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Synthesis, spectroscopic characterization and antimicrobial evaluation of novel nitrogen containing metal complexes

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Abstract

Four novel Mn(II), Co(II), Cu(II) complexes with nitrogen containing ligand (L) i.e. N, N-((Z)-ethane-1,2-diylidine) bis (2-amino benzohydrazide) have been synthesized and structurally characterized by elemental analysis, spectral, thermal (TG/DTG), molar conductance and magnetic susceptibility measurements. From the spectroscopic and magnetic studies, it has been concluded that all complexes have a six-coordinated octahedral geometry. The Schiff base and their metal complexes have also been screened for their antibacterial and antifungal activities by using a modified Kirby-Bauer disc diffusion method.

Keywords: synthesis, spectral, antimicrobial, complexes.

1. Introduction

Schiff bases are azomethine group (C=N) containing compounds which were first announced by Hugo Schiff in 1864 and synthesized by condensation of the primary amine with carbonyl compound [1]. Schiff bases remain the most extensively utilized organic compounds [2-7] and it has potential sites, such as nitrogen and other electron donors; it may be related to their stability, uses and applications in numerous scientific areas [1, 2, 4, 5, 8-10]. For long periods of time, coordination chemistry of Schiff base ligands has been the topic of great interest. These compounds are able to form coordination bonds with many metal ions via azomethine group (C=N) and other electron donor groups, so they have been used for the synthesis of metal complexes due to their easy formation and strong ability to bind with metal ions [4-7, 10-13] and their ability to form stable complexes to use as model compounds in service of biologically important species [12-14] where the nitrogen atom of azomethine group may be participated in the formation of constituents and interferes in normal cell processes so the (C=N) bond in azomethine derivatives is essential for biological activities [1, 15] as antitumor, antimicrobial agents (antifungal, antibacterial, antiviral and antiparasitic agents) and other biological performances [15]. These compounds have also played a great role in the chemistry development [2, 3, 5, 6, 11, 16]. These complexes were more widely studied because of their attractive chemical and physical properties and their wide range of applications in numerous scientific areas [15]. The design and synthesis of symmetrical Schiff bases derived from the 1:2 stepwise condensation of carbonyl compounds such as various aldehydes or ketones with aryl or alkyl diamines as well as their metal complexes have been of interest due to their facile synthesis, the accessibility of diverse structural modifications and The moderate electron donation easy tunable electronic properties proved Schiff bases as versatile ligands and allowed to carry out systematic reactivity studies based on ancillary ligand modifications [10, 17]. Recently, there is an increasing interest in the design and preparation of bis-hydrazones alongside with their metal complexes because of their uses as antitumor and antimicrobial agents [18, 19]. Moreover, when the hydrazono group is combined with other functional groups leads to compounds with unique physical and chemical properties [19]. Many coordination compounds have antimicrobial activity. Much interest has been focused on the hydrazone complexes to exhibit the coordinative behavior-microbial activity relationship and to their antiparasitic, fungicidal and bactericidal properties [8, 20]. And this is because the strong ability of hydrazone part in chelating metal ions [7, 8], whereas hydrazone contain two nitrogen atoms of different nature are associated and a C=N that is conjugated with alone pair of the terminal nitrogen atom [19]. The hydrazides and their derivatives are very important ligands and have attracted considerable attention recently, due to their several biological activities. Their bioactivity may be due to the presence of multi-coordination centers and having ability to form stable chelates with essential metal ions, which the organisms require in their metabolism. Usually include the biological interactions necessary for life processes [12]. In the view of the aforementioned applications, herein, we report the synthesis and spectroscopic characterization of Mn(II), Co(II) and Cu(II) complexes with the ligand (L). The present investigation also concerns with evaluating their antibacterial and antifungal properties against various pathogenic bacteria (Escherichia coli, Staphylococcus aureus) and fungal strains (Candida albicans) using a modified Kirby-Bauer disc diffusion method.

2. Experimental

Materials and methods

All the chemicals used were of AnalaR grade and procured from Sigma–Aldrich and Fluka. Metal salts (E. Merck) were commercially available as pure samples and solvents were used as received.

Synthesis of ligand (L)

The current ligand (L) has been synthesized as previously reported for similar compounds [3, 21].

Synthesis of the metal complexes

All the metal complexes (Scheme 1) were prepared as follows: 0.5 g of the ligand was dissolved in 30 mL acetone. To this solution 30 mL acetone solution of different metal salt was added dropwise in molar ratio 1:1 (metal: ligand). The reaction mixture was stirred under reflux whereupon the complexes precipitated. The precipitated solid complex was separated from the solution by filtration, purified by washing several times with ethanol and then dried under vacuum at room temperature.

Physical measurements

The elemental analysis (C, H, N) were achieved at Cairo University, Micro analytical center, using CHNS-932 (LECO) Vario Elemental Analyzer. Metal and halide ions of metal complexes were determined using the standard methods [22]. The FT-IR was performed (4000-400 cm-1) in KBr discs using Nenexeus-Nicolidite-640-MSA FT-IR Thermo-Electronic Co. The UV–visible absorption spectra were measured in DMF using 4802 UV/vis double beam spectrophotometer. Molar conductivities of studied complexes were estimated in DMF (10^{-3} M) using a CON 6000 conductivity meter. Magnetic susceptibilities of studied complexes were measured at room temperature by the modified Gouy method using magnetic susceptibility Johnson Matthey balance. The effective magnetic moments were calculated using the equation $\mu_{eff} = 2.828 (\chi_m T)^{1/2}$ BM., where χ_m is the molar susceptibility corrected for diamagnetism of all tomes in the compounds. Thermal analyses (TGA/DTG) were carried out by using a Shimadzu DTG/TG-50 thermal Analyzer with heating rate 10 °C/ min in nitrogen atmosphere with a flowing rate 20 ml/min in the temperature range 25-800 °C using platinum crucibles.

In vitro antimicrobial studies

Antimicrobial activity of the tested samples were determined using a modified Kirby-Bauer disc diffusion method [23]. Disc diffusion method for bacteria and fungi tested by using approved standard methods developed by National Committee for Clinical Laboratory Standards (NCCLS) [24]. Agar disc plate using agar medium was used to study the activity of synthesized ligand and its complexes with Ampicillin and fluconazole as reference drugs for bacteria and fungi respectively. The ligand (L) and its complexes were examined against *Staphylococcus aureus* (ATTCC 25923) as Gram-positive strain, *Escherichia coli* (ATTCC 25922) as Gram-negative strain and *Candida albicans* (ATCC 10231) as fungi, (the strains were provided by Microbiology Department, Faculty of Pharmacy, Misr University for Science and Technology, 6th of October City, Giza, Egypt). Each studied activity compound was dissolved at a different concentration of (10 and 20 mg/mL) in DMF. Discs of 6 mm were impregnated with the samples solution and allowed to dry at room temperature, testing was performed on sabouraud dextrose agar for antifungal strain and on nutrient agar for antibacterial strains. For each strain plates were inoculated by dipping two sterile cotton swabs into 0.5 McFarland (approximately 10^4-10^6 colony forming unit (CFU/mL) suspension of the used microorganisms and streaking the plate surface in three directions. After the plate was allowed to dry for 20 min, the petri dishes were subsequently incubated at 35-37 °C and 30 °C about 24-48 h for (gram positive, gram negative) bacteria and fungi, respectively. Standard discs of Ampicillin (antibacterial agent), Fluconazole (an antifungal agent) [25] were used as positive controls for antimicrobial activity and filter paper disc impregnated in DMF was used as negative control. The activity was measured in millimeters [23].

3. Results and Discussion

Physical properties

All the metal complexes are colored solids and are stable towards air. Elemental analysis and analytical data of the complexes (Table 1) suggest that the metal to ligand ratio in all the complexes is 1M:1L (Scheme 1). All complexes are slightly soluble in alcohols, insoluble in most common organic solvents, but soluble in polar solvents such as DMF and DMSO solvents.

Molar conductivity

The molar conductivity of complexes (1-3) in DMF solutions (10^{-3} M) at room temperature indicates that they are non-electrolytes, complex (4) shows Λ_m value (65 Ω^{-1} cm^2 mol^{-1}) indicating that it is 1:1 electrolyte [3].

No.	Compound	Color	Empirical formula	Yield (%)	Decomp. Temp./°C	Elemental Analysis. Calc. (F) (%)					$(\Lambda_m)^a$
						C	H	N	M	halogen	
	Ligand	Brown	$C_{16}H_{16}N_6O_2$	50	170-175	59.26(60.22)	04.90(04.73)	25.90(25.40)	---	---	
1	$[Mn(L)Cl_2].2H_2O$	Brown	$C_{16}H_{20}N_6O_4Mn.2Cl$	70	150	39.50(38.24)	04.10(03.98)	17.20(16.85)	11.30(10.97)	14.60(14.20)	37
2	$[Co(L)(OAc)_2].2H_2O$	Pale brown	$C_{20}H_{26}N_6O_8.Co$	90	136	44.70(45.54)	04.80(04.41)	15.60(15.05)	11.00(11.30)	---	5
3	$[Cu(L)(OH)_2]$	Dark brown	$C_{16}H_{18}N_6O_4.Cu$	90	180	45.50(46.60)	04.20(03.93)	19.90(19.47)	15.06(15.20)	---	24
4	$[Cu(L)(OH)(H_2O)]ClO_4$	Dark brown	$C_{16}H_{19}N_6O_8Cu.Cl$	30	---	36.78(36.61)	03.60(03.01)	16.00(15.53)	12.10(12.16)	06.80(07.10)	65

Table 1. Analytical and physical data for the ligand and its metal complexes.

Spectral studies

FT-IR Spectra

The important IR frequencies of the Schiff base and its Mn(II), Co(II) and Cu(II) complexes are presented in (Table 2). In order to study the binding mode of the ligand to the metal ion complexes, the IR spectrum of the free ligand was compared with the spectra of the metal ion complexes. A strong band is observed for the free ligand at around 1606 cm^{-1} characteristic of the azomethine (C=N) stretching vibrations, This band in the metal ion complexes is shifted to lower frequencies 1586–1515 cm^{-1}, suggesting coordination of the azomethine (C=N) nitrogen atom to the metal ion [18, 19]. In addition to this, a medium intensity broad band observed at around 3380 cm^{-1} in the ligand is due to υ(NH) of free amine groups [18], this band in the metal ion complexes is shifted to lower frequencies 3317–3250 cm^{-1}, suggesting coordination of the amine (NH_2) nitrogen atom to the metal ion [3, 26]. A further evidence for these coordination modes is the appearance of new band at 536-455 cm^{-1} due to υ(M–N) stretching vibrations [3]. Aquo and hydroxo complexes exhibit a strong as well as broad band at 3439–3419 cm^{-1} due to υ(OH) vibration. This observation has been further confirmed by the appearance of new medium intensity band in the 593–528 cm^{-1} region, assignable to υ(M-O) [22], and also is confirmed from elemental and thermal analysis [21] and this band υ(OH) sometimes obscures the NH stretching vibrations which occur in the same region for all complexes [26]. The acetato complex display bands at 1605 and 1425 cm^{-1} due to unidentate coordination of acetate ion [21]. It is worth to mention that, the region of $υ_{asym}(COO^-)$ overlapped with υ(C=N) [21]. The presence of uncoordinated perchlorate anion in complex (4) is inferred from the bands around 1106 and 1092 cm^{-1} [21]. Therefore, and according to the IR spectra, it is concluded that the ligand behaves as a tetradentate one with the lone electron pairs of two amino groups and the lone electron pairs of two azomethine groups.

No.	Compound	υ(OH+NH)	υ(C=N) Azomethine	υ (M-O)	υ (M-N)	υ Anion (NO_3^-, OAc^-, CLO_4^-)	$λ_{max}$(nm) (d–d) Transition	$μ_{eff}$(B.M.)
	Ligand	3446(b),3380(sh)	1606(b)	---	---	---	---	---
1	$[Mn(L)Cl_2].2H_2O$	3419(b),3317(sh)	1570(sh)	593(w)	535(w)	---	545	6.01
2	$[Co(L)(OAc)_2].2H_2O$	3434(b),3260(sh)	1586(b)	528(w)	455(w)	1605(sh),1506(m),1425(m)	450,540, 670	5.60
3	$[Cu(L)(OH)_2]$	3435(b),3260(sh)	1570(b)	593(w)	536(w)	---	445, 540, 660	1.80
4	$[Cu(L)(OH)(H_2O)]ClO_4$	3439(b),3250(sh)	1515(b)	539(w)	---	1106(m),1092(m)	445,540, 660	1.97

Table 2. Selected infrared spectral bands (cm^{-1}), magnetic moment (BM) and electronic spectral data (nm) of ligand (L) and its metal complexes.

Electronic spectra and magnetic measurements

The nature of the ligand field around metal ion and the geometry of the metal complexes have been deduced from the electronic spectra and magnetic moment data of the complexes (Table 2).

Manganese(II) complex

The electronic spectrum of the Mn(II) complex (1) exhibits only one d-d transition band at 545 nm suggesting an octahedral geometry around Mn(II) ion [27, 28], the octahedral geometry was proposed to the complex referring to its elemental, spectroscopic, analytical data and its empirical formula. The room temperature magnetic moment of complex (1) is 6.01 BM., is well within the range usually observed for octahedral Mn(II) (5.34-6.02 BM.) [27, 28].

Cobalt(II) complex

The electronic spectrum of the Co(II) complex (2) displays three bands at 670, 540, 450 nm. the observed bands are assigned to the transitions ${}^4T_{1g}(F)\rightarrow{}^4T_{2g}(F)$, ${}^4T_{1g}(F)\rightarrow{}^4A_{2g}(F)$ and ${}^4T_{1g}(F)\rightarrow{}^4T_{2g}(P)$, respectively suggesting an octahedral geometry around Co(II) ion [29-31]. The magnetic susceptibility value is found to be 5.6 BM., which is an indicative of octahedral geometry [29-31].

Copper(II) complex

The electronic absorption spectra of the Cu(II) complexes (3,4) exhibit spectral bands which have one trend around 660, 540, and 445 nm corresponding to ${}^2B_{1g}\rightarrow{}^2A_{1g}$, ${}^2B_{1g}\rightarrow{}^2B_{2g}$, ${}^2B_{1g}\rightarrow{}^2E_g$ transitions, which suggests distorted octahedral geometry around Cu(II) ions [21, 26]. Their room temperature magnetic moment is in the range 1.80–1.97 BM. (fall within the range normally observed for octahedral Cu(II) complexes which is 1.7–2.2 BM.) corresponding to one unpaired electron in an octahedral geometry. These values clearly support the monomeric nature of the complexes and also show the absence of metal–metal interaction [10, 13, 21, 26, 30, 32].

Thermal studies

TG/DTG measurements have yielded much insight in the composition and the thermal stability of compounds. The thermal properties of ligand and its metal complexes were investigated by thermogravimetric analysis (TG/DTG), under a nitrogen atmosphere from RT to 800 °C. The nature of proposed chemical change with temperature and the percent of metal oxide obtained are given in (Table 3). The results obtained from thermogravimetric analysis were in agreement with the suggested theoretical formulae from the elemental analyses [21, 22]. The thermal behavior of perchlorate complex (4) has not been investigated, due to its explosive nature [22].

Manganese(II) chelate

The TG/DTG curve of Mn(II) complex (1) show weight loss in the temperature range of 38-150 °C (Cal/Found% 7.40/7.56) with one DTG peak at 61 °C that is related to the release of two molecules of the outside coordination sphere water. Gradual decomposition is observed in temperature range 150-735 °C, the DTG curve for complex (1) shows peaks at 291, 408, 563 and 604 °C, leaving Mn metal as final residue for the complex (Figure 1).

Cobalt(II) chelate

The TG/DTG curve of Co(II) complex (2) showed weight loss in the temperature range of 30-136 °C (Cal/Found% 6.70/7.26) associated with DTG peaks at 52 °C that are assigned to the release of two molecules of the outside coordination sphere water. Gradual decomposition is observed at temperature range of (136-420 °C). DTG curve showed peaks at (252 and 345 °C) respectively, leaving CoO contaminating with carbon as final residue (Figure 1). It is worth to note that the slight higher value found and the theoretical residue value percentages show that the residues are contaminated with some carbon atoms. These data are confirmed from the calculations and IR spectra.

Copper(II) chelate

The TG/DTG curves of Cu(II) complex (3) showed no weight loss up to 180 °C indicating that there is no water and/or any adsorptive solvent molecules. Gradual decomposition is observed continued at temperature range of (180-800 °C), associated with DTG peaks at (328, 462 and 597 °C), leaving Cu metal as final residue. Due to the explosive nature of perchlorate complex, the thermal behavior of copper perchlorate complex (4) has not been investigated.

NO.	Compound	Temp/°C		Mass. loss %		Leaving species
		DTG	TGA	Cal.	(F.)	
1	$[Mn(L)Cl_2].2H_2O$	61	38-150	07.40	07.56	$-2H_2O$
		291,408,563,604	150-735	81.30	81.18	Decomposition
			At 735	11.30	11.26	≡ Mn
2	$[Co(L)(OAc)_2].2H_2O$	52	30-136	06.70	07.26	$-2H_2O$
		252,345	136-420	72.70	72.14	Decomposition
			At 420	20.60	20.60	≡ CoO+3C
3	$[Cu(L)(OH)_2]$	328,462,597	180-800	84.94	85.00	Decomposition
			At 800	15.06	15.00	≡ Cu

Table 3. **TG/DTG** data for the investigated complexes.

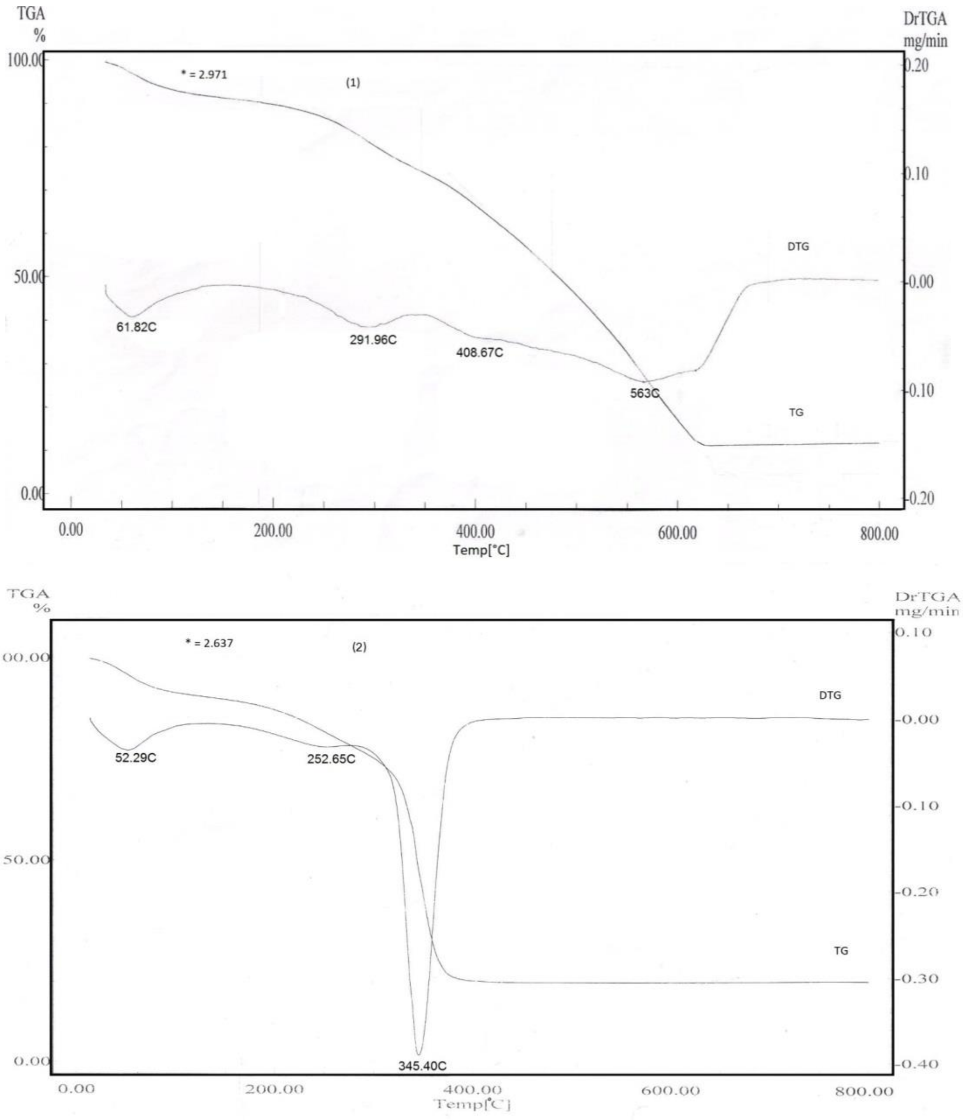


Fig.1. TG/DTG curves of the complexes (**1**,**2**).

Antibacterial activity

The free ligand and its metal complexes were tested against bacterial species by measuring the bacteriostatic diameter. The results were showed in Figure 2. The results indicate that most of the tested complexes showed moderate antibacterial activity and the ligand has lower antibacterial activity against the same microorganisms under identical experimental conditions. Generally, complexes have different activities for both two concentrations (20 mg/ml and 10 mg/ml) with inhibition range of 10-17 mm with *S.aureus* it is clear that for complex (4) at low concentration (10 mg/ml) gave higher activity with *S.aureus* compared with that the high concentration (20 mg/ml), complex (1) showed high activity with high concentration and the other complexes (2,3) have no activity for both two concentrations. For *E.coli*, the highest activity value was 13 mm with high concentration (20 mg/ml) for complex (1). Complex (4) showed high activity with high concentration (20 mg/ml). Complexes (1,4) have no activity with low concentration (10 mg/ml) and the other complexes (2,3) have no activity for both two concentrations. All these activity values are compared with that of positive control of commercial and the most available drug Ampicillin (26 mm and 22 mm with 20 mg/ml and 10 mg/ml respectively). The higher activity of some complexes could be explained according to Tweedy and chelation theory, chelation decreases the polarity of the metal ion due to partial sharing of positive charge with donor groups and delocalized electrons of chelate ring system formed during coordination [33, 34]. Antibacterial activity of metal complexes is also affected by many other factors, such as the chelate effect of the ligand, the nature of the donor atoms, metal ion, counter ions that neutralize the complex, the total charge on the complex ion and the geometric structure of the complex. All these factors play important role in the increase of the lipophilicity of the central metal atom which increases the hydrophobic character and liposolubility of the complex that facilitates the permeation through the lipid layer of the bacterial membrane [33, 35]. Due to the complexity of biological system it is difficult to estimate exact mechanism for such activities.

Antifungal activity

Candida albicans was used as fungal species by measuring the fungistatic diameter with the free ligand and metal complexes. The results were showed in Figure 2. The results indicate that the ligand has no antifungal activity with both two concentrations against the same microorganisms under identical experimental conditions which used to test the antifungal activity of metal complexes. However, *Candida albicans* was affected by the complex (4) with inhibition zone 11 mm with high concentration (20 mg/ml) and also affected by the complex (2) with inhibition zone 10 mm with low concentration (10 mg/ml) compared with the positive control drug used for both two concentration (20 mg/mL and10mg/mL) Fluconazole, which gave inhibition values 30 and 26 mm, respectively. Complexes (1,3) have no activity with both two concentrations used. It was clear that the fungal activity affected with the nature of each metal ion. The result may be explained due to the presence of chloride ions in complex (4) which may be combined with membrane proteins and enzymes. The increase of antifungal activities may be due to the formation of hypochlorous acid that decompose to HCl and O_2 which destroy the cellular components and the microbes [34, 35].

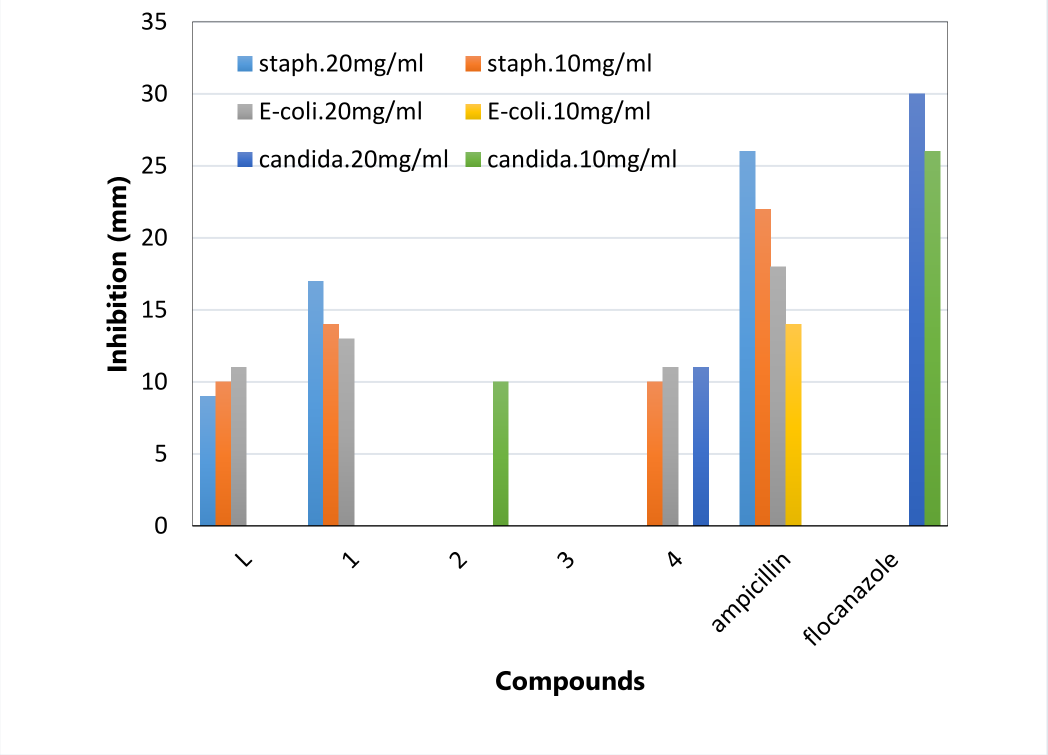
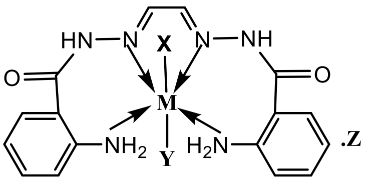


Fig.2. In vitro antimicrobial activity of the free ligand and its metal complexes.



	M	X	Y	Z
(1)	Mn	Cl	Cl	2H2O
(2)	Co	OAc	OAc	2H2O
(3)	Cu	OH	OH	---
(4)	Cu	H2O	OH	ClO4

Scheme1. Suggested structures of the complexes.

4. Conclusion

In this work the synthesis and spectroscopic characterization of new four transition metal Mn(II), Co(II), Cu(II) complexes of nitrogen containing ligand are reported. The analytical and physicochemical data confirmed the composition and the structure of the newly obtained compound. The results obtained can be summarized as follows:

1- The ligand shows an interesting coordinating ability to metals ions behaves as neutral tetradentate one.

2- All the metal complexes are mononuclear adopt octahedral geometry.

3- The antimicrobial (antibacterial and antifungal) studies of the prepared compounds screened against pathogenic bacteria and fungal strains proved that these compounds exhibit moderate antibacterial and antifungal activity.

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