}^4A_{1g}$, and ${}^6A_{1g} \rightarrow {}^4A_{2g}$, respectively, that are depicted to an octahedral geometry about manganese(II) ion. Mn(II) complex shows a magnetic moment value at 5.92 B.M ⁹.

The three bands were shown for Ni (C₃₀H₂₁N₇O₄Cl₂) complex in the range of 446, 578 and 625 nm, responsible to ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$, ${}^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ and ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$ d-d transitions. Magnetic moment value of Ni(C₃₀H₂₁N₇O₄Cl₂) at 3.12 Bohr magneton.

TABLE 3: UV-VIS ABSORPTION SPECTRAL DATA OF METAL (II) COMPLEXES AT 300 K

Complex	λ_{max} (nm)	Band assignments	Geometry	Magnetic moment (B.M.)
Ni(II)complex	357, 370, 450, 582, 630	INCT, LMCT ${}^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$, ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$	Distorted octahedral	3.14
Cu(II)complex	345, 370, 510, 609	INCT, LMCT ${}^2B_{1g} \rightarrow {}^2E_{1g}$ ${}^2B_{1g} \rightarrow {}^2A_{1g}$		1.92
Mn(II)complex	295, 370, 525, 560, 695	INCT, LMCT ${}^6A_{1g} \rightarrow {}^4A_{1g}$, ${}^6A_{1g} \rightarrow {}^4A_{2g}$, ${}^6A_{1g} \rightarrow {}^4E_g$, ${}^4A_{1g}$		5.90
Co(II)complex	360, 370, 470, 560, 630	INCT, LMCT ${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$, ${}^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$, ${}^4T_{1g}(F) \rightarrow {}^4T_{2g}(P)$		3.62

¹H NMR: ¹H NMR spectral studies of derived ligand (C₂₀H₁₃N₄O₄) indicated signals at 6.40– 7.90 ppm corresponding to aromatic protons at 8.23 ppm, which is attributed to azomethine moiety (C=N). These signals afford structural arrangement for the prepared ligand ¹⁰.

ESR Studies: The Electromagnetic Resonance spectrum of the derived Cu(C₃₀H₂₁N₇O₄Cl₂)

compound gives an idea of the exacted geometry and the nature of ligating sites around the central metal ion ¹¹.

X-band of the ESR spectrum for the Cu(C₃₀H₂₁N₇O₄Cl₂) complex was recorded at a particular frequency range of 9.1 GHz at 77 K, suggesting that this complex has a distorted octahedral geometry.

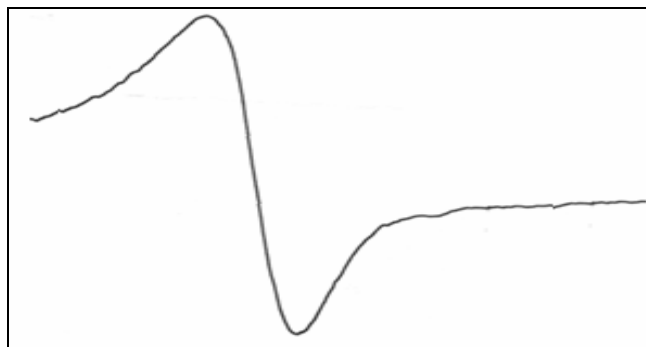


FIG. 3: EPR SPECTRA OF CU(C₃₀H₂₁N₇O₄Cl₂) SCHIFF BASE COMPLEX

Gel Electrophoresis Assay: Doxyribonucleic acid cleavage actions of the prepared complexes have been completely monitored by changing supercoiled pUC18 DNA (Form I to Form II to Form III) via a suitable gel-electrophoresis method. **Fig. 4** depicts the exact cleavage pattern. The Doxyribonucleic acid cleavage competence of derived compounds was the binding affinity of complex to the Doxyribonucleic acid molecule. In this case, the control did not illustrate noticeable cleavage of DNA (lane 1 & 2). **Fig. 4**, derived complexes are noted to illustrate the nuclease activity in the existence of H₂O₂ (Lanes 3–6). These results point out that the central metal ions played an imperative role by cleaving isolated Doxyribonucleic acid ¹².

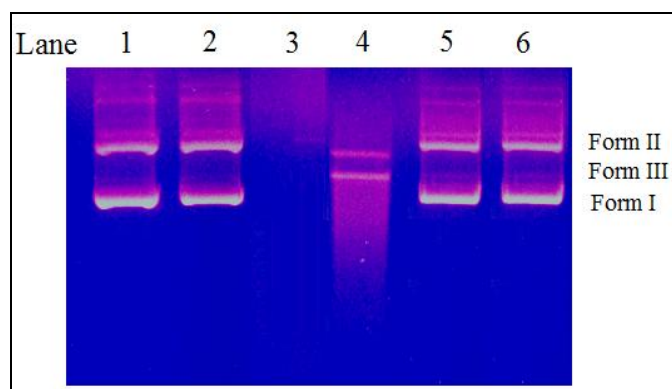


FIG. 4: DNA CLEAVAGE CHANGES IN THE AGAROSE GEL ELECTROPHORETIC PATTERN OF PUC18DNA INDUCED BY H₂O₂ AND METAL COMPLEXES. LANE 1-DNA ALONE; LANE 2- DNA ALONE + H₂O₂; LANE 3-DNA + CU COMPLEX + H₂O₂; LANE 4-DNA + NI COMPLEX+ H₂O₂; LANE5-DNA + CO COMPLEX LANE 6-DNA+ MN COMPLEX H₂O₂

DPPH Radical Studies: DPPH radical scavenging effect is an easy and suitable process that is expansively utilized to assess the antioxidant capability of derived compounds.

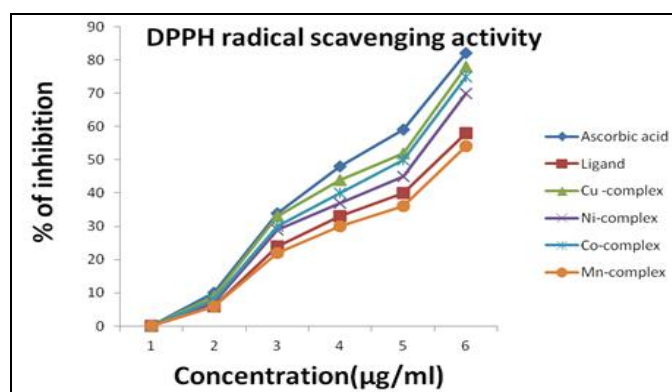


FIG. 5: DPPH SCAVENGING ACTIVITY OF C₂₀H₁₃N₄O₄ AND ITS COMPLEXES

Diphenyl picrylhydrazyl radicals are firm but prepared compounds competent of contributing H atoms and radical activity completely damaged, ensuing in a transformation of colour as of purple one to yellow. To be noted, Schiff base along with its complexes definitely diminish its attentiveness of Diphenyl-picrylhydrazyl radical in the solution and has been considered a confirmation of their antioxidant capacity ¹³. **Fig. 5** depicted the growth inhibitory result. **Fig. 6** shown IC₅₀ values for reported compounds. Actually, here for comparison, ascorbic acid molecule was used as standard.

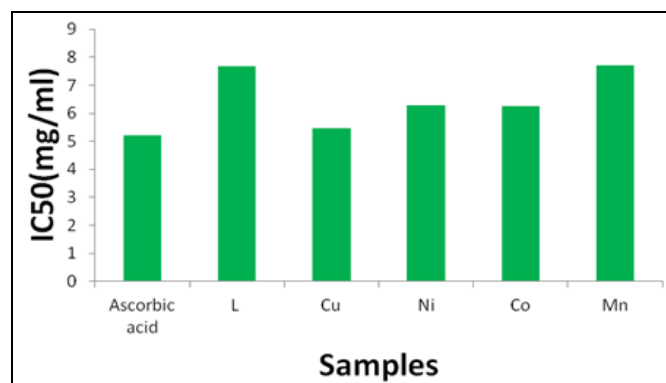


FIG. 6: DPPH RADICAL SCAVENGING CAPACITIES (IC₅₀) OF SYNTHESIZED METAL COMPLEXES

Anti-microbial Activity: The anti-microbial capacities of all reported samples have been assessed against two pathogenic strains such as *S. aureus*, *B. subtilis* (gram-positive), while gram-negative strains are *E. coli* and *K. pneumonia* species ¹⁴. The disc diffusion technique at a concentration of 50 µg/mL. Streptomycin taken as the standard drug here. The noted anti-bacterial studies of reported samples are in **Fig. 7**. Also, it was clearly noticed that all complexes showed better inhibition than ligands.

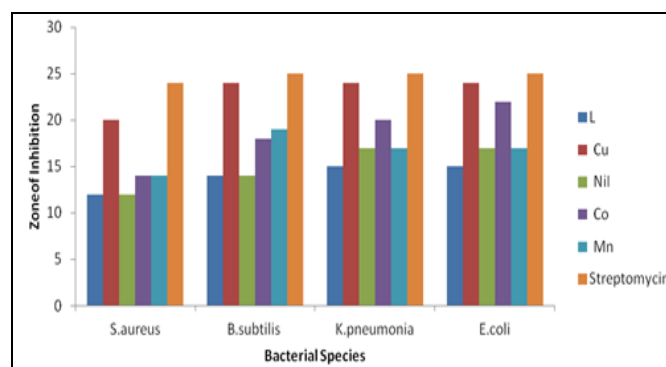


FIG. 7: ANTI-BACTERIAL ACTIVITY OF SYNTHESIZED COMPOUNDS AGAINST FOUR BACTERIAL STRAINS

TABLE 4: ANTI-BACTERIAL ACTIVITY FOR SCHIFF BASE LIGANDS AND ITS MONONUCLEAR METAL COMPLEXES

Compounds	Zone of inhibition (mm)															
	Gram-positive bacteria								Gram-negative bacteria							
	<i>Staphylococcus aureus</i>				<i>Bacillus subtilis</i>				<i>Escherichia coli</i>				<i>Klebsiella pneumoniae</i>			
	Concentration ($\mu\text{g/mL}$)															
Ligand	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100
Ni(II)complex	7	7	8	10	7	7	9	10	6	6	7	9	7	8	8	10
Cu(II)complex	9	8	10	12	9	8	11	12	6	7	7	9	9	8	9	10
Mn(II)complex	10	11	12	14	10	9	12	13	9	9	10	11	10	12	15	17
Co(II)complex	8	9	9	10	8	9	9	10	7	8	9	9	7	8	9	10
Streptomycin	10	9	12	13	10	11	12	14	8	9	9	11	9	10	11	12
	16	17	20	22	16	15	17	20	13	13	16	20	13	15	16	18

CONCLUSION: Condensation reaction of p-nitroaniline, isatin and 2, 2' bipyridyl in the ratio of 1:1:1 derived in the development of Schiff base ligand and complexes in an extraordinary yield. The analytical, molar conductance, vibrational frequencies, and electronic spectral studies suggested that the structures are in an octahedral arrangement.

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