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Synthesis, Characterization and Antibacterial Activity Study of New α-Aminonitrile Complexes with Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) Metal Ions

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Abstract

Several metal ion complexes of manganese (II), cobalt (II), nickel (II), copper (II) and zinc(II) were derived from (E)-2-(2-hydroxyphenyl)-2-((4-(phenyldiazenyl)phenyl)amino)acetonitrile ligand (L) and have been characterized using conventional techniques such as ^{1}H and ^{13}C-NMR, FT-IR and UV-VIS spectroscopy, elemental analysis, thermal analysis, flam atomic absorption and molar conductivity. The five new complexes were suggested to be of octahedral geometry and were observed to be electrolytic. The coordination pattern of the ligand was bidentate of NO-type through amine and hydroxyl groups forming stable 6-membered ring releasing nitrile group free. $[ML_2(H_2O)_2]Cl_2.XH_2O$ is the suggested general formula for the complexes that M: manganese (II), cobalt (II), nickel (II), copper (II) or zinc (II)], X = 1, 4, 2, 4 or 1 respectively, hence that each metal interacted with two ligands and two aqua molecules. All the complex compounds and the ligand were investigated for anti-bacterial activity against four bacterial genera including: *Pseudomonas spp., Escherichia coli, Proteus* spp. and *Staphylococcus aureus*. The antibacterial activity results were promising, that they ranged from 11mm to 21mm, especially the L against *Escherichia coli and Staphylococcus aureus* bacteria, meanwhile, the complexes showed very good inhibition zones against the gram positive bacteria only, except Ni(II) and Zn(II) complexes showed no activity against *Escherichia coli.* Generally, the activity was suggested due to taking in account the presence of nitrile moiety, beside complex form that possesses metal ion. The aim of this work is to design a chelating α-aminonitrile ligand to interact with some transition element salts to produce the corresponding complexes and to investigate their activities against selected bacteria.

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1. Introduction

Amino nitrile constituents represent as one of basic building blocks of many substances and products. α-aminonitriles which have bifunctional structure of amino and cyano groups which represent an important class of compounds. Although more than 170 years old, Strecker's famous three component condensation of carbonyl, amines compounds and hydrogen cyanide remains still the fastest and most widely used method to synthesize α-aminonitriles [1]. This class of compounds considered attractive due to the formation of multi-bonds in one pot, mild and simple conditions and environmentally friendly. In another significant viewpoint the direct hydrocyanation of imines can be considered as fundamental carbon-carbon bond forming processes [2, 3]. An α-aminonitriles are highly useful synthons for the synthesis of α-amino acids [4, 5], amino amides [6], amidine[7] nitrogen and oxygen-containing heterocyclic [8-10] which have grate biological activity even higher than the commercial and corresponding antibacterial and antifungal

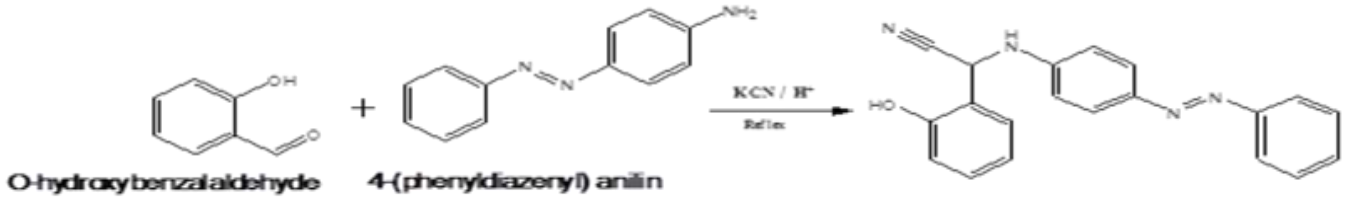
compounds [11-13]. Almost a decade ago α-aminonitrile and its derivatives were involved in the synthesis of hepatitis C virus NS3 serine protease inhibitors using diastereoselective Strecker reaction [14], (±) phthalascidin 622 (Saframycin A analogue) [15] , and novel boron-containing retinoids [16]. Generally to synthesize α-aminonitriles the three component aldehyde or ketone, amine and cyanide source mixed together, the third one is variable according to the type of reaction [17, 18]. A modified Strecker method have been achieved using catalyst [19-22] and the modification in progress, especially those using nano particles [23-25] or those using eco-friendly conditions like water or ionic liquid [26-31]. Recently organo-catalysis serves in the synthesis of α-aminonitrile [32]. Thus, α-aminonitrile simplifies the synthesis of artificial amino acids and achieves the shortest way of one pot reaction in the synthesis of stereo selective and biologically active compounds [33]. Meanwhile, some researches presented light to assist for the synthesis of α-aminonitrile [34, 35]. Since α-aminonitrile synthesized, a number of publications have appeared to investigate the biological activities like antibacterial, antifungal, insecticidal and anticancer activity [36-39]. α-aminonitrile and its complexes still under active investigation as long as the nitrile group plays an important role as a functional group [40]. In this work we decided to design an α-aminonitrile and investigate the interaction with metal ion to figure out the coordination site, hence that there is a lack in the field of α-aminonitrile complexation study. Thus, we encourage researchers to work in this field. In addition, antibacterial activity applied against some bacteria to find out the behavior of the prepared compounds towards the selected bacteria.

2. Materials and Methods

Chemicals used were utilized without further filtration or purification, tetracycline drug was used as standard antibiotic in antibacterial activity. Melting points were determined using open capillary method by hot stage Gallenkamp melting point apparatus and they were uncorrected. Elemental analysis was preceded on Euro EA (C.H.N.S) Elemental Analyzer 2000 ºC (model EA3000 by Eurovector). Metal contents were measured using flame atomic absorption using Agilent Varian AA 240 FS/ETA model device. Magnetic susceptibility measurements were obtained at room temperature using Magnetic Susceptibility Balance of Johnson Mathy Catalytic Systems Division. Thermo gravimetric analyses were measured using TGA model STA PT 1000 by Linseis Company. Infra-red spectra were recorded using fourier transform infrared spectrophotometer (model FTIR 8300) by Shimadzu. The electronic spectra were reported using ultra violet spectrophotometer (model UV-Vis-160A) by Shimadzu. Proton and carbon nuclear magnetic resonance ^{1}H-NMR and ^{13}C-NMR spectra were recorded on nuclear magnetic resonance Bruker model ultra-shield 300 MHz The four bacterial genera including: *Pseudomonas spp.*, *Escherichia coli, Proteus spp.* and *Staphylococcus aureus* were isolated clinically from human wound-burn swab as human patients sources. Tetracycline (10^{-3}M) antibiotic was used as a Gram positive control and DMSO as Gram negative control and solvent. The antibacterial activity results were promising, especially the L against *Escherichia coli and Staphylococcus aureus* bacteria which showed 19mm and 21mm respectively, meanwhile, the complexes showed interesting inhibition zones against the gram positive bacteria which ranged from 11mm to18mm, except Ni(II) and Zn(II) complexes showed no activity against *Escherichia coli.* The results reported against gram negative bacteria were not significant due to that the effect were mainly by the solvent (DMSO) effect.

2.1. Synthesis of α-aminonitrile ligand (L):

About 0.01 mole (1.22g) of o-hydroxy benzaldehyde was dissolved in glacial acetic acid 25 mL, followed by the addition of 0.01 mole (1.97g) of 4-(phenyldiazenyl) aniline. The *p*H value was adjusted near 4 by addition of conc. sulfuric acid drop by drop, the color changed to orange (Schiff base formation). A small amount of catalyst *p*-toluene sulphonic acid was added Potassium cyanide 0.02 mole (1.30g) was added to the mixture and kept under refluxed and stirring over night; the color changed to brown. The reaction mixture was transferred to crashed ice, and then made slightly alkaline by ammonia solution drop wise addition (exothermic). The solid dark green precipitate was filtered, washed with water and air-dried; yield percentage was 86% and melting point 88 ºC. The synthesis route of the ligand which considered as one pot three-component reaction was illustrated in scheme 1.



Scheme 1. Three-component one pot reaction of α-aminonitrile ligand (L)

2.2. Preparation of Complexes (C1-C5)

About 1 mmol of metal ions Mn(II), Co(II), Ni(II), Cu(II) or Zn(II) were dissolved in 10 ml of ethanol, then added drop wisely to 2 mmol of the ligand (L) which dissolved previously in 10 ml ethanol. The resulting mixture was refluxed for three hours to synthesize C1-C5 complexes respectively. The colored precipitates were collected, dried and stored in desiccator.

3. Results and Discussion

The complexes and ligand structures were characterized using the conventional techniques mentioned in this article to find out the probable structures. The symbol, chemical formula, elements composition and some physical properties of the prepared compounds were listed in Table 1.

Table 1. Some physical properties, metal and elemental analyses and yield percent of L and C1-C5.

Comp. symbol	Chemical formula	Color	Melting point °C	Yield%	Metal and elemental analysis			
					C%	H%	N%	M%
					calc. (found)	calc. (found)	calc. (found)	calc. (found)
L	$C_{20}H_{16}N_4O$	Green	129-133	86	73.17 (72.86)	4.87 (4.70)	17.07 (16.41)	---
C1	$[Mn\ L_2(H_2O)_2]Cl_2.H_2O$	Green	102-104	74.3	57.41 (56.52)	4.54 (4.40)	13.39 (12.88)	6.57 (6.02)
C2	$[CoL_2(H_2O)_2]Cl_2.4H_2O$	Brown	215-218	70.0	53.69 (53.26)	4.92 (5.11)	12.53 (12.41)	6.58 (6.49)
C3	$[NiL_2(H_2O)_2]Cl_2.2H_2O$	Dark-green	223-225	77.3	55.96 (54.55)	4.66 (4.31)	13.06 (12.71)	6.84 (6.50)
C4	$[CuL_2(H_2O)_2]Cl_2.4H_2O$	Green	200 Dec.	70.12	53.42 (53.04)	4.89 (4.55)	12.46 (11.77)	7.07 (6.39)
C5	$[ZnL_2(H_2O)_2]Cl_2.H_2O$	Pale green	230 Dec.	80.13	56.71 (55.58)	4.49 (4.13)	13.23 (12.77)	7.72 (6.78)

3.1. FT-IR Spectral Studies

The infrared spectra charts of the complexes were compared with the ligand (L) chart, figure 1, to identify the coordination sites potentially involved in chelation. Table 2 summarized the FT-IR absorption bands for all synthesized compounds including some characteristic peaks. The probable coordination site was through the available donation

groups; nitrile, secondary amine and phenolic hydroxyl. But according to the observed shifting in complexes spectra with comparison to that of the ligand, chelation was suggested bidentate of NO-type. The coordination through amine and hydroxyl groups was obvious due to peaks shifting in the involved groups. Generally according to coordination in all complexes, amino groups stretching υ(N-H) and bending δ(N-H) were shifted to lower frequencies by (113-20) cm^{-1} and (28-21) cm^{-1} respectively, while hydroxyl groups stretching υ(O-H) were shifted to higher frequencies by (28-6) cm^{-1} , hence nitrile groups (-C≡N) were shifted to lower energy region by (130-74) cm^{-1} due to removal of intra molecular hydrogen bonding with hydroxyl moiety after coordination.

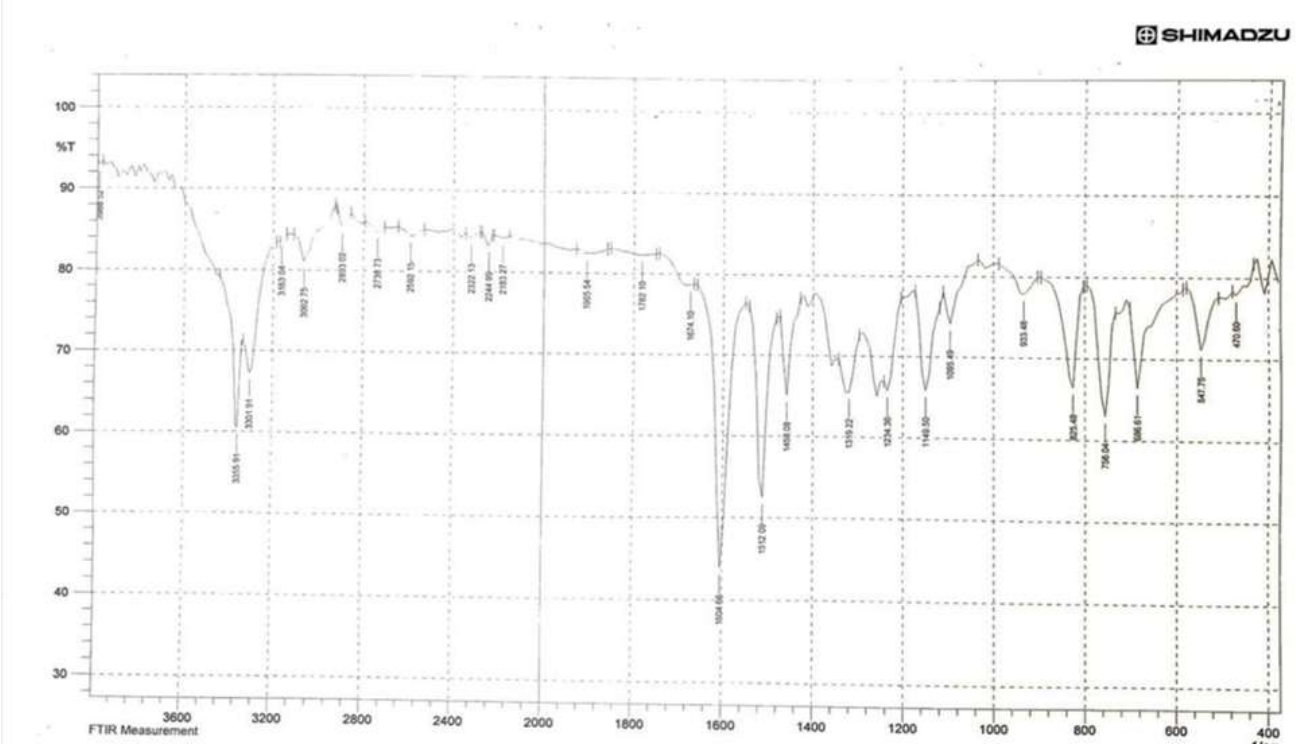


Figure 1. FT-IR spectrum of the ligand (L) regarding significant peaks.

Table 2. Characteristic infrared absorption bands of the ligand (L) and complexes (C1-C5).

Compound symbol	υ(O-H)	υ(N-H)	υ(C_{sp2}H)	υ(C_{sp3}H)	υ(C≡N)	δ(N-H)	υ(N=N)	υ(C=C)	Out of plain deformation		
									mono sub.	ortho sub.	Para sub.
L	3356s	3301s	3062	2893	2245	1512	1458	1604	686-756	756	825
C1	3370	3281	3054	2927	2115	1484	1452	1598	686-744	744	868
C2	3384	3220	3064	2947	2171	1487	1454	1596	680-758	758	840
C3	3300-3400	Masked	3064	2941	2120	1485	1454	1596	686-756	756	846
C4	3362	3223	3055	2931	2137	1491	1452	1590	690-758	758	879
C5	3300-3400	3188	3070	2929	2130	1487	1458	1596	686-758	758	842

3.2. Ultraviolet-Visible spectroscopy, magnetic moment and molar conductivity

Ultraviolet-Visible electronic transitions spectra of (C1-C5) complexes and L were determined at range of (250-1100) nm and at room temperature, using dimethyl sulfoxide solvent and of 10^{-3} M concentration. The electronic transitions L showed two peaks; the first around 300 nm due to n→π* transitions of non-bonding electrons, and the second around 250 nm which can be assigned to π → π * transitions of the azo-group and aromatic ring. These peaks also can be observed in all complexes representing ligand field region. Table 3 illustrates the electronic transitions, assignments, suggested structures and molar conductivities of the synthesized complexes. Manganese (II) complex C1 has magnetic moment value of 5.58 B.M. suggesting octahedral geometry. The d-d electronic transitions are spin forbidden. The electronic spectrum of Mn(II) complex registered bands at (19337 and 27777) cm^{-1} which can be that can be assigned to the spin forbidden transition $^6A_{1g}\rightarrow{}^4T_{1g}$, and C.T transitions of Manganese (II) ion in a spin free d^5 configuration confirming to octahedral arrangement. The diffuse reflectance spectrum of Cobalt (II) complex C2, showed three bands at (9174, 18867 and 21276) cm^{-1} which were assigned to ^4T_1g (F) $\rightarrow{}^4T_2g$ (F), ^4T_1g (F) → ^4A_2g (F) and ^4T_1g (F) → ^4T_2g (P) transitions respectively. The observed magnetic moment 4.80 B.M. favors the high spin nature and octahedral geometry of complex. Nickel (II) complex C3, showed three bands at (10348, 15996 and 23122) cm^{-1}, these bands are assigned to $^3A_2g\rightarrow{}^3T_2g$ (F), $^3A_2g\rightarrow{}^3T_1g$ (F) and $^3A_2g\rightarrow{}^3T_1g$ (P) electronic transitions respectively. The observed bands and paramagnetic nature (3.31 B.M.) of the complex suggest octahedral geometry. The Copper (II) complex C4, exhibited 1.81 B.M. magnetic moment value and appeared two bands at (17140 and 26650) cm^{-1} in the diffuse reflectance spectrum, these bands are assigned to $^2Eg\rightarrow{}^2T_2g$ and ligand field respectively, suggesting the distorted octahedral geometry of the complex. The Zinc (II) complex C5, was found to be diamagnetic in nature and did not show any band in the electronic spectrum (except ligand field), indicating no d-d electronic transitions which is expected for d^{10} system, so that octahedral geometry is expected for this complex. Molar conductivity of the prepared complexes was appeared to confirm the ionic nature; [1:2] ratio was suggested for C1-C5, that chloride ion reported as counter ion.

Table 3. Electronic transitions, molar conductance and suggested structure of the complexes.

Compound symbol	λ nm	ὐ cm^{-1}	Assignments	Conductivity in DMSO	Meff (B.M)	Suggested Structure
C1	517 360 286 266	19337 27777 34965 37593	$^6A_{1g}\rightarrow{}^4T_{1g}$ (G) C.T n → π * π → π *	25.3	5.58	Octahedral
C2	650 493 Masked 388 320 286	15384 20283 Masked 25773 31250 34965	^4T_1g(F) → ^4T_2g (F) ^4T_1g (F) → ^4A_2g (F) ^4T_1g (F) → ^4T_2g (P) C.T n → π * π → π *	53.4	4.80	Octahedral
C3	966 625 432 309 286	10348 15996 23122 32331 34892	$^3A_{2g}\rightarrow{}^3T_{2g}$(F) $^3A_{2g}\rightarrow{}^3T_{1g}$(F) $^3A_{2g}\rightarrow{}^3T_{1g}$(P) +C.T n → π * π → π *	35.0	3.31	Octahedral
C4	583 375	17140 26650	$^2Eg\rightarrow{}^2T2g$ n → π *	23.2	1.81	Distorted Octahedral
C5	410 325 317	24390 30769 31545	C.T n → π * π → π *	17.0	Diamagnetic	Octahedral

3.3. Thermal analysis

The thermal behavior of cobalt complex $[CoL_2(H_2O)_2]Cl_2.4H_2O$ was studied using thermo gravimetric analysis (TGA) as shown in figure 2. The decomposition pattern in air occurred in two steps. The compound decomposed upon time with temperature rising (0-290) ˚C, liberating gases that started by dehydration, lattice and coordinated aqua molecules evolved first then followed by chlorine molecules. In the next step during temperature elevating, a significant weight loss was observed related with ligand part as Ph-N=N-Ph molecule from each terminal of the two ligands. The complex residue $[Co(HO\text{-}Ph\text{-}CH(CN)NH)_2]$ represented a strong coordination bounding between the metal and the ligand part without oxide formation, this indicating a good stability. Table 4 illustrates temperatures ranges companying weight loss and liberating parts.

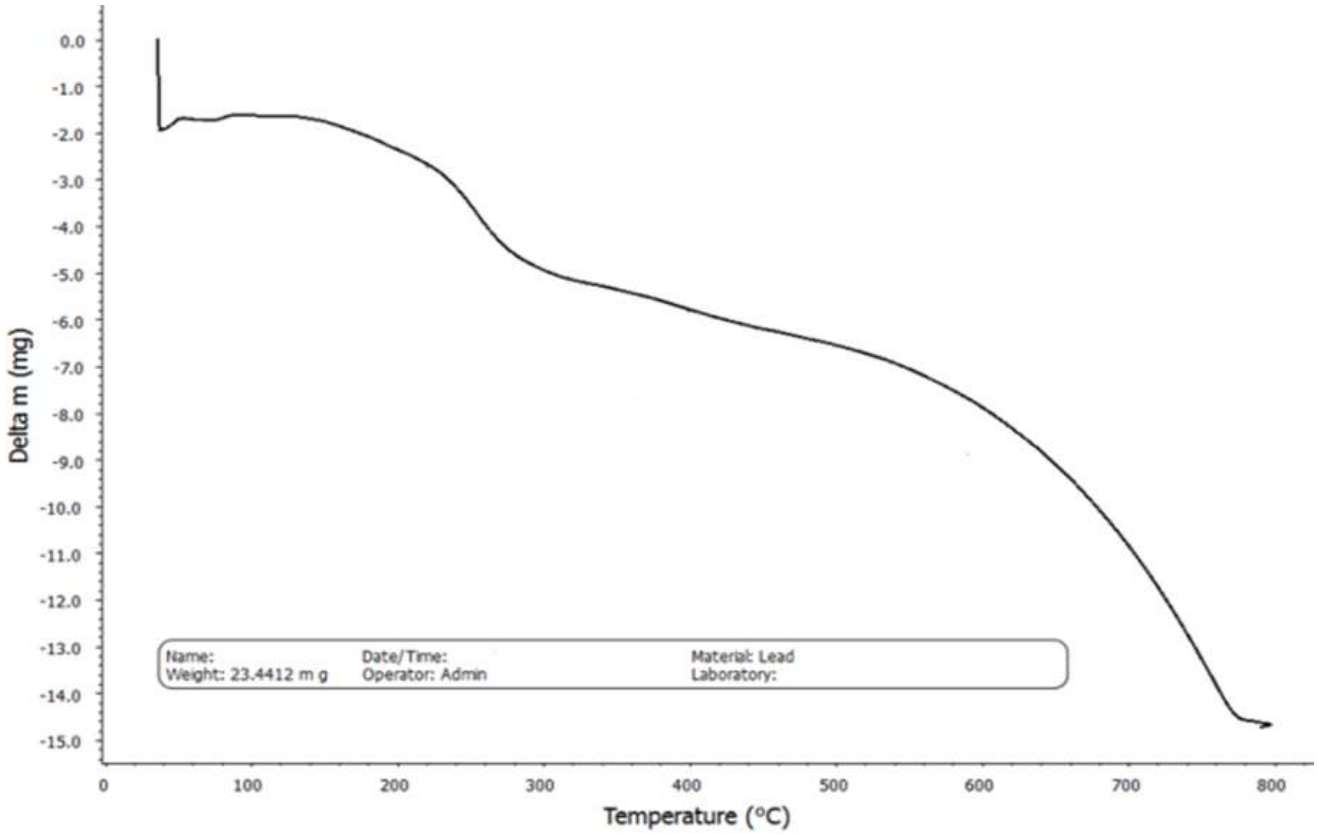


Figure 2. Thermogram of cobalt complex 23.4412 mg (C2) including three stages of weight loss percentages.

Table 4. Thermal decomposition data of cobalt complex (C2)

Complex	Temp. range ,˚C	Weight lost %		Decomposed part	Liberated part
		Found	Calc.		
C2	0-140	8.10	8.05	$[Co(H_2O)_2L_2]Cl_2$	$[H_2O]$ Lattice water
	140-290	12.79	11.96	$[CoL_2]$	$[H_2O, Cl_2]$ gases
	290-780	41.40	40.50	$[Co(HO\text{-}Ph\text{-}CH(CN)NH)_2]$	[Ph-N=N-Ph] or as benzene and N_2 gas
Ph: benzene ring					

3.4. Spectra of Nuclear magnetic resonance ^{1}H-NMR and ^{13}C-NMR of L

The 2-(2-hydroxyphenyl)-2-((4-(phenyldiazenyl)phenyl)amino)acetonitrile ligand (L) was characterized by ^{1}H and ^{13}C-NMR spectroscopy in figures (3 and 4) respectively, using deuterated dimethyl sulfoxide solvent DMSO (d6) . The ^{1}H-NMR spectrum showed four sets of peaks; the C-H signal of aliphatic carbon attached to nitrile group was appeared at δ5.99 ppm (singlet,1H) , while of aromatic occurred at the range δ(7-8) ppm for the mono- and bi-substituted aromatic rings, the azo- benzene at δ7.28 (multiplet, 5H), amino-benzene at δ 7.4 (multiplet, 4H) and phenolic-benzene at δ 7.81(multiplet, 4H). The N-H signal was appeared nearby at δ7.3 ppm (singlet, 1H). Finally the O-H signal of phenolic group was occurred at the low field near δ10.27 ppm (singlet,1H). Solvent signals were appeared at δ2.5 ppm and δ3.3 ppm as singlet for water molecules and non-deuterated DMSO respectively. The ^{13}C-NMR spectrum showed four regions of peaks; the sp^{2}-carbon bonded to electronegative oxygen or nitrogen which was deshielded to appear at range δ(140-160) ppm, while the other sp^{2}-carbon signals appeared at range δ (110-130) ppm. The sp^{3}-carbon bonded to nitrile showed signal at δ 44 ppm, meanwhile, sp-carbon of C≡N was shifted at δ 180 ppm. Table 5 summarizes the ^{1}H and ^{13}C-NMR chemical shifts and the assignments of L.

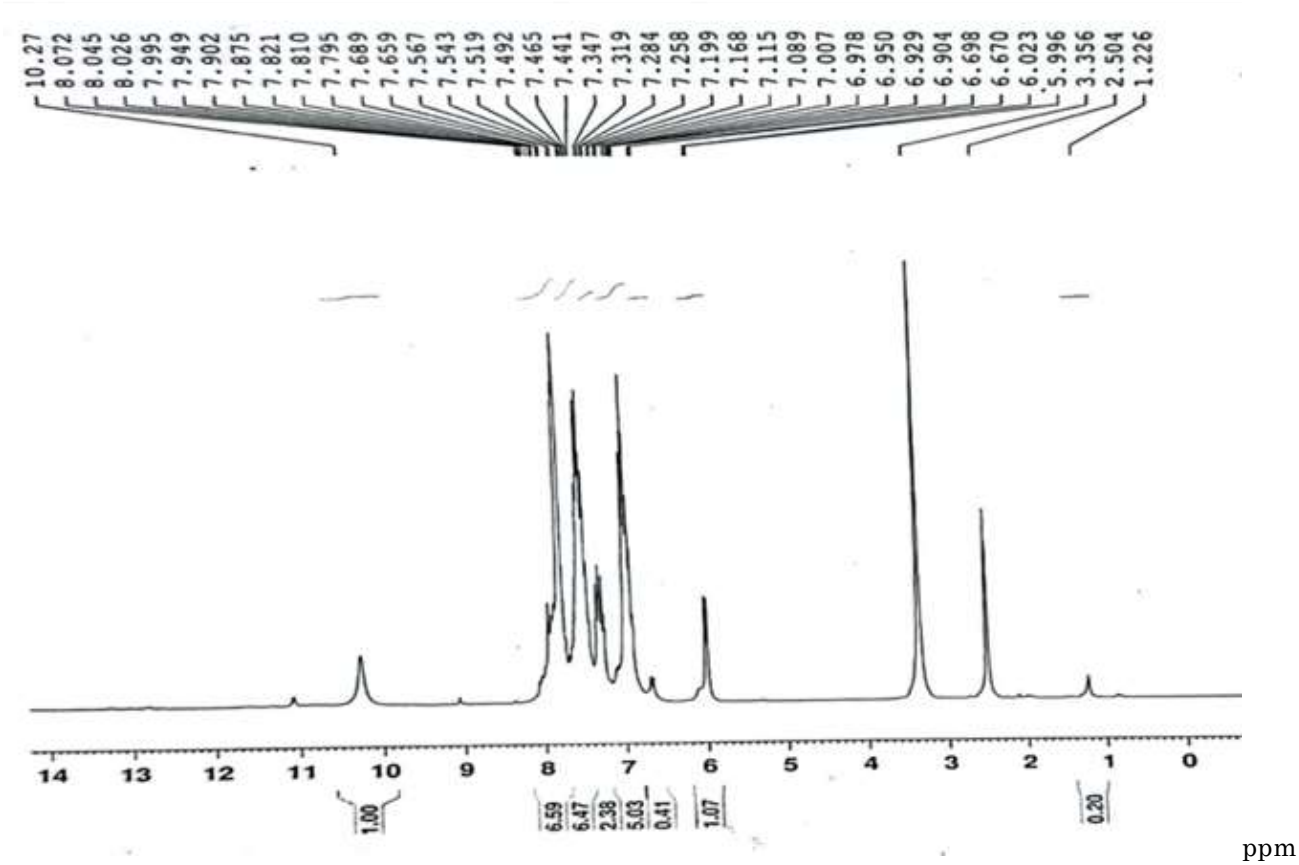


Figure 3. ^{1}H-NMR spectrum of the ligand (L).regarding multiplicity and chemical shifts.

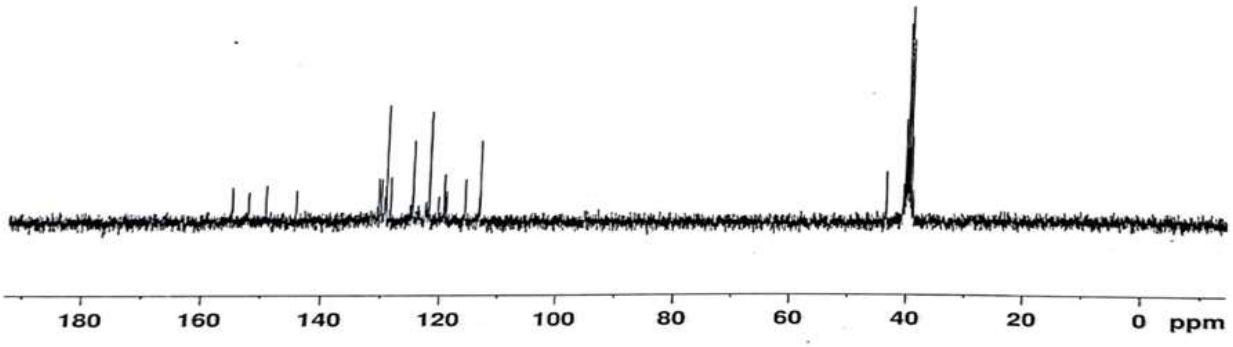


Figure 4. ^{13}C-NMR spectrum of the ligand (L) regarding multiplicity and chemical shifts.

Table 5. ^{1}H and ^{13}C-NMR chemical shifts, multiplicity and assignments of L.

L	δ(ppm)	Multiplicity	Integration	Assignment
^{1}H-NMR	5.99	Singlet	1H	CN-C-H
	7.28	Mutiplet	5H	N=N-Ph
	7.46	Mutiplet	4H	NH-Ph-N=N
	7.81	Mutiplet	4H	HO-Ph
^{13}C-NMR	44	Singlet	C-CN	sp^3-carbon
	114-129	Mutiplet	Ph-	sp^2-carbon
	144, 149, 152 and 155	Singlet	Ph-N=N-Ph, Ph-NH, HO-Ph	sp^2-carbon-electronegative
	180	Singlet	C≡N	sp-carbon

4. *In vitro* Antibacterial Activity

The α-aminonitrile ligand (L) and its complexes were scanned for their anti-bacterial activity against four bacterial species including Gram negative bacteria represented by *Pseudomonas spp.*, *Escherichia coli*, *Proteus* spp. and Gram positive bacteria represented by *Staphylococcus aureus*. Well diffusion method upon agar medium was used to find out the activity in diameter of 8mm. The incubation temperature was 37°C for 18 hours period of time. 10^{-3} M was the concentration of all compounds using DMSO as solvent and Gram negative control, while Tetracycline was as a Gram positive control. The zone of inhibition refers to the clear area devoid of bacterial growth, it is measured in millimeters (mm).The inhibition zone of the prepared compounds was compared to that of antibiotic and solvent. Table 6 illustrates the results of the antibacterial activity of the ligand and its complexes against. The results showed that complexes were highly active than the free ligand against gram negative *Pseudomonas spp.* and *Proteus spp.*.The presence of metal ion in case of overlap with the ligand orbital will reduce the polarity of the metal ion to greater extent. Thus it increases the delocalization of electrons over the whole chelating ring. This advantage improves the ability of the complexes to penetrate lipid membranes, which intern block metal binding sites in microorganism's enzymes. In addition, counting on the ligand part and nitrile group must be considered, which is known for its antibacterial and antifungal properties.

Table 6. Zone of inhibition measured in mm of DMSO, tetracycline and the synthesized compounds.

Compound	Pseudomonas spp G^-		Escherichia coli G^-		Proteus spp G^-		Staphylococcus aureus G^+	
	activity	zone (mm)	activity	zone (mm)	activity	zone (mm)	activity	zone (mm)
C1	++	12	++	11	++	16	++	12
C2	++	11	++	15	++	12	++	15
C3	++	14	---	---	++	11	++	18
C4	++	15	++	12	++	14	++	18
C5	++	12	---	---	++	15	++	12
L	---	---	++	19	---	---	+++	21
DMSO (Control)	++	12	---	---	++	11	---	---
Tetracycline	---	---	+++	25	---	---	---	---
Zone= (1-10)mm: + , (11-20)mm: ++ , (21-30)mm: +++								

5. Conclusions

The prepared α-aminonitrile ligand (L) contains different donor atoms, but the coordination in complexes was bidentate, through nitrogen and oxygen atoms of two ligands [1:2][M:L] forming stable six membered-rings, meanwhile, the six-coordination number was satisfied by two aqua molecules. The complexes of manganese, cobalt, nickel, copper and zinc were have octahedral shape, and chemical formulas $[MnL_2(H_2O)_2]Cl_2.H_2O$, $[CoL_2(H_2O)_2]Cl_2.4H_2O$, $[NiL_2(H_2O)_2]Cl_2.2H_2O$, $[CuL_2(H_2O)_2]Cl_2.4H_2O$ and $[ZnL_2(H_2O)_2]Cl_2.H_2O$ respectively, all are dichloride salts and hydrated complexes. It was expected that water molecules are involved in the coordination sphere due to using ethanol instead of absolute ethanol and without applying anhydrous conditions. Accordingly; the suggested chemical structure of the complexes are presented in figure 5. The antibacterial activity was promising and encouraging, and this could be due to the presence of nitrile group and the metal ion which were bizarre and complicated structure toward bacteria, thus, leading to variable behavior of activity according to the structure-activity relationship. L showed very good inhibition zone against *Escherichia coli and Staphylococcus aureus bacteria*, meanwhile, the complexes showed very good inhibition zones against the four bacteria, except Ni(II) C3and Zn(II) C5 complexes showed no activity against *Escherichia coli. Therefore, both C3 and C5 is not good candidate as drug while L is the one.* In conclusion, all the complexes showed good results in comparison with the ligand, this could be due to the presence of metal ion and the complex form which have two ligand molecules.

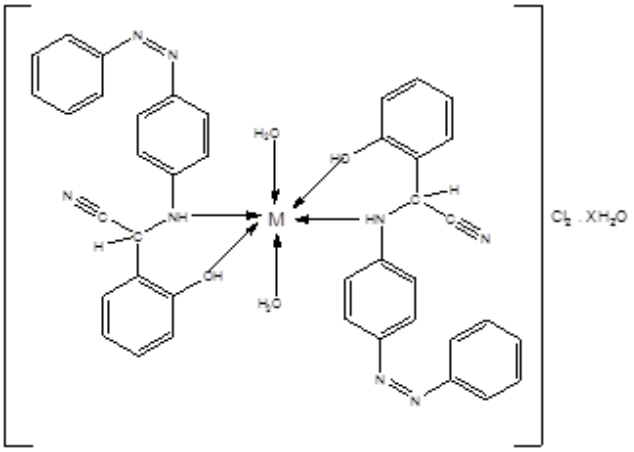


Figure 5. Suggested Structure of the complexes, M:Mn, X:1 (C1), M:Cu, X:4 (C2) , M:Ni, X:2 (C3), M:Cu, X:4 (C4), M:Zn, X:1 (C5)

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Conflict of interest: The authors declare no conflict of interest regarding this work.

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