ATIVIDADE ANTIBIÓTICA DE NOVAS ESPÉCIES DE COMPLEXOS DE METAIS DE BASE DE SCHIFF

ANTIBIOTIC ACTIVITY OF NEW SPECIES OF SCHIFF BASE METAL COMPLEXES

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RESUMO

A resistência bacteriana é um crescente desafio enfrentado pelos cientistas de design de medicamentos para encontrar novos medicamentos ou atualizar o antibiótico comumente usados. O objetivo deste estudo foi sintetizar, estruturar e destacar características biológicas de novas espécies de complexos metal-orgânicos $[(MCl_2)_2L_1]$ $\{M = Ni, Cu, L_1 = N, N'-1, 4\text{-Fenilenobis (metanililideno) bis (etano-1,2-diamina)}\}$ e $[(NiCl_2)_2L_2]$. Os ligantes da base de Schiff foram preparados em excelentes campos, adicionando tereftalaldeído a 1,2-etano-diamina ou 1,4-butano-diamina. Três complexos binucleares de metais básicos de Schiff foram sintetizados em uma reação simples de um reator por reação dos sais correspondentes de cloreto de metal ($NiCl_2$ e $CuCl_2$) com os ligantes da base de Schiff $\{L_1 = N, N'-(1,4\text{-fenilenobis (metanililideno) bis (1,2-dietilamina)})$ e $L_2 = N, N'-(1,4\text{-fenilenobis (metanililideno) bis (butano-1,4-diamina)})\}$. Os complexos de metal base schiff obtidos foram caracterizados analiticamente por um conjunto de técnicas espectroscópicas, tais como espectroscopia FT-IR, espectros de RMN de 1H e espectros de massa. Os estudos de caracterização de estruturas sugerem que os ligantes da base de Schiff se comportam como ligantes bidentados N para centros de metal de níquel e cobre que são conhecidos por atuarem como ácidos de Lewis. A atividade biológica da base Schiff dos complexos de níquel (II) e cobre (II) foi testada usando o método de diluição em caldo. Os complexos mostraram atividade antibacteriana contra todas as bactérias gram-negativas e gram-positivas (*Enterobacter cloacae* G-, *Citrobacter* G-, *Pseudomonas* G-, *Klebsiella* G-, *Staphylococcus* G + e *Streptococcus* G +) utilizadas nos ensaios. Conclui-se que os complexos de metais básicos de Schiff são um bom candidato ao medicamento antibacteriano devido à sua boa atividade contra cepas gram-negativas e gram-positivas demonstradas no presente estudo, bem como em outros estudos na literatura.

Palavras-chave: atividade antibacteriana, organometálica, complexos, síntese, espectroscópica.

ABSTRACT

Bacterial resistance is a growing challenge facing drug design scientists to find new medications or update commonly used antibiotics. The objective of this study was to synthesize, structure and highlight biological features of new species of metal-organic complexes $[(MCl_2)_2L_1]$ $\{M = Ni, Cu, L_1 = N, N'-1,4\text{-Phenylenebis(methanylylidene)bis(ethane-1,2-diamine)}\}$ and $[(NiCl_2)_2L_2]$. The Schiff base ligands were prepared in an excellent yeilds by adding terephthalaldehyde to 1,2-ethane-diamine or 1,4-butane-diamine. Three binuclear Schiff base metal complexes were synthesized in a simple one-pot reaction by reacting the corresponding metal chloride ($NiCl_2$ and $CuCl_2$) salts with the Schiff base ligands $\{L_1 = N, N'-(1,4\text{-phenylenebis(methanylylidene)bis(1,2-diethylamine)})$ and $L_2 = N, N'-(1,4\text{-phenylenebis(methanylylidene)bis(butane-1,4-diamine)})\}$. The obtained Schiff base metal complexes were analytically, characterized by a set of spectroscopic techniques such as FT-IR spectroscopy, 1H NMR spectra, and mass spectra. The structures characterization studies suggest that Schiff base ligands behave as N bidentate ligands for nickel and copper metal centers, which are known to act as Lewis acids. The biological activity of the Schiff base of nickel(II) and copper(II) complexes was tested by using the broth dilution method. The complexes showed antibacterial activity against all gram-negative and gram-positive bacteria (*Enterobacter cloacae* G-, *Citrobacter* G-, *Pseudomonas* G-, *Klebsiella* G-, *Staphylococcus* G+, and *Streptococcus* G+) that used in the trials. It is concluded that Schiff base metal complexes are a good candidate for the antibacterial drug because of its good activity against gram-negative and gram-positive strains demonstrated in the present study, as well as in other studies in the literature.

1. INTRODUCTION:

Schiff bases are an essential ligand system in developing coordination chemistry due to their tunable and chelating properties (Radha et al., 2018). Chelating ligands containing O, N, and S donor atoms are well known as having a broad biological activity (Shi et al., 2007). Moreover, this activity can be increased when such ligands are bonded to a metal ion (Chohan et al., 2007; Tsapkov et al., 2008).

Intensive studies have been reported on the biological activity of Schiff bases like antibacterial (Panneerselvam et al., 2009; Rai, and Kumar, 2013), antifungal (Ramesh and Maheswaran, 2003), antitumor (Liu et al., 1992), antiviral (Kumar et al., 2010), anti-HIV (Vicini et al., 20103), herbicides (Holla et al., 2000), insecticides (Alcock et al., 1980) and anti-influenza virus (Zhao et al., 2011). In addition, a wide range of applications have been reported in food and dye industries, analytical chemistry, catalysis, agrochemical, and anti-inflammable and antiradical (Kuddushi et al., 2018). Furthermore, anti-corrosion properties were also investigated (Shaju et al., 2014).

The importance of Schiff bases and their metal complexes was also demonstrated in electrochemical (Neelakantan et al., 2010) and redox catalysis (Vedanayaki et al., 2010; Canpolat, and Kaya, 2004). The role of chlorophyll, hemoglobin, carbonic anhydrase, vitamin B12, xanthine oxidase, and hemocyanin, illustrates the intimate linkage between inorganic chemistry and biology (Singh and Bhatnagar, 2004).

This study aimed to explore the antimicrobial activity of Schiff base metal complexes against six different strains of gram-negative and gram-positive bacteria (*Enterobacter cloacae* G-, *Citrobacter* G-, *Pseudomonas* G-, *Klebsiella* G-, *Staphylococcus* G+, and *Streptococcus* G+).

2. MATERIALS AND METHODS:

2.1. Chemical materials

All chemicals were of analytical reagent grade, purchased from Sigma-Aldrich and used without further purification. FT-IR spectra (KBr

matrix) ($4000\text{--}200\text{ cm}^{-1}$) were recorded on an FT-IR Spectrometer Model C103 (Shimadzu). ^1H NMR spectra were obtained on a Varian 400 NMR spectrometer and recorded in DMSO- d_6 . Chemical shifts (δ) are expressed in parts per million downfield using tetramethylsilane as an internal reference or by using the residual protonated solvent. Mass spectra of the compounds were recorded on an Agilent Technologies (HP) 5973 Network Mass Selective Detector. NMR and Mass analyses were performed at the Central Instrumental Lab., School of Chemistry, College of Science, University of Tehran, Enghelab St., Tehran, Iran.

2.2. Ligands synthesis

The condensation reaction (Vinita et al., 2013) was employed to synthesize Schiff base ligands L_1 and L_2 . Initially, terephthalaldehyde (0.13 g, 1 mmol) and 1,2-ethane-diamine (0.12 g, 2 mmol) or 1,4-butane-diamine (0.17 g, 2 mmol) were dissolved in 25 mL methanol in a 50-mL round-bottomed flask equipped with a condenser (Equations 1 and 2). The reaction mixture was stimulated by droplets of acetic acid, refluxed, and stirred for 4 h, then cooled, filtered, and dried in a desiccator over anhydrous CaCl_2 and recrystallized from methanol. Orange powder of L_1 was obtained (Yield = 0.20 g, 80%, m.p. = $265\text{--}267\text{ }^\circ\text{C}$) or white powder of L_2 (Yield = 0.25 g, 83%, m.p. = $241\text{--}243\text{ }^\circ\text{C}$).

Equation 1

Equation 2

2.3. Schiff base metal complexes synthesis

$NiCl_2$ and $CuCl_2$ salts were reacted with Schiff base L_1 or L_2 to form the following solid complexes $[(NiCl_2)_2L_1]$, $[(CuCl_2)_2L_1]$ and $[(NiCl_2)_2L_2]$ (**Scheme 1**). These complexes were prepared by addition of (2 mmol, 0.25 g) $NiCl_2$ or (2 mmol, 0.26 g) $CuCl_2$ in about 25 mL of hot ethanol to a solution of (1 mmol, 0.21 g) L_1 or (1 mmol, 0.27 g) L_2 Schiff bases in about 15 mL ethanol with continuous stirring. The reaction mixture was refluxed for 4 h. Colored complexes were filtered, washed with methanol, and dried in a vacuum desiccator (Mishra et al., 2015; Salehi et al., 2016; Nagesh and Mruthyunjayaswamy, 2015).

2.4. Biological activity

The antibacterial activity of the chemical compounds $[(NiCl_2)_2L_1]$ (**1**), $[(CuCl_2)_2L_1]$ (**2**) and $[(NiCl_2)_2L_2]$ (**3**) was *in vitro* screened using the disc diffusion method (CLSI, 2010). The strains were treated with compounds **1**, **2**, **3** and a ligand, which were dissolved in DMSO. The biological activity of these compounds was tested against a set of pathogenic gram-negative and gram-positive bacteria, respectively, *Enterobacter cloacae* G-, *Citrobacter* G-, *Pseudomonas* G-, *Klebsiella* G-, *Staphylococcus* G+, and *Streptococcus* G+. Sterilized needles were used to transfer the required microorganisms to the nutrient agar. Amoxicillin and Azithromycin (10 mg mL⁻¹) were used as reference drugs, DMSO was used in the measurement as a control. The diffusion was done by placing plates at room

temperature for 1 h and incubated at 37 °C for 24 h. A scale was used to measure the inhibition diameters of the clear zone. Minimum inhibitory concentration (MIC) studies of the prepared Schiff base complexes lead to promising antimicrobial activity. The Microwell dilution technique (CLSI, 2012) was employed to determine the minimum inhibitory effect of the Schiff base, and its metal complexes and Muller-Hinton (Ansari et al., 2011) broth was used as culture media. Complexes (**1-3**) were prepared with 1000 mg mL⁻¹ and the discs were incubated at 50°C for 30 min to increase solubility.

3. RESULTS AND DISCUSSION:

Schiff base metal complexes described above (**Scheme 1**) were synthesized by the direct reaction between the ligands (L_1 , L_2) and ($NiCl_2$, $CuCl_2$) in 1:2 molar ratio in ethanol. All the complexes showed a high level of stability at room temperature (25 °C) while the solubility in some organic solvents was limited, except in DMSO. The solubility difficulties of complexes **1-3** did not allow us to perform single-crystal X-ray diffraction analysis in this study for structure elucidation. Thus, all ligands and their complexes were diagnosed according to the results of a set of accurate analysis.

3.1. $[(NiCl_2)_2L_1]$ (**1**)

A metathesis reaction between $NiCl_2$ and L_1 (**Scheme 1**) led to the formation of complex (**1**) in good yield (0.18 g, 85 %, m.p. 243-245 °C).

3.2. $[CuCl_2)_2L_1]$ (**2**)

Similar product (**2**) with an excellent yield (0.19 g, 90 %, m.p. 237-239 °C) was obtained starting from $CuCl_2$ and L_1 .

3.3. $[(NiCl_2)_2L_2]$ (**3**)

The complex **3** was prepared by following the same described route for complexes **1** and **2**, resulting in a moderate product yield (0.17 g, 62 %, m.p. 246-248 °C).

3.4. FT-IR spectra

FT-IR spectra analysis of the Schiff base ligands L_1 and L_2 show strong bands, respectively, at 1643 and 1635 cm⁻¹, assigned to azomethine $\nu(C=N)$ group confirming the success of ligand synthesis. The shift toward higher or lower frequencies is a common trend in coordination chemistry due to the association of the metal ions

with the azomethine group with a symmetric ring. The nature of the metal ion and the associated atoms and bonds can shift the frequencies toward higher or lower values. Spectra of compounds **1**, **2** and **3** also showed the disappearance of the band attributed to carbonyl (C=O) group of aldehydes, the starting material, confirming the success of ligand synthesis. Schiff bases metal complexes (**1-3**) were also characterized by the appearance of bands at the region 2939, 3387, and 3302 cm^{-1} respectively, due to the $\tilde{\nu}(\text{C-H})$ aromatic vibrations.

3.4.1 $[(\text{NiCl}_2)_2\text{L}_1]$ (**1**)

The following IR bands were observed in **Figure 1** at $\tilde{\nu}$ equal to: 3541 (w), 2939 (w), 2839 (s), 2110 (m), 1697 (w), 1643 (s), 1550 (w), 1442 (s), 1350 (m), 1296 (s), 1211 (m), 1111 (m), 1049 (w), 1002 (m), 964 (m), 918 (w), 871 (w), 833 (s), 686 (s), 524 (w), 462 (w), 393 (s), 316 (m), 270 (m). In comparison with the spectra of the Schiff bases ligand, the $\tilde{\nu}(\text{C=N})$ band exhibits an upward shift to 1697 cm^{-1} , which is in agreement with the azomethine group that coordinated to the metal ion, as previously reported (Das et al., 2014). The strong band that appeared at 1643 was assigned to C=C stretching. Moreover, medium intensity bands that appear at 3541 (w)-2839 (w) cm^{-1} assigned to $\tilde{\nu}\text{NH}_2$ group proved the M-NH₂ coordination (Khan et al., 2016). Furthermore, the appearance of medium intensity bands at the range 678- 316 cm^{-1} attributed to $\tilde{\nu}(\text{M-N})$ vibrations is another evidence that confirms the coordination between the ligand and the metal atoms through the azomethine nitrogen atom. The strong and medium two bands appeared at 1442 (s) and 1350 (m) were attributed to CH₂ deformation vibrations (Khan et al., 2017; Bhattacharyya et al., 2015).

3.4.2 $[\text{CuCl}_2)_2\text{L}_1]$ (**2**)

The following IR bands were observed in **Figure 2** at $\tilde{\nu}$ equal to: 3302 (m), 3232 (m), 3124 (w), 3024 (w), 2916 (m), 2854 (m), 1697 (s), 1635 (s), 1450 (s), 1365 (s), 1280 (s), 1203 (s), 1111 (m), 1010 (s), 972 (m), 840 (s), 678 (m), 532 (s), 432 (s), 362 (w), 339 (w), 277 (m). The compound $[(\text{CuCl}_2)_2\text{L}_1]$ (**2**) showed two strong bands that shifted upward assigned to the $\tilde{\nu}(\text{C=N})$ group of the azomethine function, as previously reported (Das et al., 2014). The intense band that appeared at 1635 was assigned to C=C stretching. Comparing with the previously reported complexes (Khan et al., 2016), the bands observed at 3302 (m), and 3024 (w) cm^{-1} clearly confirm the M-NH₂ coordination. Methylene (CH₂)

deformation vibrations are responsible for the two bands appeared at 1450 (s) and 1365 (s). IR spectrum of compound $[\text{CuCl}_2)_2\text{L}_1]$ can provide further support to confirm the coordination by the presence of bands at the range from 678 (m) to 277 (m) cm^{-1} , related to $\tilde{\nu}(\text{M-N})$ vibrations (Khan et al., 2017; Bhattacharyya et al., 2015).

3.4.3 $[(\text{NiCl}_2)_2\text{L}_2]$ (**3**)

The following IR bands were observed in **Figure 3** at $\tilde{\nu}$ equal to: 3595 (w), 3387 (w), 3024 (w), 2939 (s), 2839 (s), 2060 (w), 1697 (s), 1635 (s), 1458 (s), 1342 (s), 1296 (s), 1211 (s), 1095 (w), 1049 (w), 1002 (m), 964 (m), 918 (w), 825 (s), 740 (w), 678 (w), 516 (s), 385 (w), 331 (w), 270 (w). The $\tilde{\nu}(\text{C=N})$ at 1697 (s) cm^{-1} for compound $[(\text{NiCl}_2)_2\text{L}_2]$ (**3**), at higher frequencies compared to the spectrum of the Schiff base ligand confirms the coordination to the metal ion (Das et al., 2014). Aromatic C=C stretching originated the band at 1635. Moreover, the prove of M-NH₂ coordination can simply be highlighted by the appearing of bands at 2939 (s)-2060 (w) cm^{-1} , following previously reported complexes (Khan et al., 2016). Two sharp bands appeared at 1458 (s), and 1342 (s) were attributed to (CH₂) deformation vibrations. Further comparison with reported structures (Khan et al., 2017; Bhattacharyya et al., 2015) proves the M-NH₂ coordination by another clear evidence, which is the appearance of $\tilde{\nu}(\text{M-N})$ vibrations in the range from 678 (w) to 270 (w) cm^{-1} .

3.5. ¹H NMR spectra

¹H NMR spectra of the complexes (**1** and **2**) were recorded at room temperature in DMSO and interpreted using TMS as an internal reference, while ¹H NMR spectrum could not be obtained for compound (**3**) due to its poor solubility in DMSO. The spectrum of the DMSO as a solvent has been zeroed. The ¹H NMR spectra of compounds (**1**) and (**2**) are given in **Figures 4** and **5**, respectively. The behavior of compounds (**1** and **2**) in solution is an important indicator of the complexation since the characteristic signals of the free ligand have suffered noticeable changes after complexation. ¹H NMR spectra of azomethine group of all complexes show signals shifted to the down field as a result of the coordination between the metal ion and the ligand. This shifting toward downfield is caused by the deshielding of protons as it is well-known; its electron density is reduced after coordination (Silva et al., 2008). The chemical shift detected for the NH₂ group in the ligand was not observed in any of the complexes (**1**) and (**2**).

3.5.1 [(NiCl₂)₂L₁] (1)

The ¹H NMR (400 MHz, DMSO, 25°C) peaks are observed at δ/ppm equal to: 8.21 (s, 2H, CH=N-), 8.00-7.49 (m, 4H, Ar), 2.50, 3.33 (8H, CH₂), 1.09 (d, 4H, NH₂). The formation of the Ni Schiff base complex [(NiCl₂)₂L₁] was further confirmed by the NMR spectrum. The ¹H NMR spectrum given in **Figure 5** showed multiple small signals in the aromatic region (7.49 - 8.00 ppm) related to the phenyl aromatic protons and the integration of these signals corresponds to the composition of the compound. The spectrum also showed the appearance of two triplets in the region between 2.50 and 3.33 ppm attributed to the methylene groups. Chemical integrations show that the singlet at (8.21 ppm) is related to azomethine.

3.5.2 [(CuCl₂)₂L₁] (2)

The ¹H NMR (400 MHz, DMSO, 25°C) peaks are observed at δ/ppm equal to: 10.14 (d, 2H, CH=N-), 8.12-7.80 (m, 4H, Ar), 2.50, 3.40 (8H, CH₂), 1.21 (t, 4H, NH₂). The ¹H NMR spectrum of the compound [(CuCl₂)₂L₁] confirmed the formation of the complex. The two signals appearing at 2.50 and 3.40 ppm are attributed to the methylene groups. Also, multiple independent signals are observed around 8.12-7.80 ppm related to aromatic protons of the phenyl group.

3.6. Mass spectra

The mass spectra were collected at 70 V cone voltage for all complexes (**1**, **2** and **3**) to reduce the dissociation of ligand. Chemical elements have different isotopes that occurred naturally. Due to isotopic abundance, for some fragments the number of peaks was higher than the calculated. The voltage and the nature of the substitution groups relatively can affect the peak intensity (Keypour et al., 2014). Some water molecules suggested being involved with complexes structures to justify mass spectra results as it is very often in such syntheses to obtain chemical structures with water molecules such as [(NiCl₂)₂L₁].7H₂O, [(CuCl₂)₂L₁].6H₂O and [(NiCl₂)₂L₂].3H₂O due to wet solvents that used in syntheses such as EtOH and MeOH.

3.6.1 [(NiCl₂)₂L₁]

In the mass spectrum of the complex [(NiCl₂)₂L₁].7H₂O, the molecular ion peak (M⁺) was observed at m/z 599.60. The spectrum shows

two other main peaks (577.60 and 551.60) (**Figure 6**), corresponding to the ions of [(NiCl₂)₂L₁].5H₂O and [(NiCl₂)₂L₁].4H₂O respectively. Later [(NiCl₂)₂L₁] complex losing NH₄Cl and NH₃ leaving ions at m/z (calc. 401.96, found 400.20, and calc. 373.92, found 381.30) respectively.

3.6.2 [(CuCl₂)₂L₁]

In the mass spectrum of [(CuCl₂)₂L₁] complex, the peak at 594.60 m/z is due to the molecular ion [(CuCl₂)₂L₁].6H₂O⁺. The peaks at 551.60 m/z is related to [(CuCl₂)₂L₁].4H₂O fragment. The intensity of these peaks is clear evidence of the stability of these fragments. The mass fragmentation pattern of the Cu(II) complex is depicted in **Figure 7**. The spectrum of [(CuCl₂)₂L₁] complex shows a peak at m/z (calc. 448.91, found 451.50), indicating the loss of NH₃, 4H₂O, and Cl. The ion at m/z 451.50 loses CH₃CH₂NH₄Cl, leaving an ion at m/z (calc. 313.97, found 313.30), which further loses two CH₃CH₃ molecules to leave an ion at m/z (calc. 231.91, found 236.30). Two intense peaks observed at low mass (57.20 and 83.10 m/z) are due to trace of unreacted starting material such as CuCl₂ fragments.

3.6.3 [(NiCl₂)₂L₂]

The mass spectrum of [(NiCl₂)₂L₂].3H₂O complex shows a molecular ion peak at m/z 591.60. Two main peaks, at 577.60 and 551.60 m/z (**Figure 8**) attributed to [(NiCl₂)₂L₂].2H₂O and [(NiCl₂)₂L₂].H₂O fragments and their intensity prove the fragments stability. The molecular ion of [(NiCl₂)₂L₂] complex loses NH₄Cl and Cl leaving an ion at m/z (calc. 460.02, found 466.50), which by its turn, loses NH₃, Cl, and HCl giving an ion at m/z (calc. 386.08, found 381.50).

3.7. Biological activity

The prepared metal complexes (**1**, **2**, **3**) and L₁ ligand were *in vitro* screened for their antibacterial activity by the disc diffusion and microdilution methods, according to the European Committee on Antimicrobial Susceptibility Testing and Clinical and Laboratory Standards Institute guidelines (Ansari et al., 2011). The biological activity of these compounds was tested against a set of human pathogenic gram-negative and gram-positive bacteria, respectively, *Enterobacter cloacae* G-, *Citrobacter* G-, *Pseudomonas* G-, *Klebsiella* G-, *Staphylococcus* G+, and *Streptococcus* G+. The minimum effective concentration of compounds was also

investigated and compared with Amoxicillin and Azithromycin as reference drugs (10 mg mL⁻¹) (**Table 1**). At low concentration, approximately, all complexes displayed destruction activity to all tested bacteria. **Table 1** shows the inhibition zone of Gr- bacteria by Schiff base metal complexes and L₁ ligand. Metal complex (**2**) inhibited *Enterobacter cloacae* G- growth significantly in comparison with the ligand L₁ using the same strain. This clearly indicates that the metal complex (**2**) has a destructive effect on bacteria activity. Among the prepared compounds, complexes **1** and **3** were found to be less active than L₁ against *Enterobacter cloacae* G- with MIC (minimum inhibitor concentration) expressed in terms of generated inhibition zones (13 and 14 mm) values, respectively, but showed higher inhibition than the reference drug (**Figure 9**). Moreover, compounds (**1-3**) and L₁ are more effective against *Enterobacter cloacae* G- than the reference drugs.

Inhibition percentages for the compounds (**1-3**) and L₁ presented in **Figure 10** exhibited an excellent effect (inhibition zones of 4, 28, 45, 48 mm) against *Citrobacter* G- compared to other isolated strains and the reference drugs. As seen in **Figure 10** and **Table 1**, inhibition activity increases significantly for the L₁ ligand after coordination with metal ions (Gündüzalp et al., 2016; Parlakgümüş et al., 2016).

Compounds (**1-3**) and L₁ ligand were shown to have promising antimicrobial activity against *Pseudomonas* G-. **Figure 11** and **Table 1** clearly showed a significant increase in the inhibition activity of L₁ Schiff base ligand after coordination to metal ions (Ansari et al., 2011). The anti-*Pseudomonas* G- inhibition activity of L₁ was (0 mm) and became (25, 30, 15 mm) for compounds **1**, **2** and **3**, respectively. Also, in comparison with reference drugs, this experiment clearly proves the significant distraction action of the metal complexes antibiotic *versus* organic antibiotic (Gündüzalp et al., 2012).

As expected *Klebsiella* G- showed a strong resistance to complexes **1-3** and L₁ (**Figure 12**) as it is well- known its resistance to a wide array of antibiotics. Complexes **1-3** and L₁ in addition to the reference drugs did not show any antibacterial activity against *Klebsiella* G- and the inhibition zones were 0 mm for all tested compounds (€Ozbek et al., 2013).

All four tested compounds showed significant activity against the selected gram-positive pathogens (*Staphylococcus* G+, and *Streptococcus* G+), the L₁ ligand activity against

Staphylococcus G+ increases significantly after coordinating to metal ions. Complex **3** showed maximum inhibiting activity against the organisms here study. Complex **2** did not show any antibacterial activity against *Streptococcus* G+, which could be related to the experimental condition. The results (**Table 2** and **Figures 13** and **14**) show that the compounds affect more the *Staphylococcus* G+ and less the *Streptococcus* G+ (Muche et al., 2017).

4. CONCLUSIONS:

The prepared complexes showed promising results in the conducted biological tests. Highly air and thermal stable Schiff base metal complexes [(NiCl₂)₂L₁], [(CuCl₂)₂L₁] and [(NiCl₂)₂L₂] with a melting point ranged between (237-248 °C) were synthesized in one step reaction pot by reacting tetradentate ligands (Eqn. 1) with different metal chlorides. Complexes (**1**, **2**, **3**) showed high chemical stability in DMSO for at least 72 h. The compounds were chemically characterized using a set of analytical techniques such as IR, ¹HNMR spectroscopy, and MS spectrometry. The biological properties of the complexes were determined according to the broth microdilution technique by employing a range of gram-negative and positive bacteria strains such as *Enterobacter cloacae* G-, *Citrobacter* G-, *Pseudomonas* G-, *Klebsiella* G-, *Staphylococcus* G+, and *Streptococcus* G+. It was established out that complexes are very effective inhibition activity ranged between (13-50 mm) against gram negative and gram positive pathogens. The comparison results revealed better antibacterial activity of the synthesized complexes than reference drugs (Amoxicillin and Azithromycin). These results encourage further studies of these species to identify the activity of the compound toward new pathogens.

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Scheme 1

Figure 1. FT-IR spectrum of $[(\text{NiCl}_2)_2\text{L}_1]$ complex.

Figure 2. FT-IR spectrum of $[(\text{CuCl}_2)_2\text{L}_1]$ complex.

Figure 3. FT-IR spectrum of $[(\text{NiCl}_2)_2\text{L}_2]$ complex.

Figure 4. ^1H MNR spectrum of $[(\text{NiCl}_2)_2\text{L}_1]$ complex.

Figure 5. ^1H MNR spectrum of $[(\text{CuCl}_2)_2\text{L}_1]$ complex.

Figure 6. Mass spectrum of $[(\text{NiCl}_2)_2\text{L}_1]$ complex.

Figure 7. Mass spectrum of $[(\text{CuCl}_2)_2\text{L}_1]$ complex.

Figure 8. Mass spectrum of $[(\text{NiCl}_2)_2\text{L}_2]$ complex.

Figure 9. Inhibition zones for *Enterobacter* generated by L_1 , 1, 2 and 3 are 13, 15, 20 and 13 mm.

Figure 10. Shows inhibition zone of *Citrobacter* by the effects of (L_1 , 1, 2 and 3) was (4, 28, 45 and 48 mm) respectively.

Figure 11. Shows inhibition zone of *Pseudomonas* by the effects of (L_1 , 1, 2 and 3) were (0, 25, 30 and 15 mm) respectively.

Figure 12. Showing inhibition zone of *Klebsiella* by the effects of (L_1 , 1, 2 and 3) was (0, 0, 0 and 0 mm) respectively.

Figure 13. Showing inhibition zone of *Staphylococcus* by the effect of (L_1 , **1**, **2** and **3**) were (38 mm, 40 mm, 42 mm and 50 mm).

Figure 14. Showing inhibition zone of *Streptococcus* by the effect of (L_1 , **1**, **2** and **3**) was (22, 0, 0 and 42mm).

Table 1. The effects of chemical compounds (inhibition zone) on Gr-bacteria.

Test No.	Types of isolates	Antibiotics		Chemical compounds			
		Amc*	Azm**	L ₁	1	2	3
1	<i>Enterobacter</i> G-	0	0	15mm	13mm	20mm	14mm
2	<i>Citrobacter</i> G-	12mm	10mm	4mm	28mm	45mm	48mm
3	<i>Pseudomonas</i> G-	0	0	0mm	25mm	30mm	15mm
4	<i>Klebsiella</i> G-	0	0	0mm	0mm	0mm	0mm

* Amc = Amoxicillin **Azm = Azithromycin

Table 2. The effects of chemical compounds (zone inhibition) on Gr+ bacteria.

Test No.	Types of isolates	Antibiotics		Chemical compounds			
		Amc*	Azm**	L ₁	1	2	3
1	<i>Staphylococcus</i> G+	0 mm	28 mm	38 mm	40 mm	42 mm	50 mm
2	<i>Streptococcus</i> G+	0 mm	0 mm	0 mm	22 mm	0 mm	42 mm

* Amc = Amoxicillin **Azm = Azithromycin