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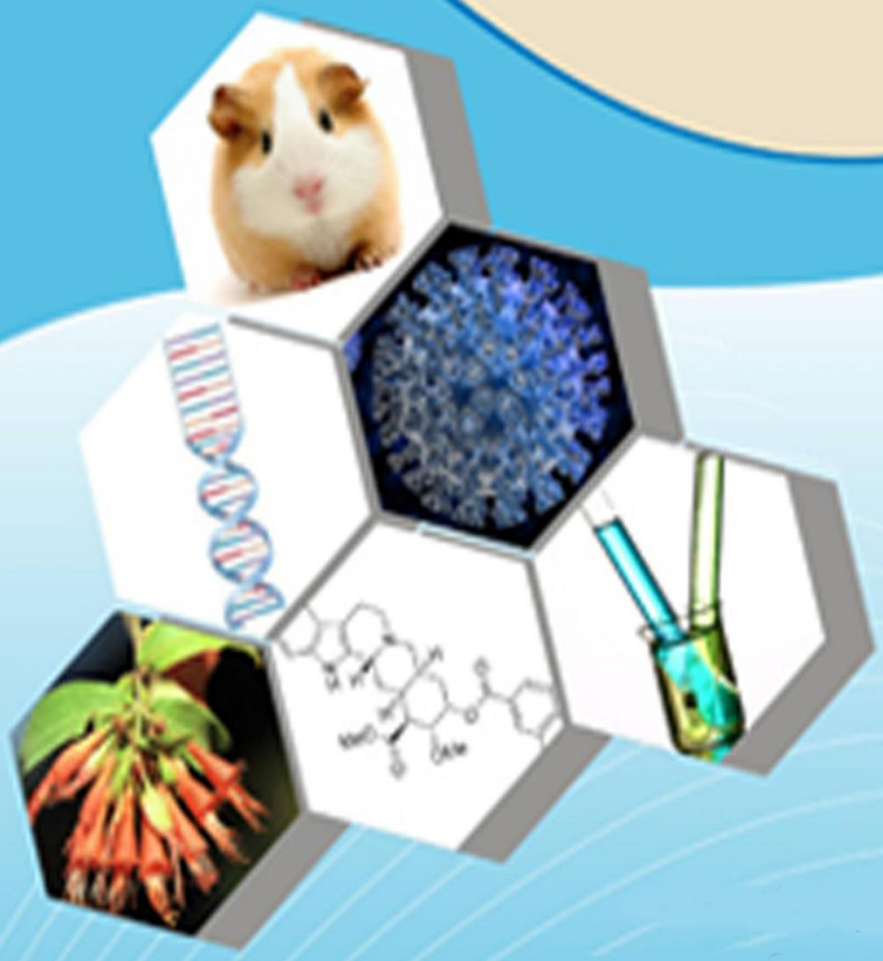

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Antimicrobial Evaluation of Novel Metals Complexes of Isonicotinamido-2-hydroxy-5-methoxybenzalaldimine

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Abstract

The emergence of antimicrobial-resistant bacteria has increased the need for new, more effective medications to treat illnesses. The interaction of several agents with metal ions has been proven to increase their antibacterial activity. Polydentate ligand complexes of metal ions have been the subject of much study because of their intriguing spectroscopic, magnetic, and biological features. The microwave synthesis method was used to create new isoniazid-based compounds and their transition metal complexes (cobalt (II), copper (II), nickel (II), and zinc (II). Multiple spectroscopy methods (including FT-IR, UV/visible electronic, mass, and 13C NMR and 1H NMR spectra) were used to completely describe all of the produced compounds, including the free ligand and their metal complexes. We also used a combination of spectroscopic data including CHN, XRFA, and AAS to determine the exact ligand to metal ratio and shape. Candida albicans (ATCC 10231), Aspergillusniger (ATCC 16404), Escherichia coli (ATCC 25922), and Staphylococcus aureus (ATCC 29213) were used in agar-well diffusion assays to assess the antibacterial activity of the produced ligands and their complexes in vitro.

Keywords: Antimicrobialactivity;MetalcomplexesandSchiffbase

1. Introduction

There are a number of variables, such as the rise of multidrug-resistant microbial infections and the emergence of new infectious illnesses, that make it difficult to effectively manage infectious diseases. New antimicrobial agents are needed despite the availability of a number of chemotherapeutics and antibiotics for use in medicine. Since many bacteria have developed resistance to existing classes of antimicrobial medications, there is also an immediate need for novel molecules with antimicrobial activity, most likely through new mechanisms of action. Many biological activities rely on metal ions, including the catalytic activity of metalloenzymes, the control of nucleic acid replication, and the transport of oxygen via hemoglobin's iron-porphyrin complexes. Its role as an oxygen transporter is linked to iron's reversible control over oxygen molecules [1]. Since amine (or Schiff base) compounds readily form stable complexes with the vast majority of transition metal ions [2;3], they play an important role in inorganic chemistry. Condensation of a carbonyl molecule with a primary amine (aldehydes or ketones) produces an azomethine group (-N=CHR) that is characteristic of these compounds [4]. The unsaturated double bond and the poor electronegativity of nitrogen in the azomethine group (>C=N) provide for a good donor and Schiff base forming active ligands because of the presence of a lone pair of electrons at the nitrogen atom. How well a ligand bonds is determined by the steric and electronegativity properties of the atoms involved in the coordination process. Chelates' structural characteristics provide extra steadiness to the complexes, especially those with a five- or six-membered ring. Therefore, an additional component in providing stability will be the existence of a functional group next to > C=N with a replaceable hydrogen atom.

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To slow down their decomposition or polymerization, aryl groups should be attached to the nitrogen or the carbon of the C=N double bond [5]. Anti-inflammatory, anti-malarial, anti-tumor, and antibacterial properties were among the many biological actions shown by Schiff bases [6,7], [8,9], [10–15], and [16–19].
Some Schiff bases demonstrated enhanced activity following coordination/chelation with some metal ions, therefore researchers focused on complexing these compounds to identify new ones with an enhanced pharmacological profile. Some of these complexes may be used in a wide variety of inorganic, organic, and medicinal applications.

key functions in orgometallic synthesis and catalysis [20-24], as well as industrial and analytical applications. Isonicotinic acid hydrazide (INH) is a first-line drug for treating Mycobacterium TB and for preventing tuberculosis infection in people with human immunodeficiency virus [25,26]. Numerous divalent ions may be chelated with isonicotinic acid hydrazide [27]. Attaining and characterizing the fungicide and bactericide properties of metal ion mixed ligand complexes with hydrazone and isoniazid derivatives [28-30].

Researchers in the field of chemistry now have a pressing responsibility to find ways to enhance chemical processes that will result in less pollution of the natural world [31]. The use of microwaves to aid in the synthesis of organic and inorganic chemicals is a relatively young but rapidly growing topic in synthetic organic chemistry. Microwave irradiation has been shown in experiments to accelerate the rate of certain chemical processes and increase product yields in comparison to traditional heating methods, hence these findings provide the basis for this novel approach. In a number of cases, microwave chemical synthesis [32-34] may shorten the time it takes to complete a reaction from hours at reflux temperature under conventional conditions to minutes or even seconds. Therefore, the purpose of this research was to find new antibiotics that can be utilized to treat illnesses caused by multi-drug-resistant bacteria. Using microwave-assisted chemical synthesis, we produced isoniazid-based compounds and their transition metal complexes (cobalt, copper, nickel, and zinc) and investigated their antibacterial properties.

2. Methods

2.1 Chemistry

Allthechemicalsandsolventsusedinthesynthesisof Schiffbaseandtheircomplexesofhighestpurityandt heywerepurchasedfromSigma Aldrich(UK)andFluka(UK)andusedwithout anyfurtherpurification.

2.2 Instruments

2.3 We used a microwave closed system (Milestone start E 2450 MHz, Italy) to synthesize chemicals (ligand and metal complexes). Pre-coated aluminum plates (silica gel 60778, Fluka analytical, UK) were used for thin layer chromatographic (TLC) examination. Both short-wave (254 nm) and long-wave (366 nm) UV light were able to identify TLC spots. Open capillary tubes were used together with a melting point instrument (Electrothermal SMP30, Stuart, UK) to get accurate readings. Using a Varian FT-IR spectrophotometer (Cary-Varian 660, Australia), infrared spectra of the produced compounds were obtained between 4,000 and 400 cm1. Using Starna quartz cuvettes of one centimeter in diameter, a Cary spectrophotometer (5000, UVVISNIR, Varian, Australia) was used to record UV-visible spectra. Using tetramethylsilane as an internal standard and a BrukerAvance 400 MHz NMR Spectrometer (Bruker, France), we obtained proton nuclear magnetic resonance (1H-NMR) and carbon nuclear magnetic resonance (13C-NMR) spectra in dimethyl sulfoxide (DMSO-d6). The Shimadzu QP-2010 plus was used to capture EI mass spectra at 70eV. Using Shimadzu TGA-50H thermal analyzers, the thermogravimetric analysis (TGA and DTG) was determined in a dynamic nitrogen environment (30 ml/min) at a heating rate of 10°C per minute. All testing was conducted at either the Microanalytical Center at Cairo University in Giza, Egypt, or the National Medical Research Center in Zawia, Libya.

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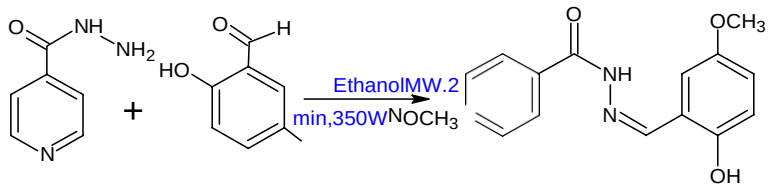
2.4 Generalprocedureforsynthesisof*N*-isonicotinamido-2-hydroxy-5-methoxybenzalaldimine(ligand):

As shown in Scheme 1, equimolar amounts of both starting materials (0.5 g isoniazid and 0.55 g 2-hydroxy-5-methoxybenzaldehyde) were weighed and triturated to form a homogeneous mixture using clean and dry Teflonvessels withthe addition of 3-4 drops of ethanol. The reaction mixture was subjected tomicrowave irradiation at350 - 600 Watt power for about 2 minutes with maximum heating of 60^{o} C. The optimum reaction time wasdetermined based on the reaction completion using TLC and the appropriate solvent system. The reaction mixturewas allowed to cool and the crude solid product was collected through vacuum filtration and washed with threevolumes of acetone, dried over anhydrous magnesium sulphate, evaporated and finally recrystallized from theappropriate solvent (ethanol). The achieved crystals were dried and their melting points were determined. Thechemicalpuritywasinvestigated byTLCusingchloroform/ethanol(90:10) asmobile phases.



Scheme1:SynthesisofcomplexesofN-isonicotinamido-2-hydroxy-5-methoxy-benzalaldimine(ligand)

2.5 Generalprocedureforsynthesisofmetalcomplexes

Metal complexes were prepared by weighing and triturating equimolar amounts of Schiff base ligand and metalchloride or acetate metal salts using clean and dry Teflon vessels. The reaction mixture was subjected to microwaveirradiationat600-800Wattpowerforabout3-5minutesusing0.5mlofdryethanolasasolventafter milling.The optimum reaction time was determined based on the reaction completion using TLC and the appropriate solventsystem. The crude product achieved after filtration under vacuum was washed several times with hot ethanol andfinally dried and their melting points were determined. Metal salts used were $ZnCl_2$, $CoCl_2.6H_2O$, $CuCl_2.2H_2O$ and$Ni(CH_3COO)_2.4H_2O$.

3. Biology

3.1 Evaluationofantimicrobialactivities

The Schiff base ligands and their metal complexes were evaluated for their *in vitro* antibacterial activity againstseveralstrainsofmicroorganisms:*Staphylo coccusAureus*(ATCC29213),*EscherichiaColi*(ATCC25922),*Candida Albicans*(ATCC 10231) and *Aspergillus Niger* (ATCC 16404). Strains were obtained from the AmericanTypeCultureCollection(ATCC)andwerecognizedbasedontheAmericantypeofcellculturecollection(ATCC) by agar-well diffusion method. Bacteria were inoculated into nutrient broth (Difco), incubated for twentyfour hours and fungi kept inoculated in malt extract broth (Difco) for forty eight hours. In the agar-well diffusionmethod, Malt Extract Broth (Difco) and Mueller Hinton Agar (Oxoid) were sterilized in a flask and cooled to 45–50°C,andweredistributedintotwentymillilitteraliquotstosterilized*petri*dishesafterinjectingtenmicrolitter

cultures of bacteria which was prepared as stated earlier and were keep to solidify. The dilution plate method wasutilized to quantify the microorganisms (10^5 bacteria ml^{-1} and fungi 10^3-10^4 ml^{-1}) for twenty four hours [35]. Wellswere dug in the culture plates by using a sterilized cork borer (7 mm diameter). The synthesized compounds weredissolved in dimethyl sulfoxide (0.2 ml) and then added to the wells aseptically. The *petri* dishes were left at 4° C fortwo hours and then the plates were incubated at 30° C for bacteria (18–24 hours) and at 25° C for fungi (72 hours). Atthe end of the incubation period the inhibition zones produced on the medium were assessed as millimeters (mm).The control samples were only loaded with only DMSO. Blank tests showed that the concentration of DMSO usedhasnotanyaffectandtheantimicrobialactivity obtainedwillbecorrelatedtothesynthesizedcompounds.Tetracyclineand Amphotericin B asantimicrobial agentswereused asreferences.

4. Results

4.1 Chemistrypart

4.1.1 Physicalproperties

Somephysicalpropertiesofthe ligand and theircomplexesareshown inTable1.

Compounds	**Color**	**Yield (%)**	**M.p. (^{0}C)**	**M.WT**	**Physical Appearance**	**Solubility**
$C_{14}H_{13}N_3O_2$ (HL)	Light yellow	83.9	197±0.8	289.29	Crystal	DMF,DMSO,THF, Hot(EtOH,MeOHandCHCl$_3$)
[Zn(L)$_2$]	Orange	80	>300*	605.94	Powder	DMF,DMSO
[Co(L)$_2$].1.5H$_2$O	Brown	69	>300*	626.48	Powder	DMF,DMSO
[Ni(L)H$_2$O](ac)	Brown	74	>300*	406.02	Powder	DMF,DMSO
[Cu(L)$_2$].6H$_2$O	Violet	78	>300*	712.16	Powder	DMF,DMSO

*****Melting points morethan300^{0}C

Table1: Physicalpropertiesoftheligandanditscomplexes

^{1}Hand^{13}CNMRspectraoftheSchiffbase:

The **^{1}H-NMR** (400 MHz, DMSO-d6): δ [ppm]

12.3 (s, 1H, –NH), 10.5 ppm (s, 1H, –OH), 8.68

ppm (s, 1H, N=CH),8.80ppm forH1(d,J_{H1-H2}=5Hz),7.85H2(d,J_{H2-H1}=5Hz),7.20-

6.85(m,3H,ArH),3.75ppm (s,3H,OCH$_3$).The

^{13}CNMR(400MHz,DMSO-d6):δ[ppm]161.34(C4,*C=O),148.10(C15,*C=N),55.44(C12,*CH$_3$O),152.12,

151.49,150.34,140.04,121.50,118.95,118.64,117.31,111.55forC7,C10,C1,C3,C2,C6,C9,C8andC11,

respectively.Figure1showsa ^{1}H-NMRspectrumoftheligand.

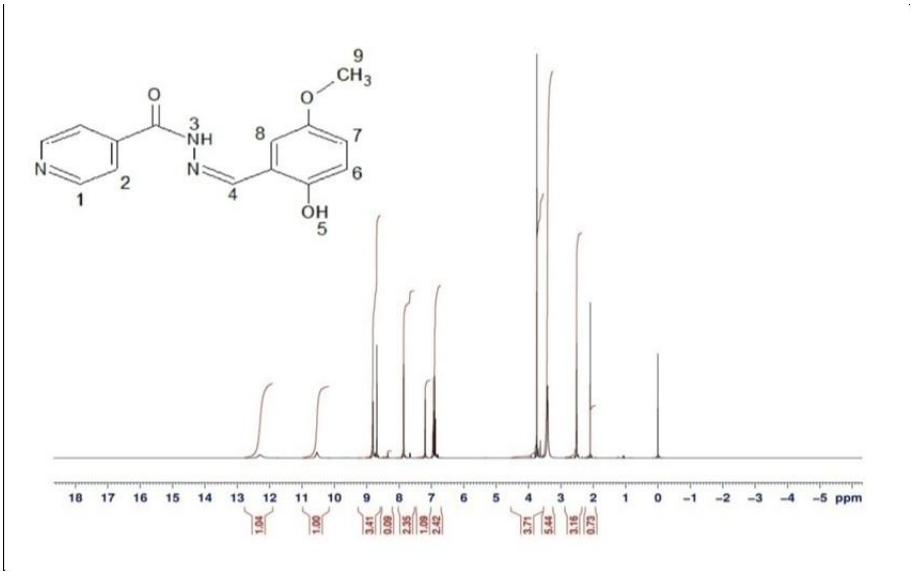


Figure1: ^{1}H-NMRspectrumoftheligand

4.1.2 IRspectra

ThemainstretchingfrequenciesoftheIRspectraoftheligandanditscomplexesareshowninTable2

Ligand/complexes	$\nu_{(C=O)}$ Amide	$\nu_{(C=N)}$ Azomethine	$\nu_{(OH)}$ Phenolic	$\nu_{(C-O)}$ Phenolic
$C_{14}H_{13}N_3O_2$(HL)	s1652	s1578	3380 m	1261s
$[Zn(L)_2].1.5H_2O$	s1679	s1542	Disappeared*	1268s
$[Co(L)_2]$	s,1608s1679	s1539	Disappeared*	s1267
$[Ni(L)H_2O](ac)$	s1595	s1535	Disappeared*	19s12
$[Cu(L)_2].6H_2O$	S,1562m1584	s1523	Disappeared*	s1251
s:strong,m:medium				

Table2:IRstretchingfrequenciesofvariousfunctionalgroupsofligandsanditsmetalcomplexes

*The peak in the Ligand spectra at 3380 cm^{-1} due to the deformation of OH group and disappeared in the complexes.ThisindicatesdeprotonationofphenolicOH,oncoordinationwithmetal ion.

4.1.3 Electronicspectralanalysis

The electronic spectra of the free ligand showed two strong absorption bands in the Ultraviolet-Visible region (294-361 nm), allocated to the transitions π→π* and n →π *. These transitions are found only in the spectra of thecomplexes.Buttheyareshiftedandstronglydisplacedinallcomplexes,verifyingcoordinationofmetalionstothe

ligand.Thespectraofthecomplexesalsoshowednewban dsthatwereattributedtotheformedligandcomplexes(Ta ble3).

Ligand⁄complexes	**Frequencies nm⁄cm^{-1}**	**Assigning**	**Geometry**
$C_{14}H_{13}N_3O_2$(HL)	294⁄34013 361⁄27700	π→π* n →π*	……..
[Zn(L)$_2$]	⁄33898295 ⁄27624362 ⁄22522444	π→π* n →π* chargetransfer	octahedral
[Co(L)$_2$].1.5H_2O	⁄34965286 ⁄28011357 ⁄21691461	π→π* n →π* d→dtransition	octahedral
[Ni(L)H_2O](ac)	⁄31847314 ⁄26595376 ⁄22727440 ⁄21551464	π→π* n →π* $^3A_2$→$^3T_1$(P) $^3A_2$→$^3T_1$(F)	tetrahedral
[Cu(L)$_2$].6H_2O	⁄35460282 ⁄28818347 ⁄22624442	π →π *n→π* $^2T_{2g}$→ ^2E_g(D)	octahedral

Table3: Electronicspectraldataandgeometriesfortheligandandtheircomplexes

4.1.4 Elementalanalysis,X-rayfluorescenceanalysisandatomicabsorptionspectroscopy.

Elementalanalysisofthefreeligandanditsmetalcomplexesalongwith X-rayfluorescenceanalysisandatomicabsorptionspectroscopyare listed in Table4.

Compounds	Formula weight	ElementalanalysisCHN Found(Calcd.)%			XRF (%)	AAS (%)
		C	H	N	Cl	M
[HL]. $H_2OC_{14}H_{15}N_3O_4$	289.29	55.64 (58.13)	5.07 (4.83)	14.08 (14.53)	…….	…….
[Zn(L)$_2$]$C_{28}H_{24}N_6O_6Zn$	605.94	55.26 (55.50)	3.96 (3.99)	13.81 (13.87)	<LLD	9.02 (10.79)
[Co (L) $_2$].1.5 H_2O $C_{28}H_{27}N_6O_{75}Co$	626.48	55.53 (53.68)	4.25 (4.04)	13.88 (13.41)	<LLD	10.19 (9.41)
[Ni(L)H_2O](ac) $C_{16}H_{17}N_3O_6Ni$	406.02	47.81 (47.33)	4.06 (4.22)	11.20 (10.35)	……	13.75 (14.46)
[Cu (L)$_2$].6H_2O $C_{28}H_{36}N_6O_{12}Cu$	712.16	47.86 (47.22)	3.48 (4.00)	11.27 (11.80)	<LLD	7.03 (8.92)
LLD: LowerLimitofDetection=0.01ppm						

Table4: Theresultsofelementalanalysis,AASandXRFoftheligandsandtheircomplexes:

4.1.5 Massspectra

ThemostimportantpeaksintheEImassspectraldataofallcomplexeswithligandarelistedinTable5.

Compounds	m/z	Fragments	R.I(%)
$C_{14}H_{13}N_3O_3$	271 107	$[M]^+$ $[C_7H_7O]^+$	83.90 100
$[Zn(C_{14}H_{12}N_3O_3)_2]$	608 605	$[M+3]^+$ $[M]^+$	2.87 0.53
$[Co(C_{14}H_{12}N_3O_3)_2]$	601 600	$[M+1]^+$ $[M]^+$	51.43 81.90
$[Ni(C_{14}H_{12}N_3O_3)H_2O]^+$	346	$[M]^+$	49.01
$[Cu(C_{14}H_{12}N_3O_3)_2]$	605 604	$[M+1]^+$ $[M]^+$	86.79 66.04

Table5: Massfragmentationoftheligandanditsmetalcomplexes

4.1.6 Thermalanalysis(TGAandDTG)

Thermogravimetric analysis of the ligand and its complexes were used to obtain information about the thermalstability of these new complexes. In addition, to make a decision whether the water molecules (if it is available) areoutside or inside the inner sphere coordination of the central metal ion. The results of the thermal analysis of theligandand itsmetalcomplexes aregiven inTable6.

Compounds	TGrange (^{0}C)	DTG $_{max}$ (^{0}C)	n*	Mass loss Found (calcd)	Assignment
$C_{14}H_{13}N_3O_3$(HL)	58-141	103	1	5.79(6.22)	Lossof1H_2O(hydration)
[Zn$(L)_2$]	.....	.....		.....	.....
[Co$(L)_2$].1.5H_2O	-22632	49,192	2	3.87(4.30)	Lossof1.5H_2O(hydration)
[Ni(L)H_2O](ac)	180-328	275	1	4.89(4.43)	Lossof1H_2O(coordinated)
[Cu$(L)_2$].6H_2O	258-118	223	1	15.96(15.16)	Lossof6H_2O(hydration)
n*=numberofdecompositionsteps;L=$C_{14}H_{12}N_3O_3$					

Table6: Thermalanalysisoftheligandanditsmetalcomplexes

4.2 Biologypart

4.2.1 Evaluationofantimicrobialactivity

The antimicrobial activity studies of the ligand and its metal complexes were performed by using different fungi andbacteriaand the resultsof theinhibitionaresummarizedinTable7.

Compounds		Inhibitionzonediameter(mmpermgsample)			
		E.coli	***S.aureus***	***A.flavus***	***C.albicans***
DMSO		0	0	0	0
Standard	Tetracycline	31	28	----	----
	AmphotericinB	----	----	16	19
$C_{14}H_{13}N_3O_3$(HL)		12	16	0	9
[Zn$(L)_2$]		16	18	0	9
[Co$(L)_2$].1.5H_2O		11	10	0	9
[Ni(L)H_2O](ac)		0	0	0	0
[Cu$(L)_2$].6H_2O		12	12	0	0

Table7: Antimicrobialactivitydataoftheligandanditsmetalcomplexes

5. Discussion

5.1 IRspectra

In general, the entire synthesized amides demonstrate two absorption bands: one is the the carbonyl absorption bandnear 1640 cm^{-1} which is known as amide-I band and two is the strong band in the 1500 - 1600 cm^{-1} region which isknown as amide-II band. The origin of these two bands can be seen in hydrazones, in which the carbonyl absorptionaccountable for the amide-I band, which is probable to be lowered [36;37] occasionally by the NH group as instandard amides. The amide-I band in isoniazide derivative, however, can be seen at 1700 and 1655 cm^{-1} [38]. In theentire hydrazones, the absorptions such as 1540, 1520 cm^{-1} have been allocated to absorption of amide-II. The NHstretching absorption in free ligands occurs at ∼3300 and 3220 cm^{-1} as described in the literature [39]. The othersignificant band occurs at ∼1585-1600 cm^{-1} allocated to *ν*(C=N) (azomethine) mode [35;39]. The strong bandsallocated at 1000–1080 cm^{-1} and 1520–1575 cm^{-1} are possibly allocated to symmetric and asymmetric *ν*(C=C) +*ν*(C=N)ofthepyridineringandpyridineringdeformationsandbreathings,respectively[40;41].

The infrared spectra spectrum of the ligand exhibits a strong band at 1652 cm^{-1} due to ν(C=O) of the amide group.Thebandofthisligandhasshiftedandwaspowerfullydisplacedinallcomplexesconfirmingcoordinationthroughout the carbonyl oxygen. A band at 1578 cm^{-1} is due to ν(C=N) azomethine group which has shifted to thelowerfrequenciesfortheentirecomplexes.This suggeststheinvolvementoftheazomethinenitrogenincoordination. Another important ligand band, which allocated at about 3380 cm^{-1} owing to the phenolic–hydroxylgroup, was missing in the complexes. This proofs the phenolic–OH is deprotonated and it is on coordination withmetal. The band due to phenolic C–O stretching vibration is observed at 1261 cm^{-1} in the free ligand. In the entirecomplexes this band appears at higher or lower frequencies in 1268-1219 cm^{-1} proving the participation of thephenolic oxygen in the coordination with the metal ions. As indicated in the literature the Ni complex showsabsorption bands, one in the 1572 cm^{-1} and the other in 1425 cm^{-1} regions for symmetric $\nu(COO^{-})$ and asymmetric$\nu_{(COO^{-})}$stretching[42;43].

5.2 Electronicspectralanalysis

Theelectronicspectrumofthezinc(II)complexshowsabroadabsorptionbandat444nmwhichmaybeassignedto a charge transfer transition, due to the coordination of the ligand with metal ion [44]. The electronic spectrum ofthe cobalt (II) complexis expectedto showthree absorption bands due tothe electronic transitions, namely$^{4}T_{1g}\rightarrow{}^{4}T_{1g}(P)$, $^{4}T_{1g(}F)\rightarrow{}^{4}A_{2g}$ and $^{4}T_{1g}\rightarrow{}^{4}T_{2g}$, but bands due to these transitions usually overlap to give a broadabsorption band. The broad band allocated in the complex approximately 461 nm is in agreement with octahedralarrangements for Co (II) ion. The electronic spectrum of the nickel (II) complex shows two d-d absorption bands at440and464nm,whilethethirdd-dbandisnotobserved.Thesebandsareassignedto$^{3}A_{2}\rightarrow{}^{3}T_{1}(P)and^{3}A_{2}\rightarrow{}^{3}T_{1}$

5.3 Elementalanalysis,X-rayfluorescenceanalysisandatomicabsorptionspectroscopy

Elementalanalysisofthefreeligandandthecomplexesofthemetalinadditiontotheatomicabsorptionspectroscopy and the X-ray fluorescence analysis and demonstrated in the results section (Table 4) and are in a goodconformitywith the expectedvalues.

5.4 Massspectra

The mass spectrum of ligand showed the molecular ion peak at m/z = 271 that corresponds to its molecular formula$[C_{14}H_{13}N_3O_3]^+$,withavirtualintensityof83.90%.Thefragmentatm/z=107(R.I.=100%,basepeak)isallocatedto the $[C_7H_7O]^+$ ion. The other peaks signify fragments of the molecular ion. The EI mass spectral data of Zn(II),Co(II), Ni(II) and Cu(II) complexes show an strong molecular ion peak m/z $[M]^+$. The mass spectra of severalcompounds also exhibit a important peak matching to m/z $[M+1]^+$, $[M+2]^+$ or $[M-1]^+$ and the enduring peaks signifythe successive degradation of the complexes [46]. The peak intensity provides an thought of the stability of thefragments.

5.5 Thermalanalysis(TGAandDTG)

Thermogravimetricanalysisoftheligand anditscomplexes demonstratedgoodconformity withthe theoreticalformula as suggested from the elemental analysis. TGA of ligand, shows the initial weight loss 103°C is allocated tothelossoflatticewatermoleculewhichisallocatedtooneH2Ocorrespondingto(found5.79%;calculated6.22%).Theremainingsteps,thatoccurinsidethetemperaturerange204-799°Cattributedtodecompositionoftheligand,involvemasslossesof96.55%.TGAforCo(II)complex,demonstrates2-stagesofdecompositionwithintherangeof32-226°C,whichisattributabletothelossof1.5uncoordinatedwatermolecules(weightloss;found/calculated.3.87/4.30%),whileTGforCu(II)complexshowsonestageofdecompositionwithintherangeof32-226° C,which isattributabletothelossof6-uncoordinatedwatermolecules(weightloss;found/calculated.15.96/15.16%).Themolecularformulaeofthecomplexes Co(II)andCu(II)construedfromelementalanalysispointsouttheexistenceoflatticewater.IncaseNi(II) complex,TG shows onestageof decompositionwithinthe range of 180-328° C, which isduetothelossofonecoordinatedwatermolecule(weightloss;found/calcd.4.89/)4.43.Butinthezinc(II)complex,thereisnoconsiderableweightlossbelow180°Cproposingthenonappearance oflatticewatermolecules.Thesucceedingsteps(280-800°C)inallcomplexesmatchtotheexclusionoftheorganicpartoftheligand[47;48].Thecomplexeshavebeensynthesizedbymicrowavemethodsfromreactionof$CoCl_2.6H_2O$,$CuCl_2.2H_2O$,$ZnCl_2$and$Ni(CH_3COO)_2.4H_2O$withSchiffbase(ligand)inpresenceofethanolassolvent.Formationofthecomplexesmayhaveproceededper thefollowingequations.$MCl_2.nH_2O$ +2 L $\rightarrow$$[M(L)_2].nH_2O$+2HCl+$nH_2O$M=Co(II),n=1.5;Zn(II),n=0and Cu(II),n=6$M(ac)_2.4H_2O$+L$\rightarrow$ $[M(L)H_2O](ac).nH_2O$+ CH_3COOH+nH_2OM=Ni(II),n=0

The ligand acts as a monoanionic tridentate (O, N and O) throughout the carbonyl oxygen, phenolic oxygen andazomethine nitrogen. Composition was construed from FT-IR,elemental analyses, UV-VIS, XRF, AAS, MS andTGA. The analytical data of the complexes specify that the ligand forms a 1:2 (M : L) complex with Zn (II), Co (II)and Cu (II) and 1:1 (M: L) with Ni (II) ions. The atomic absorption and mass spectral data substantiate themonomeric structure of the metal complexes whereas the TGA studies substantiate the existence of water moleculesin some complexes and XRF data demonstrate that chloride anions are absent outside the coordination sphere in Zn(II),Co(II)andCucomplexes.Accordingtothein dicatedanalyticalandspectraldata,thecommonstru ctureformulaandstoichiometryofsynthetizedmeta lcomplexes areillustratedinFigure2.

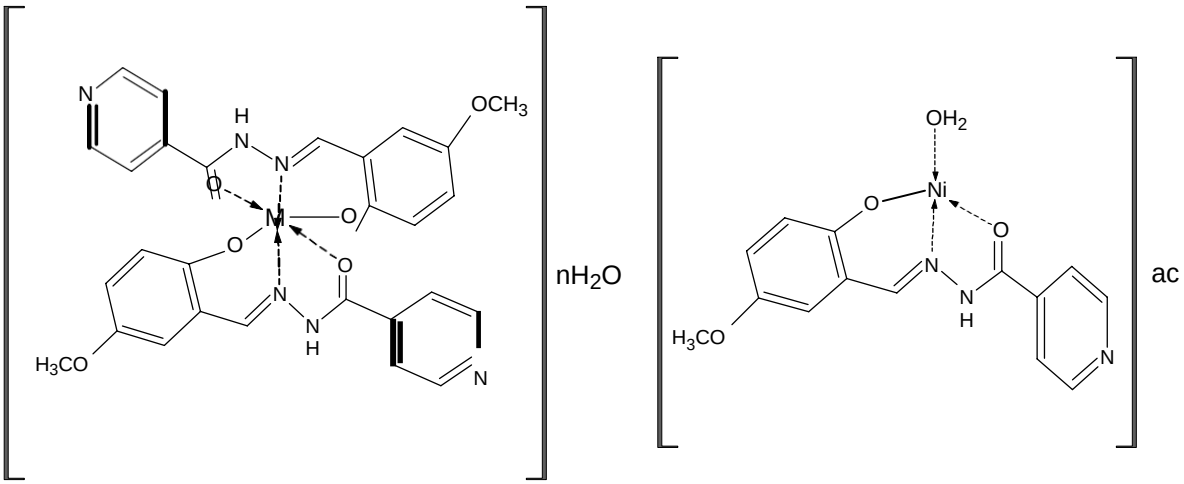


Figure 2: structural formula proposed for; (A)[M (L) $_2$].nH_2O ,(M = Co (II), n=1.5; Zn (II), n=0 ; Cu (II), n=6) and(B)[M(L)(H_2O)](ac).nH_2O,(M=Ni (II),n=0,ac=CH_3COO^-)

5.6 Biologypart

5.6.1 Evaluationofantimicrobialactivity

Microorganismsrequirethe presence ofanumberof metalsthatplayessentialbiochemicalrolesascataly sts,enzyme cofactors, activity in redox processes and stabilizing protein structures [49-51]. Metals might build upexceeding normal physiological concentrations and effecting the transport systems, and might become toxic. Theintracellular metals could cause toxic effects through forming coordinate bonds with some anions and this couldblock the efficient groups of enzymes. This formed coordinate bonds with the enzymes will be inhibiting thetransportsystemsanddisturbing cellularmembrane integrity[52;53].

It has been reported that, there are 5 fundamental mechanisms that suggest an increased level of cellular resistance tometals:(1)intra-orextracellularsequestration(2)effluxofthetoxic metalfromthecell,(3)enzymaticconversion, (4) prohibiting by a permeability barrier, and (5)

bacteria and fungi, *i.e.* chelation may improve or restrain the biochemical prospective of bioactive organic species[57]. Such generation or improvement in activity of the metal complexes can be explained based on *Overtone'sc*oncept and chelation theory. Per *Overtone's* notion of cell permeability, the lipid membrane that envelops the cellhelps the passage of lipid soluble substances owing to their liposolubility which is a vital factor that managesantimicrobial activity[58]. On chelation, the polarity of the metal ion is abridged due to the overlap of the ligandorbital and fractional sharing of the (+) charge of the metal ion with donor groups. Additionally, it enhances thedelocalization of pi-electrons over the entire chelate ring and enhances the lipophilicity of the complex. Thisincreased lipophilicity in turn enhances and this could help the penetration of the complexes into lipid membranesandultimatelyblocksmetalactivebindingsitesontheenzymesofthemicroorganisms[59;60].Moreover,coordination might escort to considerable reduction of drug – resistance [61]. In addition another factors, likeconductivity,dipolemomentandsolubilityeffectedbytheexistenceofmetalionsandmightenhancethebactericidal activity of the metal complexes in comparison to the uncomplexed compounds [62]. Low activity of thesome metal complexes, as shown in this study, may be related to their low lipophilicity which reduces penetration ofthecomplexthroughthelipidmembraneandtherefore thesecomplexescannotachievetheirtargettoblockorinhibitthegrowthof microorganisms [63;64].

In conclusion, we synthesized and characterized four new complexes of n-isonicotinamido-2-hydroxy-5-methoxybenzalaldimine with Cu(II), Co(II), Ni(II) and Zn(II). The spectroscopic data demonstrate that Schiff base acts asmonoanionictridentateligand.Schiffbaseandsomeofthemetalcomplexeswereactiveagainstsome ofrepresentative bacterial and fungal strains and complexation enhances their activity. The activity may be due toincrease in cell permeability caused by increase of lipophilicity of metal conjugates, which allows intracellular drugaccumulation and target accessibility. It is possible that intracellular reduction of metal compounds leads to highercytoplasmic concentration of metal species, which proves lethal for bacteria and fungi. This study also showssuperior antimicrobial activity of metal complexes relative to their ligands. MIC values indicate their potential forpharmacologicaluse.

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