

RESEARCH ARTICLE

Synthesis, Characterization, Antimicrobial and Antifungal Activities of Mn(II) and Zn(II) Complexes with Azo Dye of 2-aminobenzimidazole and Indole

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ABSTRACT

Azo dye namely 3-[2-(benzimidazolyl)azo]-indole (BIAI) derived from diazonium salt of 2-aminobenzimidazole and indole is reported and characterized based on IR, Proton nuclear magnetic resonance (1H-NMR), elemental analyses, mass spectroscopy, X-ray diffraction (XRD) diffraction, electronic spectra. The BIAI was utilized to form Mn(II) and Zn(II) complexes. The complexes of BIAI ligands were identified *via* distinctive techniques (infrared, UV-vis, mass spectroscopies, molar conductance, Nuclear magnetic resonance (NMR) spectroscopy, X-ray diffraction (XRD) diffraction and magnetic sensitivity). The Mn(II) and Zn(II) complexes were characterized as octahedral complexes and non-electrolytic under the common formula $[M(BIAI)_2Cl_2]$. The BIAI and its complexes showed interesting antimicrobial activity against two sorts of bacterial strains, *Staphylococcus aureus*, *Escherichia coli* and *Aspergillus niger* fungi. The metal chelates of the BIPA ligand are non-electrolytes, according to molar conductance data. BIAI is coordinated to metal ions as a bidentate manner, according to IR spectra, with NN donor atoms of azomethine-N and methoxy-O. The BIPA ligand exhibited important chromic properties under pH changes.

Keywords: Benzoimidazole, Biological activity, Bidentate, Indole, pH sensor.

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INTRODUCTION

Azo compounds possess a lot of applications like antibiotics (prontosil), antifungals, dyes for fiber, sensors, optical storage, and ink-jet printers.^{1,2} Organic components bind to inorganic components to produce inorganic-organic hybrid substances that possess properties of organic and inorganic substances, which are of great importance.³⁻⁵ Azo ligands got attention as studied modules of substances utilized for forming organic-inorganic hybrids materials.⁶

Benzimidazole derivatives are nitrogenous heterocyclic molecules that possess large important biological properties such as antiviral, anticancer, antiulcer, antioxidant, antibacterial, hypotensive, and analgesic.⁷ The hybrid of benzimidazole and coumarin, with their D-ribofuranosides derivatives, showed important properties against anti-hepatitis C virus activity.⁸ Aryl-benzimidazoles were synthesized as antimycobacterial agents and benzimidazole derivatives exhibited potential

antitubercular activity.⁹ Azo dyes of benzimidazole and their complexes exhibited photoisomerization under irradiation in the liquid state.¹⁰ Azobenzimidazole dyes showed good antibacterial activities at low concentrations.¹¹

Photoisomerization is caused by the presence of an azo chromophore in azo dyes, which allows for a wide range of applications such as optical information storage and sensor optics.^{12,13} Photochromism of metal complexes containing azobenzene as side group showed reversible changes in the color and environment of dye upon irradiation.¹⁴

Wherefore, we are interested in biological activities and chromic features for azo ligands of benzimidazole and indole and their complexes. In this study, we did the synthesis, characterization and biological properties of the novel azo dye of 2-aminobenzimidazole and indole that is 3-[2-(benzimidazolyl)azo]-indole (BIAI) and its complexes of Mn(II) and Zn(II) ions.

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METHODS AND METHODOLOGY

The materials were bought from different companies, indole, 2-aminobenzimidazole, $\text{MnCl}_2 \cdot 6\text{H}_2\text{O}$ and ZnCl_2 from Sigma-Aldrich and sodium hydroxide and sodium nitrite from Alfa Aesar. The IR spectra were performed on Shimadzu using KBr disk. The Nuclear Magnetic Resonance (NMR) spectra were recorded by Bruker Avance II 400 MHz using Dimethyl sulfoxide (DMSO) (d_6) solvent. CHNS-932 did the elemental analysis of C.H.N. The UV-vis spectra were done by Shimadzu.

Synthesis: 3-[2-(benzimidazolyl)azo]-indole (BIAI)

The new dye, namely 3-[2-(benzimidazolyl)azo]-indole (BIAI) was prepared by reacting the mixture of 2-aminobenzimidazole (0.5 g, 0.0037 mol) dissolving in 10 mL of water and 3 mL of hydrochloric acid, with 0.26 g, 0.0037 mol of sodium nitrite under cooling to produce diazonium salt. The diazonium salt was reacted with an aqueous solution of indole (0.44 g, 0.0037 mol) and 0.6 g of NaOH under cooling to produce BIAI ligand. The solution of BIAI ligand was filtrated and was washed with water. The yield was 87%, red powder.

Synthesis of Mn(II) and Zn(II) complexes of BIAI ligand

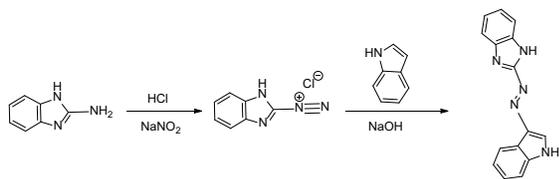
Hot aqueous solution (10 mL) of $\text{MnCl}_2 \cdot 6\text{H}_2\text{O}$ or ZnCl_2 (0.001 mol) was added to 15 mL ethanol having (0.6 g, 0.002 mol) of BIAI ligand under heating for 2 hours. The reaction solution was filtrated and was washed with a little bit of water.

RESULTS AND DISCUSSION

The azo ligand of 3-[2-(benzimidazolyl)azo]-indole (BIAI) was synthesized by reacting diazonium salt of 2-aminobenzimidazole with indole under cooling as shown in Scheme 1.

Many indicators in analytical chemistry are azo dyes as acid-base indicators like methyl orange. The BIAI ligand color changed from orange under the basic condition to dark red in the acidic condition. The UV-vis spectra of BIAI dye as shown in Figure 1, are similar in neutral and basic mediums due to exist azo mode in these mediums. While the spectrum of BIAI ligands in an acidic medium exhibited a band with a large red shift due to the presence of the hydrazine mode of BIAI dye.

The Mn(II) and Zn(II) complexes of BIAI ligand were identified *via* reacting BIAI ligand with salt chlorides of Mn(II) and Zn(II) as shown in Scheme 2, at mole ratio equal to 2:1 represents BIAI ligand to metal ion as depicted in Figure 2 for



Scheme 1: Preparing of 3-[2-(benzimidazolyl)azo]-indole (BIAI)

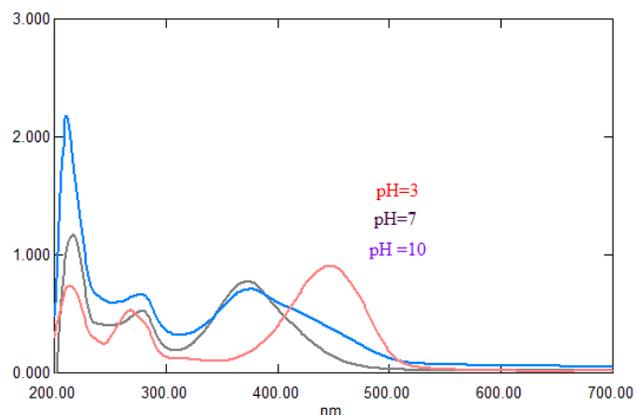
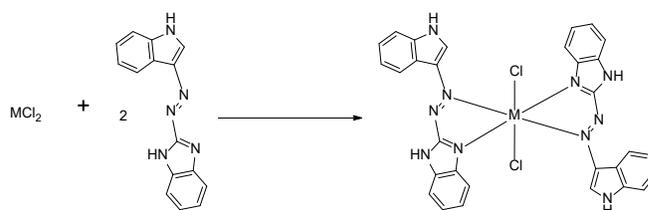


Figure 1: Sensitivity of BIAI dye under neutral, acidity and basic mediums



Scheme 2: Synthesis of Mn(II) and Zn(II) of BIAI ligand

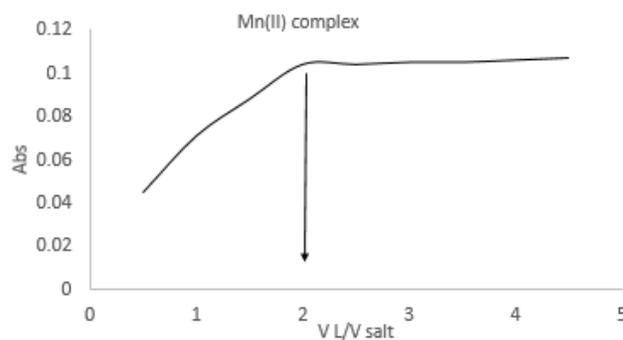


Figure 2: The mole ratio of BIAI ligand and Mn(II) ions

manganese complex.

The synthesized complexes of Mn(II) and Zn(II) for BIAI ligand were identified by elemental analysis, molar conductivity, mass spectra, FTIR, UV-vis, spectroscopy, as well as *via* X-ray diffraction (XRD) technique.

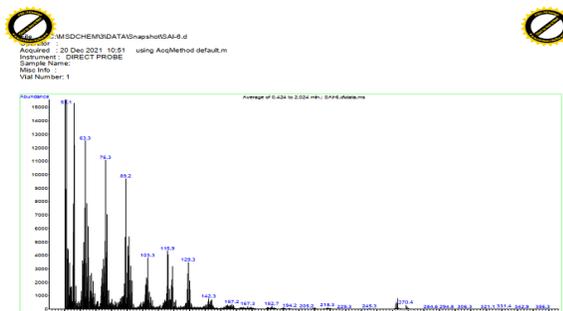
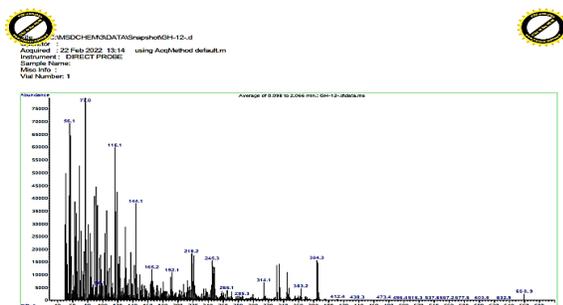
The results of elemental analyses (C, H, N) and the melting points are listed in Table 1. The obtained results are in agreement with the theoretical data for the suggested formulas.

The molar conductivity of the manganese and zinc complexes was performed within the dimethylformamide solvent (DMF) at room temperature. The conductivity values of the complexes were 9 and 7 $\text{Scm}^2 \cdot \text{mol}^{-1}$ for manganese and zinc, respectively. As a result, these coordination compounds consider non-electrolytic.

Mass spectrometry was used to identify the presence of the ligand 3-[2-(benzimidazolyl)azo]-indole. The signal

Table 1: Physical properties and elemental analysis data of BIAI ligand and its complexes

compounds	C exp, (theo)	H exp, (theo)	N exp, (theo)	color	M.p	yield	Molar conductance Scm ² .mol ⁻¹
BIAI	68.93 (68.95)	4.16 (4.24)	26.78 (26.80)	brown	162	91	-
Mn complex	55.38 (55.57)	3.35 (3.42)	31.51 (31.60)	reddish brown	284	70	9
Zn Complex	54.37 (54.69)	3.28 (3.37)	21.21 21.26	Dark brown	280	81	7

**Figure 3:** Mass spectrum of BIAI Ligand**Figure 4:** Mass spectrum of Zinc complex

(M/Z) at 261.3 as shown in Figure 3, represents the ligand mass according to the formula C₁₅H₁₁N₅. The signals for the manganese and zinc complexes were 648.5 and 658.8, respectively, in conformity with the formulas C₃₀H₂₂N₁₀Cl₂Mn and C₃₀H₂₂N₁₀Cl₂Zn as shown in Figure 4 for zinc complex.

¹H-NMR spectra

NMR spectroscopy is useful for establishing the ligands' nature and structure and the complexes in solutions. The NMR spectra of BIAI ligand and zinc complex were recorded in DMSO, (d₆) solvent.

As shown in Figure 5, the H-NMR spectrum of BIAI ligand showed eight signals in the aromatic area with an integration equating to 9 protons and two signals at 11.05 ppm and 10.70 ppm attributable to the NH of the imidazole ring and the NH of the indole, respectively. The chemical shifts of the various types of protons in BIAI ligands are as follows: 11.05 (1H, NH imidazole, s), 10.70 (2H, NH, indole, s), 8.30 (1H₂, d), 8.27 (1H₁, s), 7.63 (1H₅, t), 7.59 (1H₂^{*}, d), 7.43 (2H_{4,4}^{*}, d), 7.26 (1H₅^{*}, t), 7.19 (1H₃^{*}, t), 7.12 (1H₃, t). The spectrum of zinc complex was compared to that of the parent BIAI azo dye ligand. The spectra of zinc complex is similar to that of BIAI ligand, with the exception of shifting for chemical shifts with an integration equivalent to 18 protons in the aromatic region and two signals at 11.05 and 10.70 ppm due to NH of imidazole ring and NH of indole, respectively, as shown in

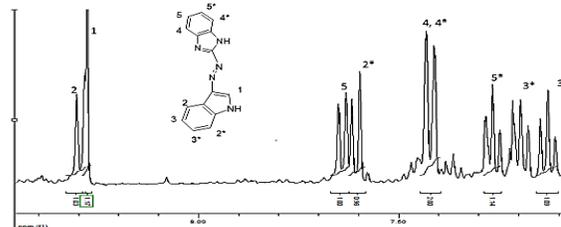
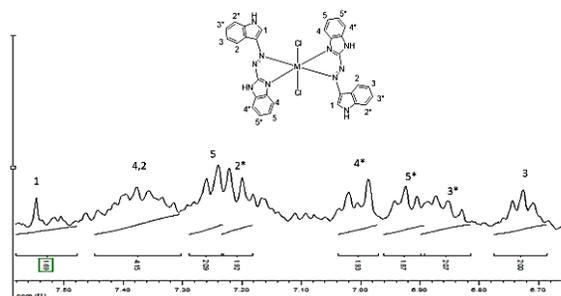
**Figure 5:** NMR spectrum of BIAI ligand in DMSO (d₆)**Figure 6:** H-NMR spectrum of zinc complex of BIAI ligand

Figure 6. The chemical shifts of different types of protons of zinc complex are 10.898 (2H, NH imidazole, s), 10.633 (2H, NH indole, s), 7.547 (2H₁, s), 7.377 (4H_{2,4}, m), 7.239 (2H₅, d), 7.200 (2H₂^{*}, d), 7.006 (2H₄^{*}, d), 6.924 (2H₅^{*}, t), 6.853 (2H₃^{*}, t), 6.728 (2H₃, t).

Infrared Spectra

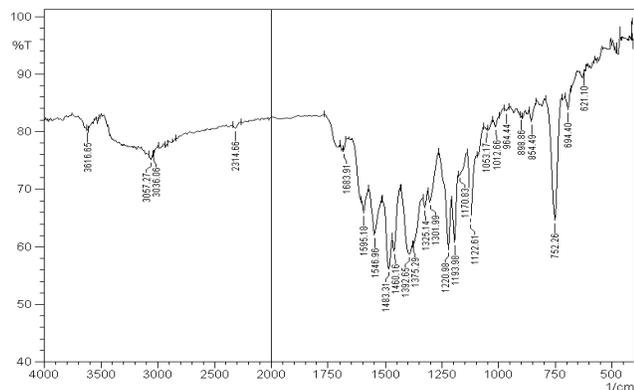
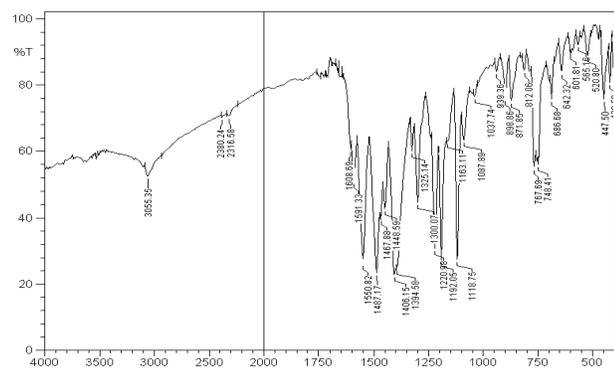
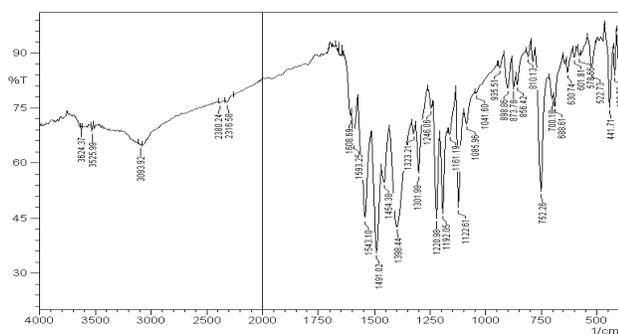
Infrared spectroscopy is an important strategy to the characterization of active functions of compounds. Where the azo-indole ligand, the 3-[2-(benzimidazole) azo]-indole ligand, showed the number of characterizing peaks reported in Table 2. The N-H groups of benzimidazoles and indoles, which occurred at (3600 and 3550) cm⁻¹ in the spectrum of the 3-[2-(benzimidazolyl) azo]-Indole ligand, did not suffer a significant alteration in the complexes, indicating that these functionalities are present but do not participate in the coordination process.

As well as other functions such as aromatic CH at 3060 cm⁻¹ and C=C at 1492 and 1626 cm⁻¹ as well as bending aromatic CH at 746 and 702 cm⁻¹, which did not suffer a change when the ligand coordinated to metals in complexes.

The 3-[2-(benzimidazolyl) azo]-indole ligand appeared assimilation of C=N at (1651) cm⁻¹, which endured a alter in area and escalated within the spectra of the complexes, which shows its consistency with the metal particles within the complexes, as its recurrence diminished by 14–51cm⁻¹

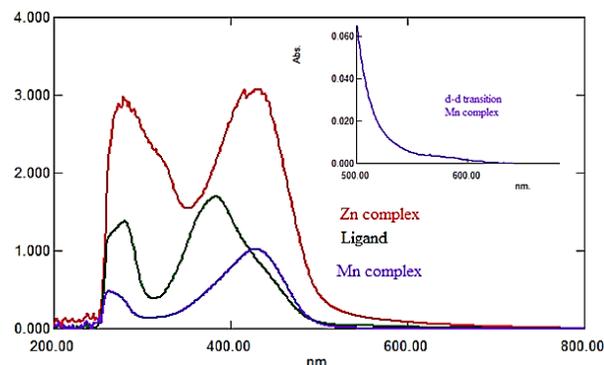
Table 2: Important Infrared frequencies of BIAI ligand and its complex

Compound	N-H imidazole	N-H indole	C-H aromatic	C=N	C=C	N=N	M-N	M-O
BIAI ligand	3616	3500	3057	1638	1595	1546	-	-
Mn (II) complex	3620	3550	3055	1608	1591 1550	1487	520	447
Zn (II) complex	3553	3300	3076	1612	1546	1487	528	420

**Figure 7:** Infrared spectrum of BIAI Ligand in KBr pellet**Figure 8:** Infrared spectrum of manganese complex of BIAI ligand in KBr pellet**Figure 9:** Infrared spectrum of zinc complex of BIAI ligand in KBr pellet

in consistency. The ligand also appeared the assimilation of the N=N gather at $(1560) \text{ cm}^{-1}$, which suffered an alteration in area and escalated within the spectra of the complexes, which shows its consistency with the metal particles within the complexes, as its recurrence diminished by 42 cm^{-1} when planning. The infrared spectra of synthesized compounds are listed in figures 7-9.

The UV-vis spectra of BIAI ligand and its complexes were

**Figure 10:** UV-Vis spectra of BIAI ligand and its complexes in DMSO solvent at laboratory temperature

done in DMSO solvent at laboratory temperature, depicted in Figure 10. The BIAI ligand exhibited a band at 279 nm due to $\pi \rightarrow \pi^*$ and a large band at 384 nm due to $n \rightarrow \pi^*$. The spectrum of zinc complex was similar to BIAI ligand, which exhibited a band at 277 nm and a band at 433 nm, which suffered a redshift of around 49 nm compared to the parent ligand. This behavior agrees with zinc complexes spectra, which do not have d-d transition.

The manganese (II) complex exhibited two bands at 293 and 428 nm due to electronic transitions of BIAI ligand. The other band was at 570 nm, which is attributed to $6A_{1g} (4T_{2g})$ a comping with magnetic sensitivity equal to 5.81 B.M. These data of electronic transitions and magnetic sensitivity agree with octahedral geometry for the Mn(II) complex of BIAI ligand.¹⁵

The crystal structures of BIAI ligand and its complexes were studied by using XRD within the angular range of 20–80. The X-ray spectra of the ligand and its complexes were included in Figures 11-13. The crystal size was calculated by Debye-Scherrer equation using Xpert HighScore program. The BIAI ligand showed the XRD peaks at 2θ equals to 13.703° , 16.015° , 17.75° , 23.917° , 27.322° , 31.883° , 45.63° , 56.68° , 66.444° , 75.438° . The XRD signals of the manganese complex are 13.381, 19.87, 26.229, 27.579, 31.754, 45.63, 56.615, 75.567, and the XRD signals of zinc complex are 12.932, 19.87, 22.825, 26.808, 27.45, 31.818, 45.566, 56.68, 75.438.

The spectra of ligand and its complexes showed sharp signals indicating these compounds' crystal structure. It also notes a difference between the X-ray spectra of ligands and its complexes, which indicates to form a new complex. All spectra of BIAI ligand and its complexes showed nanocrystal sizes less than 100 nm, indicating these compounds' nanocrystal structure.

Biological Activities

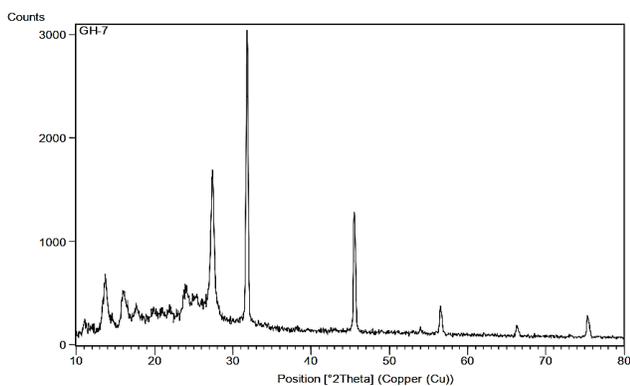


Figure 11: XRD spectrum of BIAI ligand

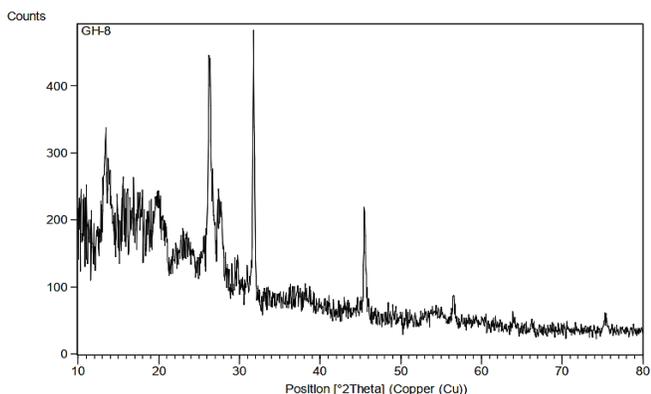


Figure 12: XRD spectrum of manganese complex of BIAI ligand

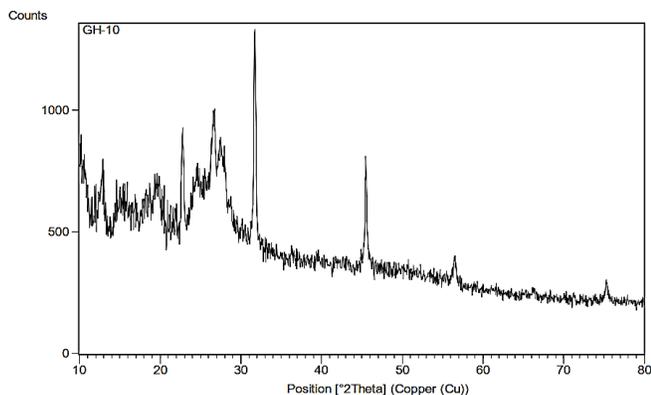


Figure 13: XRD spectrum of zinc complex for BIAI ligand

Many azo dyes possessing heterocyclic rings and their complexes displayed a tall development hindrance against numerous microbes and fungi.¹⁶⁻¹⁸ Therefore, we were fascinated by identifying the impact of BIAI ligand and its chelate complexes of Mn (II) and Zn(II) on two sort of microbes, *S. aureus* (positive gram) and *E. coli* (negative gram) and *A. niger* as a sort of fungi. The antimicrobial inhibition of BIAI ligand and its complexes against bacteria organisms are recorded in Table 3.

The BIAI ligand appeared high growth inhibition zone against *E. coli*, it was in the range of 40-100 mm at 100 mg/mL. While values of the inhibition zone of these compounds

Table 3: Inhibition zone (mm) results of BIAI and its complexes against *E. coli* and *S. aureus* bacteria

Compound	<i>S. aureus</i>	<i>E.coli</i>
BIAI ligand	0.1	1
Mn (II) complex	0.3	0.7
Zn (II) complex	0.2	0.4

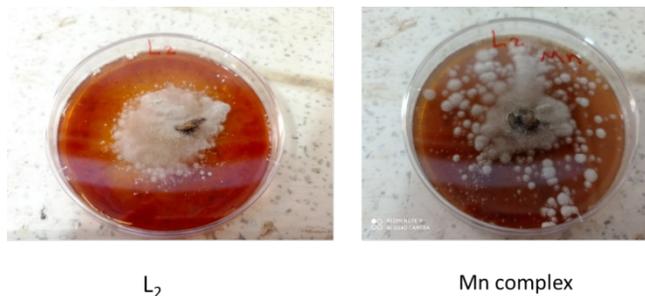


Figure 14: Biological activity BIAI ligand and Mn(II) complex on *Aspergillus niger*

against *S. aureus* were moderate in the range of 10-30 mm lower than biological activity against *E.coli*.

The BIAI ligand and its complexes displayed fabulous antifungal action against *A. niger*. It was near to be idealize as shown in Figure 14. As a result, these compounds are considered a candidate as drugs against fungi.

CONCLUSION

Diverse physicochemical techniques were utilized to characterize and affirm the BIAI dye and its complexes of Mn(II) and Zn(II). The complexes had the same molar proportion of 2:1 based on their stoichiometry (BIAI ligand: metal ions) in an impartial state. Antifungal and antibacterial activities were detailed for BIAI dye and its complexes, especially against fungi. The BIAI ligand was discovered to have excellent pH sensor characteristics.

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