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Research Article

SYNTHESIS AND CHARACTERISATION OF SOME NEW IMIDAZOLE-2-CARBOXALDEHYDE IMINE BASE METAL (II) COMPLEXES AND THEIR ANTIMICROBIAL ACTIVITY

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ABSTRACT

Novel Metal (II) complexes derived from 2-benzothiazolamine and imidazole-2-carboxaldehyde were synthesized. These compounds were characterised by FT-IR, Elemental analysis, Mass, ^{1}H NMR, TGA, Molar conductance, Electronic spectra, Magnetic moment measurements and SEM. The analytical data has shown the metal to ligand ratio is 1:1. IR spectral studies revealed that binding sites of ligand with metal ions through the azomethine nitrogen and imidazole nitrogen atoms. Thermal analysis confirms there are no coordinated water molecules present in the metal complexes. Schiff base and its metal complexes were screened against the bacterial strains such as gram positive and gram negative and fungal strain aspergillus niger to know the anti microbial activity of these compounds, in which metal complexes have shown greater activity than free ligand.

Keywords: Schiff base, Metal complexes and Antimicrobial activity.

INTRODUCTION

There has been considerable interest in the synthesis of ligands containing an imidazole moiety because of its biological significance in a variety of metalloproteins, especially heme proteins. There are huge number of Schiff base ligands[1-3] have been reported by virtue of their potential applications in industry, pharma, drug and catalysis. Subsequently, considerable importance has been given to these ligands. Metal complexes containing sulphur, nitrogen chelating ligands have gained much attention because of their compelling physico-chemical properties, distinct biological activities[4-6] and models of metallo enzyme actve sites. Imine based metal complexes have a wide variety of biological applications such as anti microbial[7], antitumour[8] and antioxidant[9] activities. These complexes are also used as catalysts[10]. Imidazole moiety played a vital role in a variety of metallo proteins, especially heme proteins because of its biological significance. Schiff bases based on benzothiazoles are bicyclic ring systems with nitrogen and sulphur heterocycles[11] play an important role in life science and in the synthesis of speciality and fine chemicals. An intensive study on 2-amino benzothiazole was done in 1950s. It was concluded that 2-amino benzothiazole scaffold is one of the previlaged structure in medicinal chemistry and cytotoxic on cancer cells[14,15]. Rupinder kaurgill and Dr. P. M. S Bedi synthesised 2-amino benzothiazole derivatives and evaluate In Vitro cytotoxic activity against different human cancer cell lines namely Lung A-549, Prostate PC-3 and lukemia[16]. It is a well known approach to design new drug like molecules which are combination of 2-amino benzothiazole with other heterocyclic compounds allows achieving new pharmacological profile, action, toxicity lowering. Accordingly, in the present work a new Schiff base ligand derived from the condensation of 2-amino benzothiazole with imidazole-2-carboxaldehyde, and its Cu(II), Co(II), Ni(II)and Zn(II) metal complexes were synthesised.

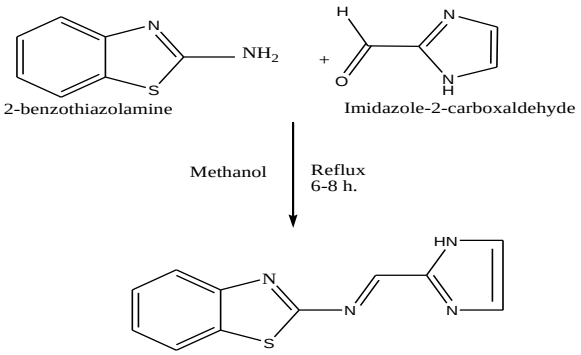
II. EXPERIMENTAL

MATERIALS

All the chemicals were purchased from sigma aldrich. The solvents and reagents used were of analytical grade.

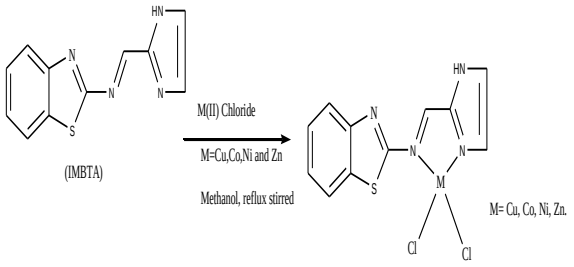
Synthesis of Ligand [L (IMBTA)]

A solution of Imidazole-2-carboxaldehyde (5 mmol) was added dropwise to the methanolic solution of 2-benzothiazolamine with continuous stirring and refluxed for 6-8 h. An yellowish white amorphous precipitate is formed, which was then recrystalized from EtOH and dried in vacuo over anhydrous $Cacl_2$. The purity of the compound was tested with TLC. Yield 75%.



Synthesis of metal complexes

A solution of metal(II) (Cu, Co, Ni and Zn) chlorides (5mmol) dissolved in methanol was added to the methanolic solution of Schiff base (5mmol). The resulting mixture was stirred and refluxed for 3-5 hours. The coloured shining amorphous metal complex was precipitated in the rb flask, which was washed with ether, ethanol and dried.



Physical measurements

The percentage of the elements such as C, H and N present in the ligand and its metal complexes were determined by using Perkin Elmer elemental analyzer. The stretching frequencies of ligand and its metal complexes were recorded by using KBr pellets in the range 4000-400 cm^{-1} on prestige-21 instrument.The non-ionic nature of the metal complexes were carried out in DMSO (10^{-3}M) by using Elico Electronic Digital conductivity meter and 0.01 M KCl solution is used for calibration. The electronic spectra of ligand and its complexes were carried out in DMSO using a SHIMADZU UV-2600 spectrophotometer. The proton nmr of the ligand was recorded at 200 MHz and 300 MHz on Varian Gemini Spectrometer and TMS is used as an internal standard. To analyse molecular weight of the compounds VG AUTOSPEC mass spectrometer was used and which is performed through ESI technique. Thermogravimetric analysis of the metal complexes was carried on a Mettler Toledo Star system in the temperature range 50-1000^{o}C and heating rates were controlled by 15^{o}C min^{-1}. A Gouy balance model 7550 using $Hg[Co(NCS)_4$] as standard is operated to examine the magnetic moment values of the metal complexes. By using Polmon instrument (model No. MP-96) the melting point of the ligand and decomposition temperature of the complexes were determined. The SEM/EDX images were obtained from a Hitachi SEM analyser.

Antibacterial activity

By using disc diffusion method[17] the invitro antibacterial activities of the Schiff base ligand and its metal complexes were analysed. All the metal complexes were screened against gram positive(eg. (i) Bacillus subtilis (ii) Staphylococcus aureus) and gram negative(eg.(iii) Pseudomonas putida (iv) Escherichia coli) bacterium. One day prior to the experiment, the bacterial cultures were inoculated in broth (inoculation medium) and incubated overnight at 37^{0}C. Inoculation medium containing 24h grown culture was added aseptically to the nutrient medium and mixed thoroughly to get the uniform distribution. This solution was poured (25mL in each dish) into petri dishes and then allowed to attain room temperature. Wells (6mm in diameter) were cut in the agar plates using proper sterile tubes. Then, wells were filled upto the surface of agar with 0.1mL of the test compounds dissolved in DMSO (200µM/mL). The plates were allowed to stand for an hour inorder to facilitate the diffusion of the drug solution. Then the plates were incubated at 37^{0}C for 24h and the diameter of the inhibition zones were read.

Antifungal Screening

MIC (Minimum inhibitory concentrations) and Agar well diffusion method both were used in the present study to evaluate the antifungal activity of the metal complexes. In the present

work all the metal complexes were screened against the fungal species Aspergillus niger (A.niger). It is by serial dilution technique the minimum inhibitory concentration was determined.

Minimal Inhibitory Concentration (MIC) determination

To measure the MIC values, six concentrations of the stock, 10µl, 30µl, 50µl, 100µl, 125µl, 150µl (corresponding to 10 µg, 30µg, 50µg, 100µg, 125µg and 150µg respectively) were assayed against the test organism. The minimum inhibitory concentration was defined as the lowest concentration able to inhibit any visible growth of the micro-organism.

III. RESULTS AND DISCUSSION

Physical characteristics of the complexes

All the metal complexes were melt at higher temperatures and they were coloured, non-hygroscopic in nature and insoluble in water.

Elemental analysis

The percentage of the elements (C, H, N) present in the ligand and complexes were given in **Table.1** The experimental values were matches with the theoretical values and these results confirmed that metal to ligand ratio is 1:1. The values in brackets are calculated.

Mass spectra and Molar conductivity

The mass spectrum of the ligand exhibits the molecular ion peak at (m/z=229), which is in agreement with its formula weight (228). The mass spectral values of the metal complexes were given in **Table.2**. The molar conductivity was measured for all the metal complexes in DMSO solution (10^{-3}M) to establish the charge of the metal complexes. The low molar conductance values suggests that all the metal complexes were non electrolytic in nature[18].

Table 1:

Compound	Molecular weight	Colour	Anal. (%) found (cal)			
			C	H	N	M
Ligand $C_{11}H_8N_4S$	228	Light yellow	56.43 (57.90)	3.14 (3.50)	23.16 (24.56)	-
Cu(II)complex ($C_{11}H_8N_4SCl_2Cu$)	362.5	Green	34.85 (36.41)	2.12 (2.20)	14.98 (15.44)	16.62 (17.51)
Co(II)complex ($C_{11}H_8N_4SCl_2Co$)	358	Brown	35.92 (36.87)	2.12 (2.23)	15.04 (15.64)	15.82 (16.48)
Ni(II)complex ($C_{11}H_8N_4SCl_2Ni$)	358	Brick red	35.62 (36.87)	2.20 (2.23)	14.99 (15.64)	15.56 (16.48)
Zn(II)complex ($C_{11}H_8N_4SCl_2Zn$)	364	Yellow	34.98 (36.23)	2.08 (2.19)	14.85 (15.37)	16.06 (17.92)

Table 2: Mass and Molar conductance values of the compounds

Compound	Calculated mass	Obtained mass	Conductance ($Ohm^{-1}cm^2\ mol^{-1}$)
Ligand	228	229 [M+1]	------
Cu(II) complex	362.5	363 [M^+]	10.21
Co(II) complex	358	360[M+2]	8.9
Ni(II) complex	358	358[M^+]	11.2
Zn(II) complex	364	364 [M^+]	9.2

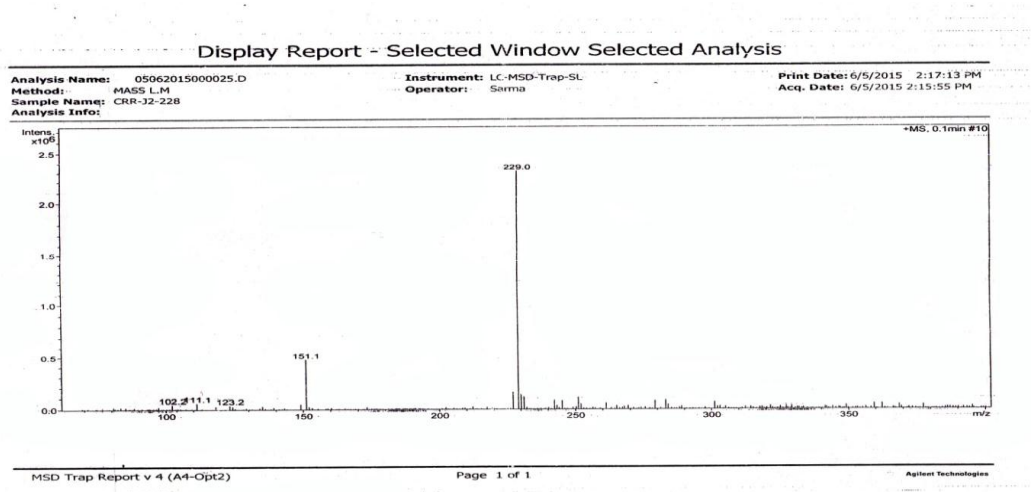


Fig. 1: Mass spectrum of IMBTA (m/z=229)

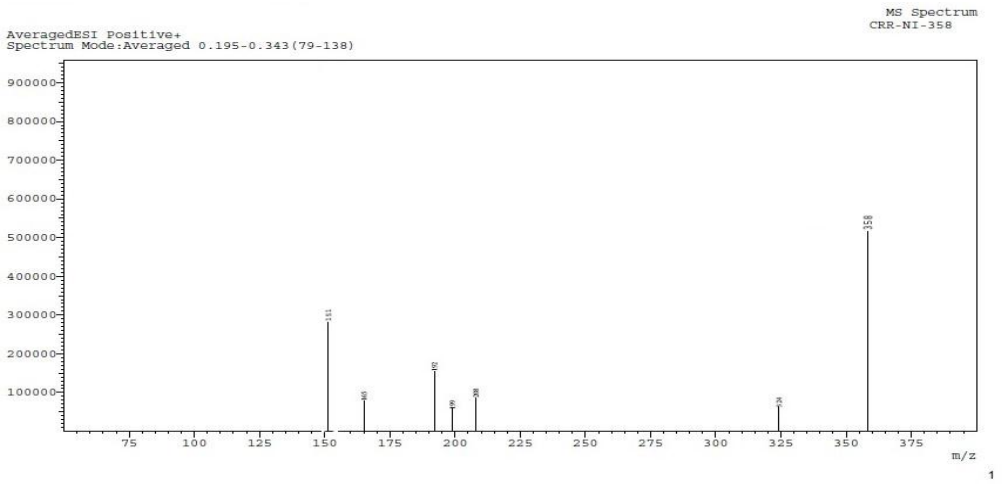


Fig. 2: Mass spectrum of Ni(II)-IMBTA

^{1}H NMR

The ^{1}H NMR spectrum of the ligand shows a singlet at 8.91 ppm, which corresponds to the azomethine proton. The signals appeared at 7.82-8.1ppm due to aromatic protons.

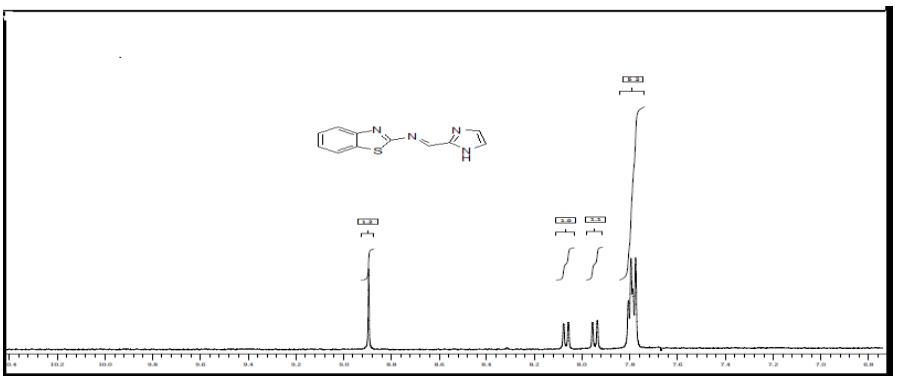


Fig. 3: ^{1}H NMR Spectrum of the Ligand(IMBTA)

UV- Vis Spectra and Magnetic moments

The UV- Vis spectra of Schiff base ligand and its metal complexes were recorded in DMSO at room temperature. The absorption bands of a ligand observed at 279 nm and 362 nm, which corresponds to π- π*(-C=C) and n- π*(-C=N) transistions respectively. These bands were shifted in the complexes due to intraligand and LMCT transistions of coordinated ligand. In addition, to this the electronic spectra of the Cu complex displayed a low intensity broad band at 434 nm in the visible region is assignable to the d-d transitions [$^2B_{1g} \longrightarrow {}^2A_{1g}$][19], which is characteristic of square planar environment. Besides this the magnetic moment value 1.76 BM for Cu(II) complex confirms the square planar geometry of this complex. The electronic spectrum of Ni(II) and Co(II) complexes shown absorption bands at 552 nm and 445 nm respectively in the visible region due to d-d transistions of metal complexes. The characteristic band at 445 nm for Co(II) complex corresponds to transistion [$^4A_{2(F)}$- $^4T_{1(P)}$] convey tetrahedral environment. The observed magnetic moment value for Co(II) complex is to be 4.24BM, which is also suggesting the tetrahedral geometry for this complex[20]. The Ni(II) complex is found to be diamagnetic, with medium intensity bands in the visible region[21]. The transistion in this region clearly indicates the square planar geometry for this complex. However, the diamagnetic Zn(II) complex shown absorption bands at 270 nm and 374 nm due to intraligand and charge transfer transistions respectively, which would have expected the tetrahedral geometry around the Zn metal center[22].

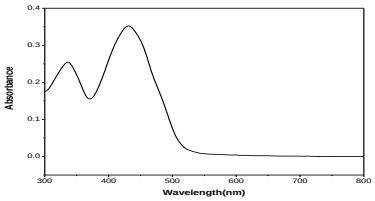


Fig. 4: UV-Vis spectrum of Cu(II)-IMBTA

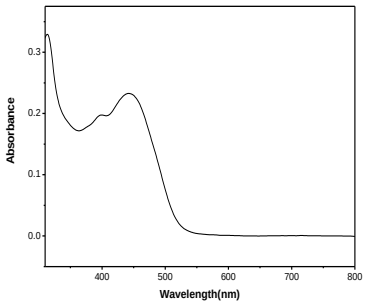


Fig. 5: UV-Vis spectrum of Co(II)-IMBTA

Infrared spectroscopy

A strong band at 1681 cm^{-1} in the IR spectra assigned to the Azomethine nitrogen of the ligand, which was shifted to lower frequency in the corresponding metal complexes of that ligand. The frequencies of Cu(II), Ni(II), Co(II) and Zn(II) complexes were 1630 cm^{-1}, 1630 cm^{-1}, 1621 cm^{-1} and 1627 cm^{-1} respectively. This indicates the coordination of azomethine nitrogen to the metal ion. It is due to the Imidazole nitrogen present in the ligand a stretching band occurred at 1619 cm^{-1}, This is also shifted towards lower frequency region in the metal complexes. However, this confirms the coordination of imidazole nitrogen to the metal ion. The bands observed in the region 3416 cm^{-1} and 1539 cm^{-1} corresponds to the –NH stretching frequencies of imidazole moiety[23]. The M-N bands of all the metal complexes were observed in the region 446-462 cm^{-1} [24]. The appearance of M-Cl bonds in the reion below 400 cm^{-1} [25].

Table 3: IR spectral data of the synthesised compounds(cm^{-1})

Compound	$\upsilon_{(CH=N)azomethine}$	$\upsilon_{(C=N)imidazole\ ring}$	$\upsilon_{(M-N)}$
Ligand	1681	1619	------
Cu(II) complex	1630	1592	462
Co(II) complex	1630	1585	446
Ni(II) complex	1621	1578	449
Zn(II) complex	1627	1591	449

Thermal analysis

It is observed that all the metal complexes do not show any weight loss upto 290°C, which suggests that there are no coordinated water molecules present in the complexes. It is between 320-510°C in TG curves, there is a significant weight loss occurred may be due to the removal of chlorine atoms as HCl gas, C, S and N in organic moiety as its oxide. It is after 600°C there is a straight line is obtained which indicates all the metals in the complexes were remained as their oxides. The Zn(II) and Cu(II) complexes do not show any weight loss upto 300°C, between 300-450°C gradual decline in the TG curve may be due to the removal of organic moiety. In the range 450-700°C the loss in weight corresponds to the remaining molecules present in the complexes. In all the cases remaining residues are metal oxides. (Fig. 6 & 7).

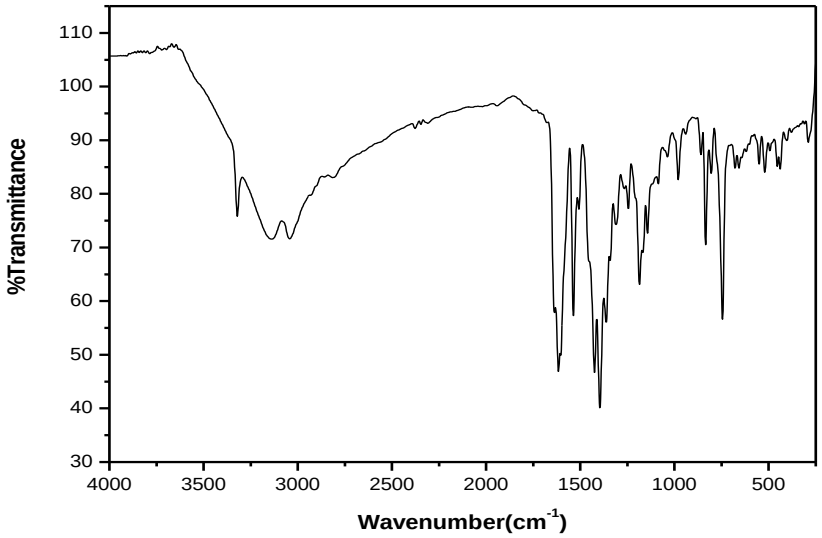


Fig. 6: IR spectrum of Ni(II)-IMBTA

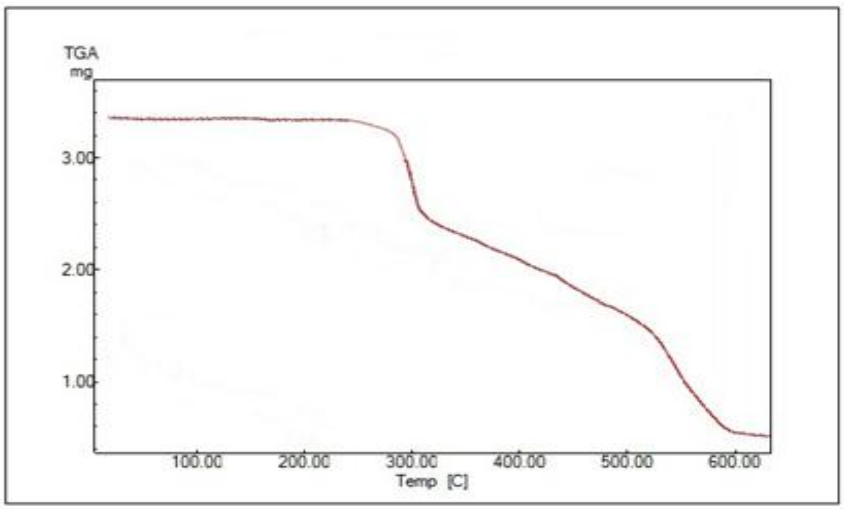


Fig. 7: TGA curve of the Ni(II)-IMBTA complex

SEM and EDX analysis

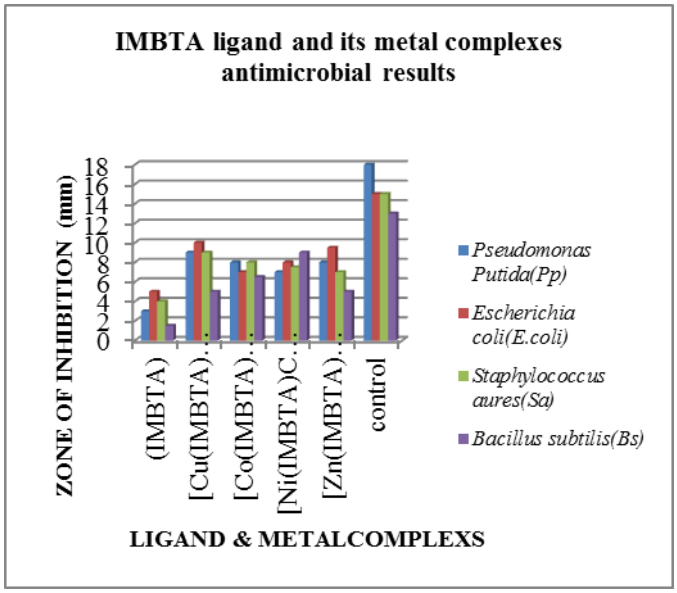
The scanning electron microscope has been used to illustrate the surface morphology and paricle size of the Schiff base metal complexes. In the present work all the metal complexes showed different morphology to that of their free ligand. The composition of the elements present in the complexes were obtained from the EDX analysis.

Antibacterial activity

The zone of inhibition of the Schiff base ligand and its metal complexes against gram positive and gram negative bacterial strains was determined by using disc diffusion method. The inhibition values are tabulated in the below **Table.4**. It is observed that all the metal complexes have shown greater activity than free ligand. This is explained on the basis of chelation theory and overtones concept[26]. The Cu(II) and Co(II) complexes have shown better activity against the **S.aureus, E.coli and P.putida** and moderate activity of Cu(II) and Co(II) complexes with **B.Subtilis** . The Ni(II) complex has shown better activity against **B.subtilis** and ligand has shown less activity against **B.subtilis** and **P.putida** bacterial strains.The Zn(II) complex has shown better activity against **E.coli** and **P.putida. Ampicillin** and **Ceftriaxone** are used as standard drugs for **gram positive** and **gram negative** strains respectively.

Table 4: Zone of inhibition (in mm)

Compound	S.aureus	E.coli	B.subtilis	P.putida
Ligand	3	5	1.5	3
Cu(II) complex	9	10	5	9
Co(II) complex	8	7	6.5	8
Ni(II) complex	7.5	8	9	7
Zn(II) complex	7	9.5	5	8
Standard	15	15	13	18



Antifungal activity

Antifungal activity of the Schiff base ligand IMBTA its metal complexes were screened against fungi *Aspergillus niger*. The activity results are presented in **Table-5** and **Table-6.** The fungal activity images of some of the metal complexes shown in **Fig-8** to **Fig-11**. Both MIC and Agar Well diffusion methods were used to evaluate the fungal activity of the compounds. From the experimental data it is found that the Cu(II) and Co(II) complexes shown significant fungal activity. Further the antifungal activity of the metal complexes have shown more activity than their free ligands.

MIC method

Minimal Inhibitory Concentration.

Table 5:

Compound/ Concentration (µg)	10	30	50	100	125	150
(IMBTA)	-	-	-	-	+	+
[Cu(IMBTA)Cl_2]	-	+	+	+	+	+
[Co(IMBTA)Cl_2]	-	+	+	+	+	+
[Ni(IMBTA)Cl_2]	-	-	-	+	+	+
[Zn(IMBTA)Cl_2]	-	-	-	+	+	+

+ = Activity Observed
= No Activity Observed

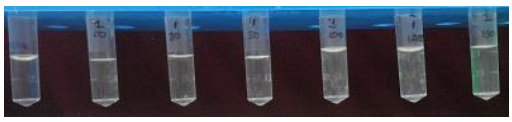


Fig. 8: Antifungal activity of [Cu(IMBTA)Cl_2]

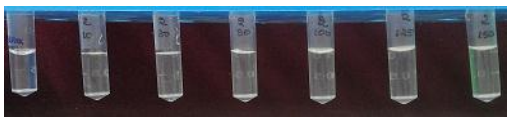


Fig. 9: Antifungal activity of [Co(IMBTA)Cl_2]

Agar Well Diffusion Assay:
Zone of Inhibition (in mm)

Table 7:

Compound/ Concentration (µg)	10	25	50	100	150
(IMBTA)	-	-	-	0.5	3
[Cu(IMBTA)Cl_2]	-	2	3	4	6
[Co(IMBTA)Cl_2]	-	3	4	5	6
[Ni(IMBTA)Cl_2]	-	1	3	4	5
[Zn(IMBTA)Cl_2]	-	1	2	3	4

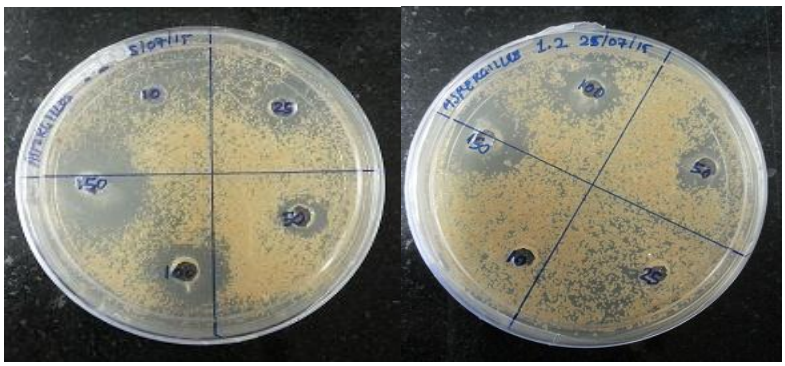


Fig. 10 : **Fig. 11:**

Fig. 10: Antifungal activity of[Cu(IMBTA)Cl_2]
Fig. 11: Antifungal activity of [Co(IMBTA)Cl_2]

IV. CONCLUSION

In the present work, a new Schiff base ligand and its metal complexes were synthesized. All the compounds were analyzed through various spectral techniques. The core intension of the present work is to check the biological activities of the compounds. All the compounds were screened against bacterial strains and fungal strain. Metal complexes have shown good antibacterial activity than ligand.

V. ACKNOWLEDGEMENTS

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