



Research Article

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Microwave assisted synthesis, characterization, and antimicrobial studies of transition metal complexes of schiff base ligand derived from isoniazid with 2-hydroxynaphthaldehyde

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ABSTRACT

New complexes of *N*-isonicotinamido-2-hydroxy naphthalaldimine with Co(II), Zn(II), and Cu(II), having formula of the type $[M(L)_2].nH_2O$ ($M = Zn^{+2}, n=2, Co^{+2}, n=1, Cu^{+2}, n=3$), and with Ni(II), having formula of the type $[M(L)H_2O](ac).nH_2O$ ($M = Ni^{+2}, n=0.5$ ac = CH_3COO^-) have been synthesized. The nature of bonding and geometry of the transition metal complexes as well as the Schiff base ligand have been deduced from NMR, IR, UV-VIS, AAS, XRF, EI mass spectrometry, elemental analysis and thermo gravimetric studies. Co(II), Zn(II), and Cu(II) metal ions formed 1:2 (M:L) complexes; whereas, Ni(II) gave a 1:1 (M:L) complex. Based on elemental and spectral studies, octahedral geometries were assigned to Zn(II), Co(II) and Cu(II) complexes and a tetrahedral geometry was assigned to Ni(II) complex. The ligand acts as a monoanionic tridentate and coordinates through the carbonyl oxygen, azomethine nitrogen and phenolic oxygen. The biological activity of the ligand and of the corresponding complexes was tested on Gram positive bacteria (*Escherichia coli*) and Gram negative bacteria (*Staphylococcus aureus*) and two fungi (*Aspergillus flavus* and *Candida albicans*). The biological activity data show that some metal complexes are more potent than of the free ligand

Key words: Schiff Base Ligand, Transition Metal Complexes, Biological Activity

INTRODUCTION

Schiff bases play an important role in inorganic chemistry as they easily form stable complexes with most transition metal ions in the periodic table[1]. Schiff bases derived from the condensation of isonicotinic acid hydrazide (isoniazid, INH) with aldehydes represent an important class of chelating ligands and their metal complexes are of great interest due to their importance in biological, pharmacological and clinical applications[2]. The hydrazone derivatives are used as fungicides and in the treatment of some diseases such as tuberculosis, leprosy and mental disorders[3]. The remarkable biological activity of acid hydrazides $R-CO-NH-NH_2$, a class of Schiff bases and their corresponding aroylhydrazones, $R-CO-NH-N=CH-R'$ and the dependence of their mode of chelation with transition metal ions present in the living system have been of significant interest in the past. The coordination compounds of aroylhydrazones have been reported to act as enzyme inhibitors and are useful due to their pharmacological applications[4]. Isonicotinic acid hydrazide (INH) is a drug of proven therapeutic importance and is used against a wide spectrum of bacterial ailments, such as tuberculosis. Hydrazones derived from condensation of isonicotinic acid hydrazide with aldehydes have been found to show better antitubercular activity than INH[1].

Microwave assisted organic synthesis (MAOS) has become a new and quickly growing area in the synthetic organic chemistry. This synthetic technique is based on the observation that some chemical reactions proceed much faster and with higher yields under microwave irradiation as compared to conventional heating. In many cases, reactions that normally require many hours at reflux temperature under classical conditions can be completed within several minutes or even seconds in a microwave oven. Recent simplifications of microwave reaction enhancement

techniques have increased safety and practical utility of the microwave oven for their use in chemical laboratories without any modification[5].

EXPERIMENTAL SECTION

Materials

Isonicotinic acid hydrazide (Isoniazid) was obtained from BDH chemicals Ltd. 2-hydroxy naphthaldehyde, and zinc(II) chloride were obtained from Sigma Aldrich. Ethanol, chloroform and nickel(II) acetate were obtained from Breckland Scientific Supplies. Copper(II) chloride and ethylacetate were obtained from T-Baker Lab chemicals. Cobalt(II) chloride was obtained from BDH limited Poole England.

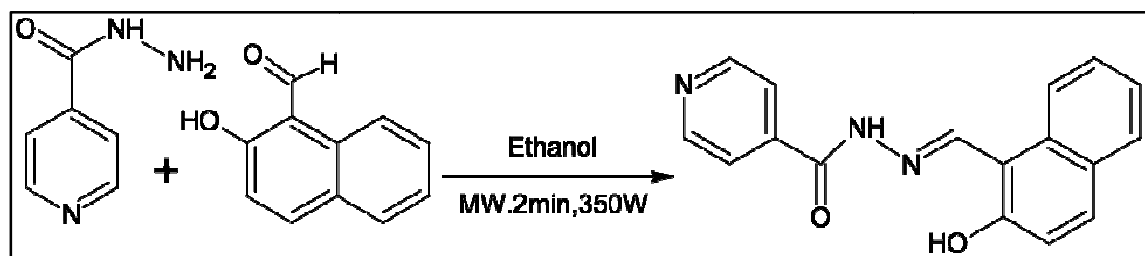
Techniques

Microwave syntheses were performed in a microwave extraction system (start E) from Milestone Company. Melting points were determined on a kofler melting points apparatus and were uncorrected. The electronic absorption spectra were recorded in a DMSO solution in the wavelength range 200–800nm using a Cary 5000 UV-VIS-NIR spectrophotometer. The IR spectra were recorded on a Varian FT-IR spectrophotometer. ^1H & ^{13}C NMR spectra were recorded in DMSO- d_6 on a Bruker's 400MHz. The mass spectra were recorded by the EI technique at 70eV using Shimadzu QP- 2010 plus. The thermogravimetric analyses (TGA and DSC) were carried out in dynamic nitrogen atmosphere (30mL/min) with a heating rate of 10°C/min using Shimadzu TGA-50H thermal analyzers. TLC analyses were performed on pre-coated aluminum plates (silica gel 60778, fluke analytical). TLC spots were visualized with UV light.

Synthesis of Schiff base (ligand)

The Synthesis of Schiff base is schematically presented in (scheme 1).

The Schiff base has been synthesized by the reaction of isoniazid (0.5g, 3.64mmole) and 2-hydroxy naphthaldehyde (0.62g, 3.60mmole) with the addition of 3-4 drops of ethanol. The reaction was carried out in a microwave for 2min at 60°C and 350W. The condensation product was washed with ethanol and water and filtered. The final product was recrystallized from ethanol to give yellow crystals (0.8g, 75.4%), mp. 271°C. The purity of the product was monitored by the use of TLC, using chloroform and ethylacetate (3.5:1.5).



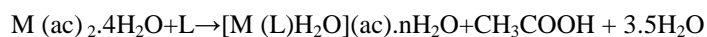
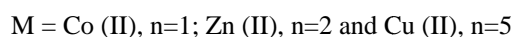
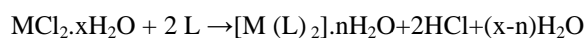
Scheme 1: Synthesis of Schiff base

Synthesis of metal complexes

The complexes were synthesized by mixing the appropriate metal chloride or acetate with the required amount of the ligand in a 1:1 or 1:2 metal to ligand ratios. The mixture was milled and few drops (~1mL) of ethanol were added. The reaction mixture was then stirred and irradiated in a microwave oven at 350W for 4min at 60°C. The final products were washed with hot ethanol, filtered and dried at room temperature. The metal salts used were ZnCl_2 , $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ and $\text{Ni}(\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$.

RESULTS AND DISCUSSION

The complexes have been synthesized by microwave methods from reaction of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, ZnCl_2 and $\text{Ni}(\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$ with Schiff base (ligand) in presence of ethanol as a solvent. Formation of the complexes may have proceeded according to the following equations.



M=Ni (II), n=0.5

The ligand behaves as a monoanionic tridentate (O, N and O) through the carbonyl oxygen, azomethine nitrogen and phenolic oxygen. Composition was deduced from elemental analyses, FT-IR, UV-VIS, XRF, AAS, MS and TGA. The analytical data of the complexes indicated that the ligand forms a 1:2 (M:L) complexes with Zn(II), Co(II) and Cu(II) and a 1:1 (M:L) with Ni(II) ions. The mass spectral and atomic absorption data confirm the monomeric structure of the metal complexes while TGA studies confirm the presence of water molecules in some complexes and XRF data show that no chloride anions are present outside the coordination sphere in Zn(II), Co(II) and Cu(II) complexes.

Physical properties

The details of physical properties of the ligand and its complexes are tabulated in (table 1).

Table 1: Some physical properties of the ligand and its complexes.

Compounds	Color	Yield (%)	M.p. (°C)	Physical Appearance	Solubility
C ₁₇ H ₁₃ N ₃ O ₂ (HL)	Dark yellow	75.4	271	crystalline	Soluble in DMF, DMSO, THF, hot EtOH/MeOH and hot (CH ₃) ₂ CO
[Zn(L) ₂].2H ₂ O	Orange	78	> 300	Powder	Soluble in DMF, DMSO
[Co (L) ₂]. H ₂ O	Brown	69	> 300	Powder	Soluble in DMF, DMSO
[Ni(L)H ₂ O](ac) 0.5H ₂ O	Dark Red	73	> 300	Powder	Soluble in DMF, DMSO
[Cu(L) ₂].5H ₂ O	Dark Brown	75	> 300	Powder	Soluble in DMF, DMSO

¹H and ¹³CNMR of the Ligand

The ¹H-NMR spectrum for ligand showed a peak at 12.47ppm (s, 1H, -OH), a peak at 9.5ppm (s, 1H, N=CH), a peak at 8.85ppm for H1 (d, J_{H1-H2} = 5Hz), a peak at 7.9ppm for H2 (d, J_{H2-H1} = 5Hz), a peak at 7.26ppm for H6 (d, J_{H6-H7} = 9Hz), a peak at 8.33 ppm for H7 (d, J_{H7-H6} = 9Hz), a peak at 7.63ppm for H10 (t, J_{H10-H11,9} = 9Hz, d, J_{H10-H8} = 2Hz), a peak at 7.43ppm for H9 (t, J_{H9-H10,8} = 9Hz, d, J_{H9-H11} = 2Hz), and a peak at 7.96 ppm for H8=H11 (d, J_{H8-H9} = 9Hz). The proton of -NH group was not observed. This may be due to the formation of intra molecular hydrogen bonding or exchangeable with solvent. The ¹³C NMR spectrum of ligand (figure 1) showed a peak at 158.23 ppm which was assigned to the carbonyl group carbon (C4); a peak at 147.97ppm that was assigned to the carbon of the azomethine group (C5), and peaks at 161.04, 150.44, 139.82, 133.09, 131.61, 128.95, 127.85, 127.83, 123.57, 121.40, 120.88, 118.83, 108.49 ppm, which were assigned to C7, C1, C3, C11, C9, C10, C15, C13, C14, C2, C8, C12 and C6, respectively.

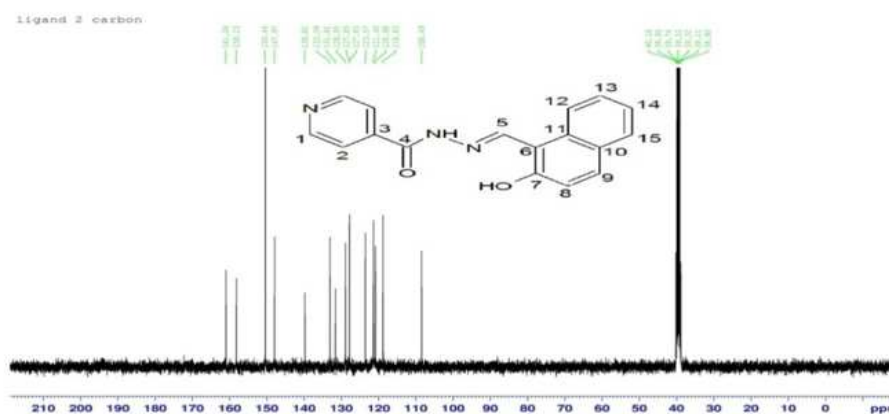


Figure 1: ¹³C NMR spectrum of the ligand

Elemental analyses of the ligand and its metal complexes along with X-ray fluorescence analysis and atomic absorption spectroscopy

The results of elemental analysis, XRF and AAS of the ligand and its complexes are given in (table 2). They are consistent with the calculated results from the empirical formula of each compound.

Table 2: The results of elemental analysis, XRF and AAS of the ligand and its complexes

Compounds	Formula weight	CHN elemental analysis Found (Calcd.)%			XRF (%)	AAS (%)
		C	H	N	Cl	M
[HL] C ₁₇ H ₁₃ N ₃ O ₂	291.00	70.31 (70.9)	4.77 (4.50)	15.07 (14.42)
[Zn(L) ₂].2H ₂ O C ₃₄ H ₂₈ N ₆ O ₆ Zn	682.03	59.46 (59.87)	4.43 (3.74)	12.47 (12.32)	< LLD	8.16 (9.59)
[Co (L) ₂]. H ₂ O C ₃₄ H ₂₂ N ₆ O ₅ Co	657.54	61.98 (62.10)	3.36 (3.78)	12.58 (12.78)	< LLD	7.92 (8.96)
[Ni(L)H ₂ O](ac)0.5H ₂ O C ₁₉ H ₁₈ N ₅ O _{5.5} Ni	435.06	54.66 (52.45)	3.56 (4.02)	9.41 (9.66)	12.55 (13.49)
[Cu (L) ₂].5H ₂ O C ₃₄ H ₃₄ N ₆ O ₉ Cu	734.00	54.90 (55.62)	3.05 (3.76)	11.13 (11.45)	< LLD	7.11 (8.65)
<i>LLD; Lower Limit of Detection= 0.01ppm</i>						

IR spectra

The IR spectra of the complexes were compared with those of the free ligand in order to determine the coordination sites that may be involved in chelation. There were some guide peaks in the spectra of the ligands, which were helpful in achieving this goal. The position and/or the intensities of these peaks are expected to change upon chelation[6]. The main bands in the IR spectra of ligand and its complexes are presented in (table 3). The IR spectrum of the ligand exhibits a strong band at 1673 cm⁻¹ due to $\nu_{(C=O)}$ of the amide group. This has shifted and was strongly displaced in all complexes indicating coordination through the carbonyl oxygen. A band at 1573 cm⁻¹ due to $\nu_{(C=N)}$ azomethine group has shifted to lower frequencies in all complexes. This suggests the involvement of the azomethine nitrogen in coordination. Another important ligand band, which occurs at about 3211 cm⁻¹, is due to the phenolic-OH group and has been found absent in the complexes. This indicates the deprotonation of phenolic-OH on coordination with metal. The band due to phenolic C-O stretching vibration is observed at 1273 cm⁻¹ in the free ligand. In all complexes, this band appears at lower frequencies, in the range 1193-1198 cm⁻¹, confirming the involvement of the phenolic oxygen in the coordination with the metal ions[7,8]. In the case of the Ni complex, the IR spectrum displays two bands assigned to the acetate group, the frequency of $\nu_{asym} (COO^-)$ appears at 1583 cm⁻¹ and frequency of $\nu_{sym} (COO^-)$ appears 1404 cm⁻¹ regions[9,10].

Table 3: Selected infrared frequencies (cm⁻¹) of ligand and its complexes

Ligand/complexes	$\nu_{(C=O)}$ Amide	$\nu_{(C=N)}$ Azomethine	$\nu_{(OH)}$ Phenolic	$\nu_{(C-O)}$ Phenolic
C ₁₇ H ₁₃ N ₃ O ₂ (HL)	1763s	1573s	3211s	1273s
[Zn(L) ₂].2 H ₂ O	1609s 1586s	1537s		1194s
[Co (L) ₂]. H ₂ O	1616m 1580s	1533s		1193s
[Ni(L) H ₂ O](ac).0.5H ₂ O	1616s 1600s	1536s		1196s
[Cu(L) ₂].5H ₂ O	1600s 1583s	1525s		1198s
<i>s: strong, m: medium</i>				

Electronic spectra

The UV-visible spectra of the complexes were recorded in DMSO solutions in the wavelength range 200–800 nm at room temperature. The spectral data of the ligand and its Co⁺², Ni⁺², Zn⁺² and Cu⁺² complexes are given in (table 4). The ligand spectrum shows three bands, assigned to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions. In the electronic spectra of the

complexes, the ligand bands are shifted to higher or lower values of the frequencies, reflecting the coordination at the metallic ions.

Table 4: Electronic spectral data and geometries for the ligand and its complexes

Ligand/complexes	Frequencies nm/cm ⁻¹	Assigning	Geometry
C ₁₇ H ₁₃ N ₃ O ₂ (HL)	277/36101 329/30395 369/27100	$\pi \rightarrow \pi^*$ $\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$
[Zn(L) ₂].2H ₂ O	275/36363 328/30487 368/27173 343/23041 458/21834	$\pi \rightarrow \pi^*$ $\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$ charge transfer charge transfer	Octahedral
[Co(L) ₂].H ₂ O	282/35460 344/29069 427/23419 451/22172 490/20408	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$ ${}^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ ${}^4T_{1g}(F) \rightarrow {}^4A_{2g}$ ${}^4T_{1g}(F) \rightarrow {}^4T_{2g}$	Octahedral
[Ni(L)H ₂ O](ac). 0.5H ₂ O	276/36231 463/21598 500/20000	$\pi \rightarrow \pi^*$ ${}^3A_2(F) \rightarrow {}^3T_1(P)$ ${}^3A_2(F) \rightarrow {}^3T_1(F)$	Tetrahedral
[Cu(L) ₂].5H ₂ O	280/35714 329/30395 368/27173 442/22624	$\pi \rightarrow \pi^*$ $\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$ ${}^2T_{2g} \rightarrow {}^2E_g(D)$	Octahedral

The electronic spectrum of the zinc(II) complex shows two absorption bands at 434 and 458nm, These transitions may be attributed to the charge transfer bands, which proves the coordination of the ligand to the metal ion[11]. The electronic spectrum of the cobalt(II) complex shows three d-d absorption bands at 427, 451 and 490nm, attributed to the d-d transitions: ${}^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$, ${}^4T_{1g}(F) \rightarrow {}^4A_{2g}$ and ${}^4T_{1g}(F) \rightarrow {}^4T_{2g}$, respectively, These transitions are consistent with the characteristic octahedral geometry. The electronic spectrum of the nickel(II) complex shows two d-d absorption bands (weak) at 463 and 500nm, while the third d-d band is not observed. These bands are assigned to ${}^3A_2(F) \rightarrow {}^3T_1(P)$ and ${}^3A_2(F) \rightarrow {}^3T_1(F)$ transitions, respectively. In fact, the band assigned to the ${}^3A_2(F) \rightarrow {}^3T_2(F)$ electronic transition is missing, which are characteristic of a tetrahedral geometry. The electronic spectrum of the copper(II) complex shows one d-d absorption band at 442nm. This band is assigned to the ${}^2T_{2g} \rightarrow {}^2E_g(D)$ transition, which is in conformity with octahedral geometry[12].

Mass spectra

The most important peaks in the EI mass spectral data of all complexes are listed in (table 5). The mass spectrum of the ligand showed the molecular ion peak at $m/z = 291$ which corresponds to its molecular formula $[C_{17}H_{13}N_3O_2]^+$. The base peak at $m/z = 169$ is due to the $[C_{11}H_7NO]^+$ ion. The different competitive fragmentation pathways of ligand give the peaks at different mass numbers at 51, 78, 106, 115 and 170. The molecular ion peaks of Zn(II), Cu(II), Ni(II) and Co(II) complexes were observed respectively at 647, 640, 367 and 644, respectively, and they were in good agreement with the molecular weight of the proposed structures. In addition to the peaks due to the molecular ion, the spectra exhibit peaks assignable to various fragments arising from the thermal cleavage of the complexes. The peak intensity gives an idea of the stability of the fragments.

Table 5: Mass fragmentation of the ligand and its metal complexes

Compounds	m/z	Fragments	R.I (%)
[C ₁₇ H ₁₂ N ₃ O ₂]	291	[M] ⁺	32.21
	169	[C ₁₁ H ₇ NO] ⁺	100
[Zn(C ₁₇ H ₁₂ N ₃ O ₂) ₂]	648	[M+2] ⁺	30.93
	647	[M+1] ⁺	33.05
[Co(C ₁₇ H ₁₂ N ₃ O ₂) ₂]	641	[M+2] ⁺	21.16
	640	[M+1] ⁺	12.03
[Ni (C ₁₇ H ₁₂ N ₃ O ₂)H ₂ O] ⁺	367	[M] ⁺	70.00
	366	[M-1] ⁺	73.33
[Cu (C ₁₇ H ₁₂ N ₃ O ₂) ₂]	645	[M+1] ⁺	88.24
	644	[M] ⁺	55.88
	643	[M-1] ⁺	64.71

Thermal analyses (TGA and DTA)

Thermal analyses (TGA and DTA) of the Schiff base ligand and its complexes were used to obtain information about the thermal stability of these new complexes, and decide whether the water molecules (if present) are inside or outside the inner coordination sphere of the central metal ion [10]. The results of thermal analysis of the ligand and its metal complexes, which are given in (table 6), showed good agreement with the theoretical formula as suggested from elemental analysis. TGA curve of ligand does not show any weight loss below 130°C suggesting the absence of lattice water molecules. The decomposition steps of the ligand, which occur within the temperature range 139-743°C, involve mass losses of 98.979% [13]. The TG curves of the complexes indicated the presence of lattice/coordinated water molecules. The thermograms of the Zn(II), Co(II) and Cu(II) complexes shows two decomposition steps within the temperature range 67-289 °C, 29-262 °C and 33-278 °C, respectively, and each exhibits a mass loss of 4.75% (calcd. 5.27%), 3.26% (calcd. 2.73%) and 12.67 (calcd. 12.25%), which correspond to the loss of hydration water molecules, for Zn(II), Co(II) and Cu(II) complexes, respectively. The TGA curve of the Ni (II) complex shows two decomposition steps within the temperature range 144-387 °C. The first step at 144-275 °C, and exhibits a mass loss 1.73% (Calcd. 2.06%), correspond to the loss of water molecules of hydration and the second step at 276-387 °C, and exhibits a mass loss 4.63% (Calcd. 4.13%), correspond to the loss of water molecules of coordination. The other decomposition steps within the temperature range 290-800°C in all complexes involve the loss of the ligand [6,10,14,15].

Table 6: Thermoanalytical results (TGA, DTA) of the metal complexes with ligand. (n*=number of decomposition steps; L=C₁₇H₁₂N₃O₂)

Compounds	TG range (°C)	DTA _{max} (°C)	n*	Mass loss (calcd.) Found %	Assignment
[Zn(L) ₂].2H ₂ O	67-289	71,227	2	(5.27)4.75	Loss of 2H ₂ O (hydration)
[Co(L) ₂].H ₂ O	262-29	47,205	2	(2.73)3.26	Loss of H ₂ O (hydration)
[Ni(L)H ₂ O](ac) 0.5H ₂ O	276- 275 144-387	212 313	1 1	(2.06)1.73(4.13)4.63	Loss of 0.5H ₂ O (hydration) Loss of H ₂ O (coordinated)
[Cu(L) ₂].5H ₂ O	278-33	58,214	2	(12.26)12.67	Loss of 5H ₂ O (hydration)

Based on the above analytical and spectral data, the structure formula and stoichiometries for metal complexes shown in (Figure 2) were proposed.

Antimicrobial activity

The antimicrobial activity of the Schiff base (ligand) and its complexes with Zn(II), Co(II), Ni(II) and Cu(II), were tested against the two bacteria, Escherichia coli (Gram negative bacteria) and Staphylococcus aureus (Gram positive bacteria), and two fungi, Aspergillus flavus and Candida albicans. Tetracycline and Amphotericin B were used as standards. DMSO was used as solvent control. The values of zone inhibition were measured in millimeter. The data of the antifungal and antibacterial activities of ligand and complexes are given in (table 7, figure 3). The data reveal that the complexes have higher activities than the free ligand. This enhancement of the activity of ligand on complexation can be explained by chelation theory. The theory states that chelation reduces the polarity of the metal atom by the partial sharing of its positive charge with donor groups and possible π-electron delocalization

over the whole ring. This results in the increase of the lipophilic character of the complex and favors the permeation of the complex through the lipid layer of cell membrane. The complex blocks the metal binding sites in the enzymes of microorganisms. Consequently, the complex disturbs the metabolism pathways in cell and as a result microorganisms die[16]. The results the antimicrobial tests indicate that cobalt complex shows maximum inhibition zone against selected microorganisms.

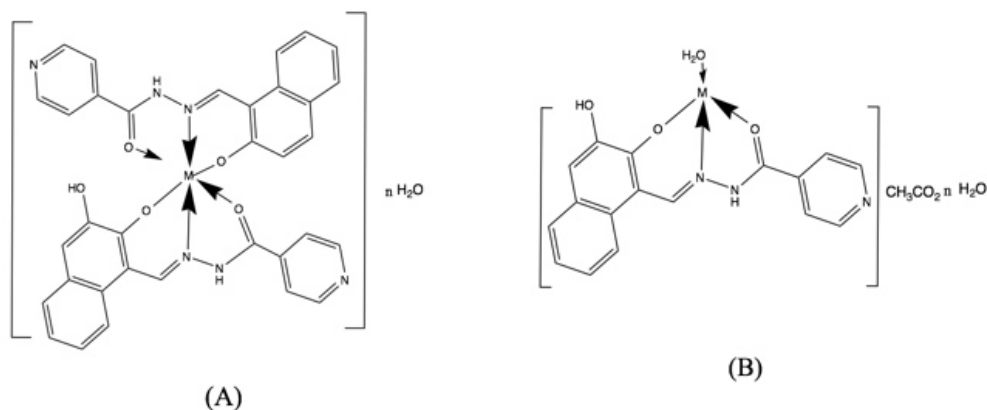


Figure 2: Structural formula proposed for;(A) $[M(L)_2] \cdot nH_2O$ ($M = Cu^{2+}$, $n = 5$; $M = Co^{2+}$, $n = 1$; $M = Zn^{2+}$, $n = 2$) and (B) $[M(L)(H_2O)](ac) \cdot nH_2O$ ($M = Ni^{2+}$, $n = 0.5$, $ac = CH_3COO^-$)

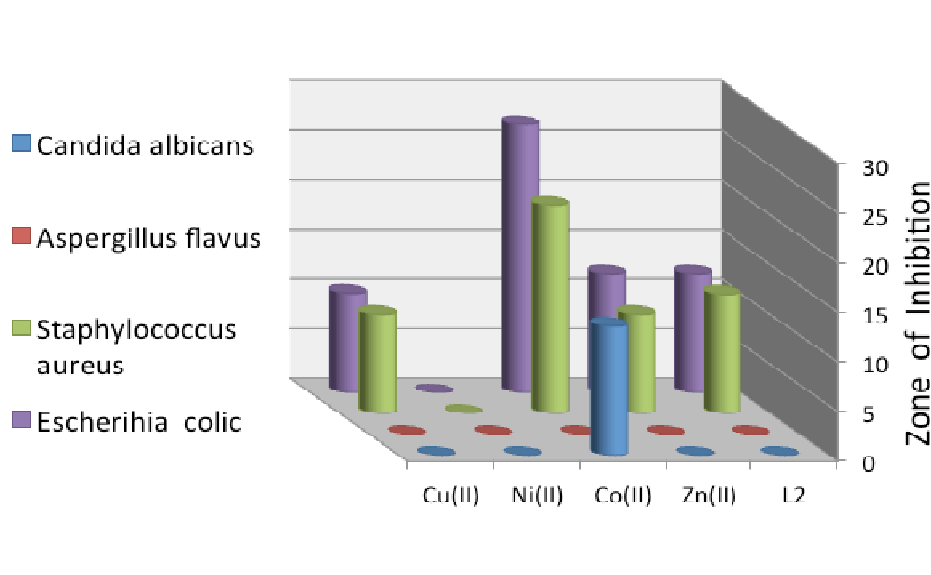


Figure 3: Zone of inhibition (mm) of the ligand and its metal complexes

Table 7: Antimicrobial activity data of the ligand and its metal complexes

Compounds	Inhibition zone diameter (mm / mg sample)			
	E. coli (G)	S. aureus (G ⁺)	A. flavus	C. albicans
Control: DMSO	0	0	0	0
Tetracycline (standard) Antibacterial agent	31	28	----	----
Amphotericin B(standard) Antifungal agent	----	----	16	19
C ₁₇ H ₁₃ N ₃ O ₂ (HL)	12	12	0	0
[Zn(L) ₂].2H ₂ O	12	10	0	0
[Co(L) ₂].1H ₂ O	27	21	0	13
[Ni(L)H ₂ O](ac).0.5H ₂ O	0	0	0	0
[Cu(L) ₂].5H ₂ O	10	10	0	0

CONCLUSION

In this study, a series of Co (II), Ni (II), Cu (II) and Zn (II) complexes with Schiff base derived from isoniazid and 2-hydroxynaphthaldehyde were synthesized and characterized. Schiff base behaves as a monoanionic tridentate ligand. On the basis of different techniques, the structural formulae of the complexes are proposed (figure 2). The Schiff base and some of the metal complexes were found to be active against some of the representative bacterial and fungal strains.

Acknowledgments

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