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Structural and Investigation of metal ligand (5-methylIsatin–Ethylenediamine) Complexes and Their Antibacterial Activity

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Abstract: *A novel Schiff base ligand was synthesized by the condensation of 5-methylisatin with ethylenediamine in ethanolic medium using acetic acid as a catalyst. The ligand was subsequently complexed with selected transition metal ions to obtain a series of metal–ligand complexes. The synthesized ligand and its metal complexes were characterized by uv absorption,NMR, infrared (IR) spectroscopy to investigate the mode of coordination and structural features. IR spectral data revealed coordination of the ligand to the metal ion through azomethine nitrogen and carbonyl oxygen atoms. The antibacterial activity of the ligand and its metal complexes was evaluated against selected Gram-positive and Gram-negative bacterial strains using standard agar diffusion techniques. The metal complexes exhibited enhanced antibacterial activity compared to the free ligand, which may be attributed to chelation and improved permeability across bacterial cell membranes. The results indicate that 5-methylisatin–ethylenediamine metal complexes have potential as promising antibacterial agents..*

Keywords: 5-methylIsatin, Ethylenediamine, Schiff base, Metal complexes, IR spectroscopy, antimicrobial activity

I. INTRODUCTION

Schiff base ligands are derived from the condensation reaction of aldehydes or ketones with primary amines (2). 5-Methylisatin (CAS 608-05-9) is a yellow-to-orange crystalline organic compound, specifically a substituted indole-2,3-dione, that acts as a key intermediate in medicinal chemistry. Schiff bases are an essential class of organic compounds with a wide variety of applications in many fields, such as analytical, biological and inorganic chemistry [3]. It is widely used to develop antimicrobial, anti-HIV, and anticancer agents due to its ability to inhibit specific enzymes and receptors. Isatin (1H-indole-2,3-dione) is an important heterocyclic compound that has attracted significant attention due to its wide range of biological activities. Schiff bases derived from 5-methylisatin are known to exhibit enhanced pharmacological properties (4-5). Ethylenediamine, a bidentate ligand, readily forms stable chelates with transition metals like Nickel(II),ferrous (II). No investigation, however, are known in the literature about the metal complexes of Schiff base driven from 5-methylisatin with ethylenediamine. And view of the importance of 5-methylisatin in many scientific fields (6-13). Coordination of Schiff base ligands with metal ions often results in compounds with improved biological efficiency (14-22). In view of increasing bacterial resistance to existing antibiotics, the present work focuses on the synthesis, structural investigation, and antibacterial and antimicrobial evaluation of 5-methylisatin–ethylenediamine metal complexes. 5-methylIsatin 5 th position methyl(1H-indole-2,3-dione) is a versatile, naturally occurring organic compound, an indole derivative, known for its unique structure and broad biological activities, making it a crucial scaffold in medicinal chemistry for developing drugs with antitumor, antimicrobial, anti-inflammatory, antiviral, and anticonvulsant properties, plus applications in dyes, agrochemicals, and research (23-29). It's a key building block (a pharmacophore) for creating complex molecules that interact with enzymes and receptors, influencing many bodily functions (30). The reaction of 5-methylisatin (5-methylindoline-2,3-dione) with ethylenediamine (1,2-diaminoethane) typically yields a Schiff base condensation product. Given that ethylenediamine is a diamine and isatin has a highly

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reactive C3-carbonyl group, the reaction commonly results in a 2:1 condensation product where two molecules of 5-methylisatin react with one molecule of ethylenediamine, forming a bis-Schiff base

II. EXPERIMENTAL

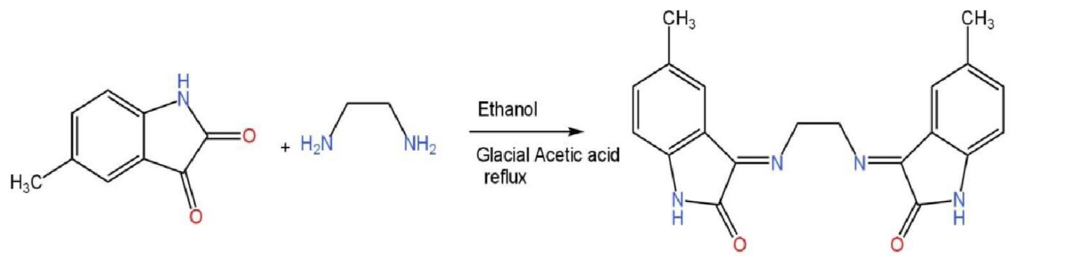
2.1 Materials

Chemicals were purchased from Sigma-Aldrich and used without further purification. Methanol, and ethanol, were of HPLC grade and were purification using standard methods described in the literature [37].

5-methylIsatin, ethylenediamine, metal salts, ethanol, and acetic acid were of analytical grade and used without further purification.

2.2 Synthesis of 5-methylIsatin–Ethylenediamine Schiff Base ligand

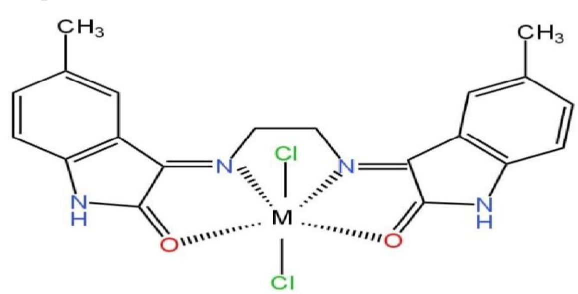
The ligands were prepared by procedure to that reported in the literature [31] Schiff base ligand was prepared by adding 5-methylIsatin 0.010 mole (1.4 gm) was refluxed with ethylenediamine 0.005 mole (0.3ml) in ethanol (20 ml) in the presence of a 3 drops of acetic acid. The reaction mixture was stirred at ambient temperature for 3 hours, on completion of reaction the product was separated as crystal which were cooled, and the resulting solid was filtered, dried, and from cooled water. The yellow colour product was recrystallized from filtered off.



5-methyl Isatin ethylenediamine (3E)-5-methyl-3-{[2-(methylideneamino)ethyl]imino}-2,3-dihydro-1H-indol-2-one

2.3 Synthesis of Metal Ligand Complexes:

The complexes were prepared by refluxing equimolar amounts ((0.01 mol) of ethylenediamine bis-5-methylisatin Schiff base ligand and hydrated of zinc acetate, Fe(II) , Ni(II), absolute ethanol(1). The resulting reaction mixtures were basified by adding the equivalent amount of sodium acetate and heated under 40 degree reflux with constant sturring for 3 h. The reaction mixtures were evaporated to a small volume and left to cool. The complexes that precipitated were filtered, washed with cool distilled water and then with ethanol several times until the washing were free from the excess sodium acetate and other soluble side products .



metal= fe(II), Ni(II)

Figure:1 Prapose the structure of metal (II)Complexes

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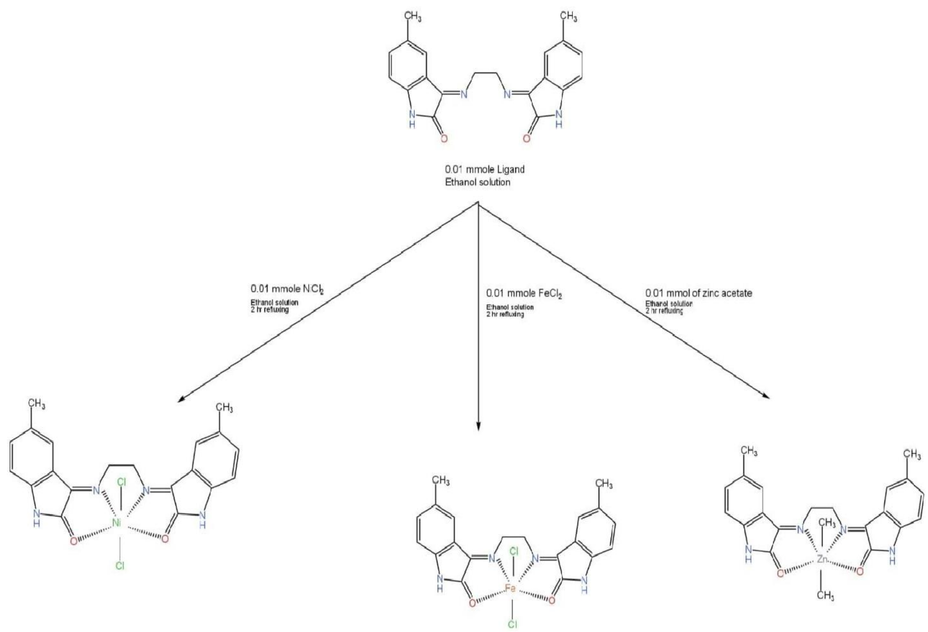
2.4 Synthesis of Ni (II) Schiff base ligand Complex:

ethylenediamine bis-5-methylisatin Schiff base ligand and hydrated of Nickel chloride metal salt, in absolute ethanol. The resulting reaction mixtures were basified by adding the equivalent amount of sodium acetate and heated under 40-degree reflux with constant sturring for 3 h. The complexes were prepared by refluxing equimolar amounts ((0.01 mol) . The reaction mixtures were evaporated to a small volume and left to cool. The complexes that precipitated were filtered, free from the excess sodium acetate and other soluble side products.

2.5 Synthesis of Zn Acetate metal Schiff base ligand Complex:

ethylenediamine bis-5-methylisatin Schiff base ligand and hydrated of Zinc acetate metal salt, in absolute ethanol. The resulting reaction mixtures were basified by adding the equivalent amount of sodium acetate and heated under 40 degree reflux with constant sturring for 3 h. The complexes were prepared by refluxing equimolar amounts ((0.01 mol) . The reaction mixtures were evaporated to a small volume and left to cool. The complexes that precipitated were filtered, washed with cool distilled water and then with ethanol several times until the washing were free from the excess sodium acetate and other soluble side products .



2.6 Synthesis of Fe (II)Metal Schiff base ligand Complex:

ethylenediamine bis-5-methylisatin Schiff base ligand and hydrated of ferric chloride metal salt, in absolute ethanol. The resulting reaction mixtures were basified by adding the equivalent amount of sodium acetate and heated under 40 degree reflux with constant sturring for 3 h. The complexes were prepared by refluxing equimolar amounts ((0.01 mol) . The reaction mixtures were evaporated to a small volume and left to cool. The complexes that precipitated were filtered, washed with cool distilled water and then with ethanol several times until the washing were free from the excess sodium acetate and other soluble side products .

III. RESULT AND DISCUSSION

3.1 IR Spectral Analysis of the Ligand

The FT-IR spectrum of the synthesized ligand was recorded in the range 4000–400 cm^{-1}. The characteristic bands confirm the formation of the Schiff base ligand and the presence of important functional groups.

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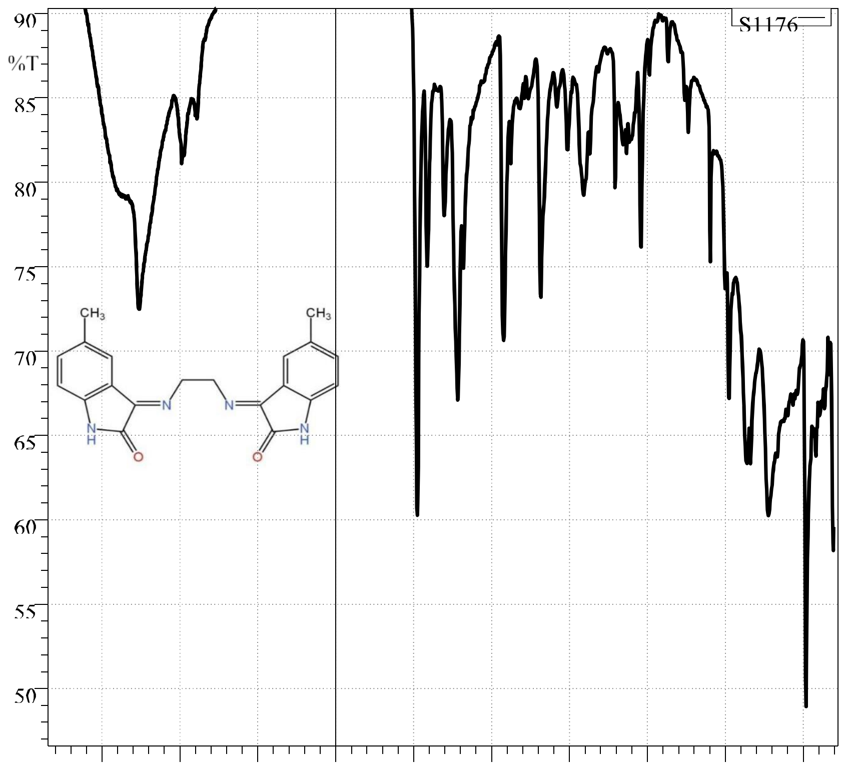
The strong band around ~1630 cm^{-1} confirms the formation of the azomethine (–C=N–) group, indicating successful Schiff base synthesis.
The carbonyl (C=O) band around ~1700 cm^{-1} shows the presence of isatin moiety.
The N–H stretching band confirms that nitrogen atoms are available for coordination in metal complexes.
Aromatic and aliphatic C–H stretching bands support the conjugated ligand structure.



Observed band (cm-1)	Assignment	Explanation
1450–1380 cm^{-1}	δ(CH_2)	Methylene group vibrations
~3350–3200 cm^{-1}	ν(N–H)	–NH group from ethylenediamine
~3050 cm^{-1}	ν(C–H)	aromatic Aromatic C–H stretching of isatin ring
~2920–2850 cm^{-1}	ν(C–H)	Aliphatic C–H stretching of –CH_2 groups
1700–1720 cm^{-1}	ν(C=O) carbonyl	Carbonyl group of isatin moiety
~1610–1640 cm^{-1}	ν(C=N) azomethine	Confirms Schiff base formation
~1250–1300 cm^{-1}	ν(C–N)	C–N stretching of azomethine linkage

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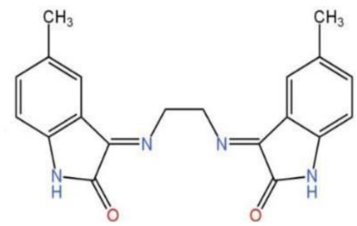
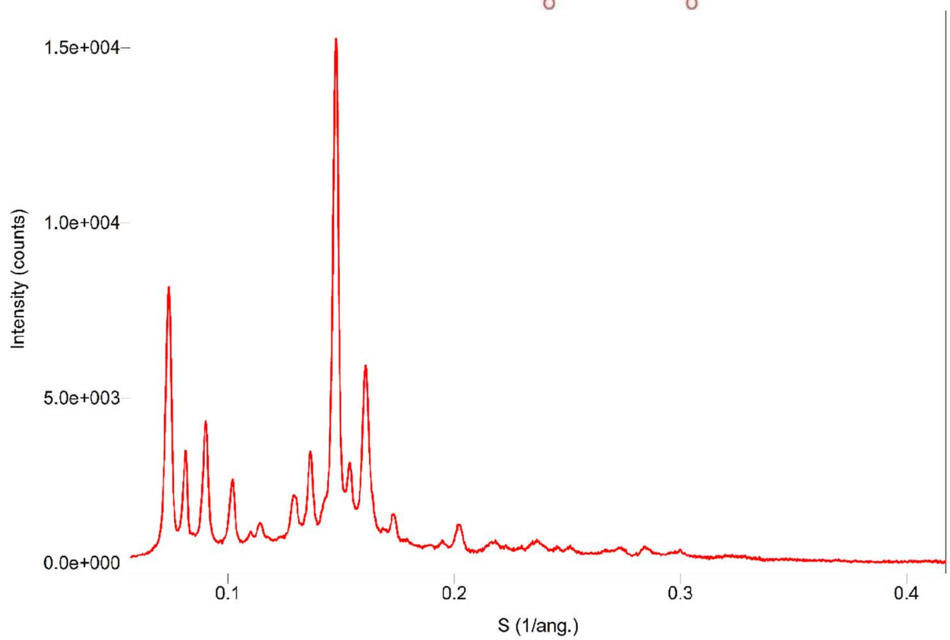
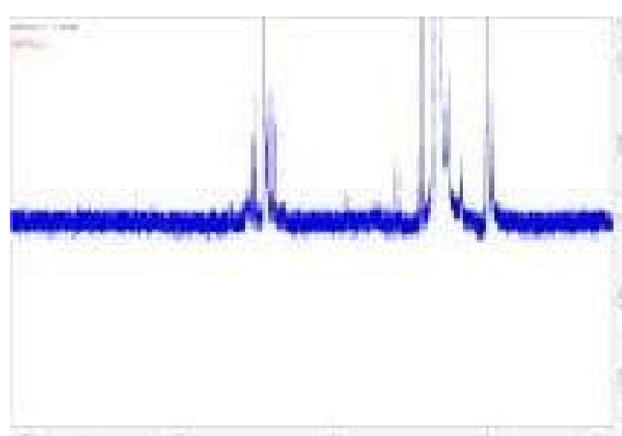
3.2 X-Ray Diffraction of Schiff base ligand :





3.3 : 1HNMR Spectrum of ligand :



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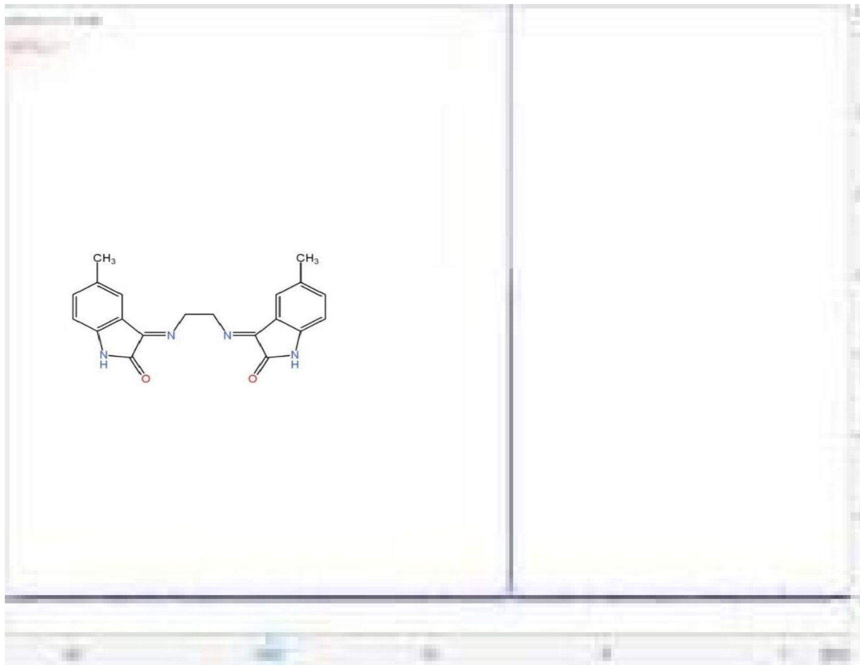

1HNMR Analysis of Schiff base ligand:

Chemical shift (δ, ppm)	Multiplicity	Assignment
~10.5–11.5 ppm	Broad singlet	NH proton of isatin moiety
~7.0–7.8 ppm	Multiple	Aromatic protons of isatin ring
~2.1–2.4 ppm	Singlet	CH_3 group at 5-position
~3.0–3.6 ppm	Triplet	CH_2– protons (if linker/amine present)

Table (1) Physical properties, FT-IR, Uv–vis, 1Hnmr, xrd and Elemental analysis data of Ligand and Metal Complexes

		Ligand (SBL)	Ni(SBL)	Zn Acetate(SBL)	Fe(SBL)
Physical property	colour	yellow	Dark yellow	Dark brown	brown
	yield	78 percent	75 percent	71 percent	63 percent
UV-vis	λmax,nm	244	252	244	304
Stoichiometry	M:L	----------	2:1	2:1	2:1
IR Spectra	ν(N–H)	~3350–3200 cm^{-1}	3145 cm^{-1} ~2820–2640	3400–3300 cm^{-1}	3400–3300 cm^{-1}

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	ν(C–H) ν(C=O) ν(C=N) δ(CH_2) M-N	~2920–2850 cm^{-1} 1700–1720 cm^{-1} ~1610–1640 cm^{-} 1450–1380 cm^{-1}	cm^{-1} 1711 cm^{-1} 1464 cm^{-1} 498–433 cm^{-1}	~2820–2750 cm^{-1} ~1550–1600 cm^{-1} ~1300–1200 cm^{-1} ~520–450 cm^{-1}	~2920–2850 cm^{-1} ~1700–1680 cm^{-1} ~1600–1580 cm^{-1} ~520–450 cm^{-1}
Xrd	2theta Intensity	26.261 6090	26.268 4933	26.388 202	15.779 2631
1Hnmr	Singlet Triplet Multiplate	~2.1–2.4 ppm ~3.0–3.6 ppm ~7.0–7.8 ppm	δ 10.5–12.0 ppm δ 7.0–8.2 ppm	δ 8.0–8.8 ppm δ 7.0–8.2 ppm	δ 8.2–8.7 ppm δ 6.9–8.1 ppm
Element	C	69.5	56.31 7.26 5.36 11.87 Ni 9.38	56.58	63.99
analysis	H O N Cl	6.12 8.83 15.46		7.29 5.38 9.93	8.77 5.68 9.95
	Ni,fe,zn			11.93 Fe 9.9	ZN 11.61

:

3.4 UV Spectral analysis ligand and metal ligand complexes:

Red line's ligand, black line: Ní complex, blue line: zinc complex, green line : Fe complex.

General observation:

1. Absorption bands below ~250 nm

The intense absorption bands observed in the region 200 – 250 nm are attributed to π → π * transitions.

These transitions arise from the aromatic ring and conjugated system present in the isatin moiety.

The high absorbance values indicate the presence of a highly conjugated chromophore.

2. Absorption bands around 260–300 nm

The bands appearing in the region 260 – 300 nm are assigned to n→ π * transitions.

These transitions originate from non-bonding electrons (n) of hetero atoms such as nitrogen and oxygen present in the carbonyl (C=O) and azomethine (C=N) groups.

Slight shifts in these bands among different spectra indicate coordination of the ligand to the metal ion.

3. Effect of metal coordination: This shift confirms the involvement of azomethine nitrogen and carbonyl oxygen in coordination with the metal ion.

UV Line Assigning

Black line (L1): SBL ligand, red line (C1): Ni complex, blue line (C2): zinc complex, green line (C3): Fe complex.

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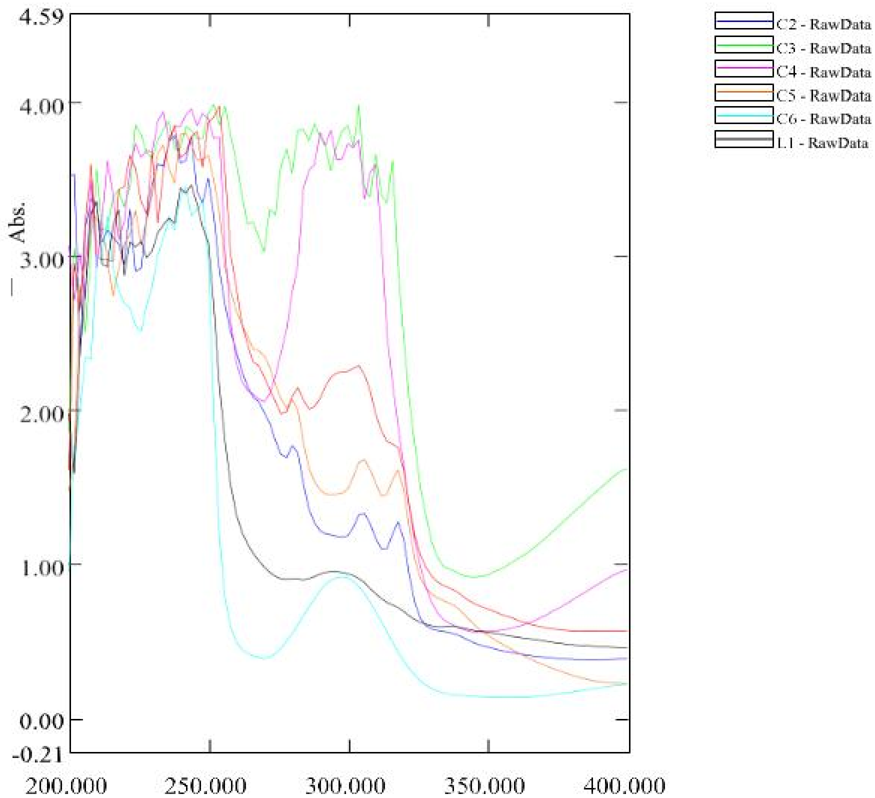

Antimicrobial Studies:

In antimicrobial activity of the three complexes and one ligand by the standard disc diffusion method [32]. The microbial activities performed against grown cultures of three selected bacteria namely, E. coli., Bacillus on agar media Overnight. grown microbial were spread on agar Petri plates and kept for about half an hour. The disc was placed and test solution was added. Dilution was made in DMA 10 mg concentration. After addition of each of the test solutions, the inoculated plates were kept at room temperature, for about an hour to enable diffusion of the test solutions and subsequently incubated at 370c. Microbial growth inhibition was determined by measuring the diameter of zone of inhibitions which was assessed at the end of 24 hrs incubation. From the zone of inhibitions test(table-1) it has found that when agar plates were supplemented with antibiotics e.g. Bacillus. the inhibition areas were 10mm.When the same agar plates were supplemented with fungal species it has been observed that the zone of inhibitions was less. From the above results it is seen that these complexes and ligand possess antimicrobial activity. The antimicrobial activity increased on the concentrations of compound increased. anti-inflammatory (33-34). It is known that in a complex the positive charge of the metal is partially shared with donor atoms present in the ligand and there may be pi electron delocalization over the whole chelating ring [35]. Manganese, cobalt, nickel, copper, and zinc are life essential metallic elements with biological activity when associated with specific proteins, participating in oxygen transport, electronic transfer reactions, or the storage of ions [36] is increases lipophilic character of the complex and Favors its permeations through the lipid layer of the bacterial membrane.

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CONCLUSION

A new Schiff base has been prepared. The spectroscopic studies have characterised the structure of the Schiff base ligand (L1) coordinates with metal ions. 5-Methyl isatin undergoes acid-catalysed condensation with ethylenediamine to form a symmetrical bis-Schiff base ligand through nucleophilic addition followed by dehydration. The Schiff base ligand coordinates to the metal ion through azomethine nitrogen and carbonyl oxygen atoms forming a stable N_2O_2 chelated metal complex. All the complexes have an electrolytic nature. (elemental analyses (C, H, and N), IR, Vis-Uv, X-ray and 1 H NMR spectroscopic), we can propose the following chemical formulae for the synthesised mixed ligand complexes.

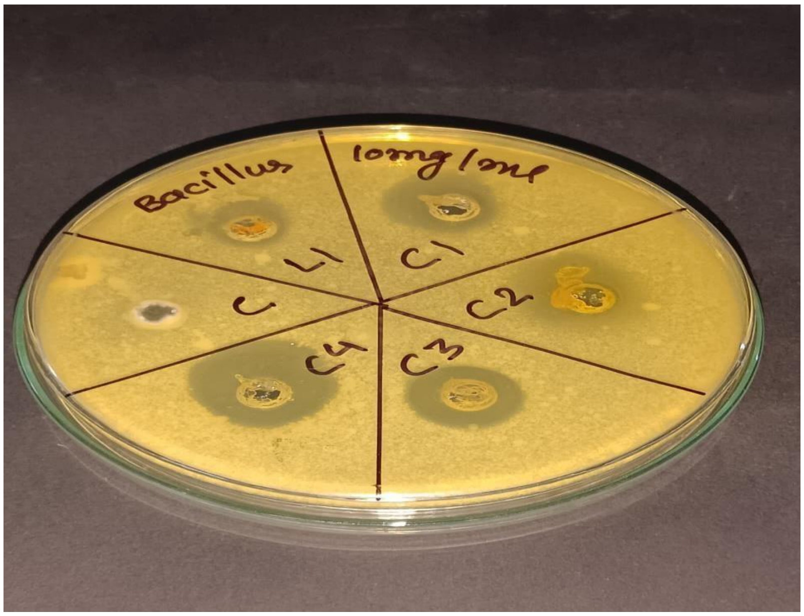

Figure :1 Antimicrobial activity of ligand and complex on bacillus

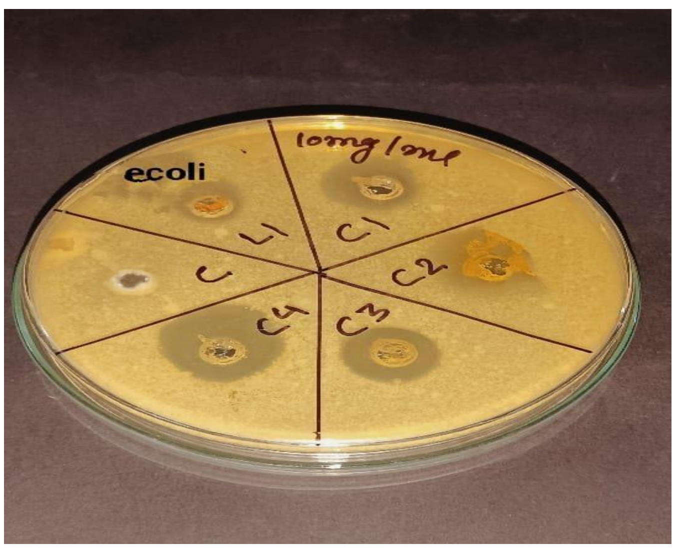

Figure:2 Antimicrobial activity of ligand and complex on E.coli

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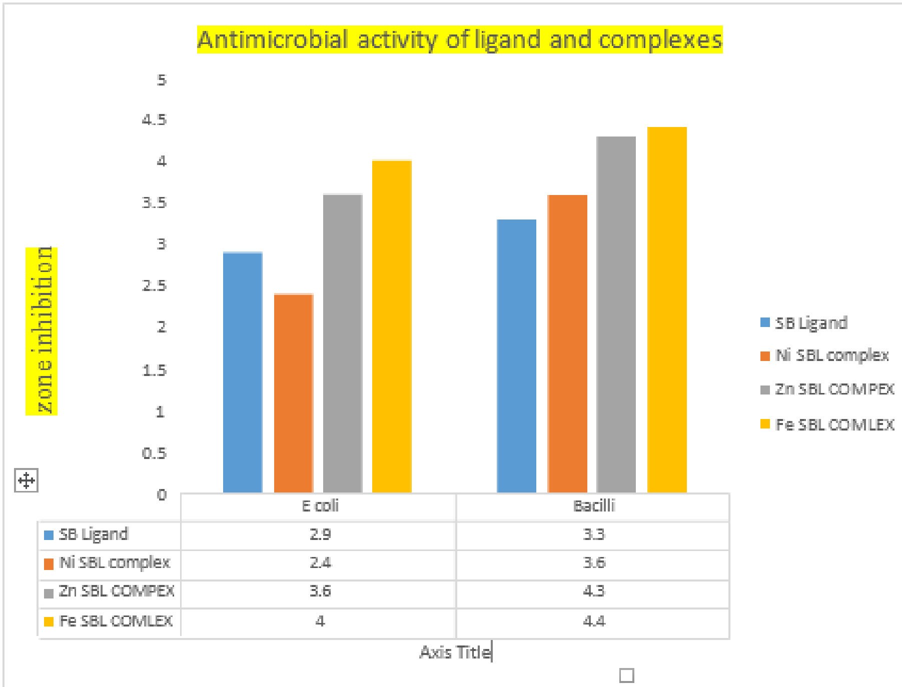

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