



Synthesis and characterization of some metal chelates derived from *N,N'*-bis[(ethylcarbamoithiyl)amino] propanediamide (EAP; L) by tribochemical reaction and its biological application

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Abstract: New metal chelates derived from EAP were synthesized in the solid state and in the absence of solvents by tribochemical reaction. The isolated ligands and metal chelates are characterized by chemical, spectral and magnetic methods. IR data suggest that the ligand (EAP) coordinates to two and/or three metal ions *via* the enolized CO, SH and the azomethine (C=N) groups with displacement of hydrogen atoms from the first two groups. The geometries of the isolated solid chelates were illustrated by spectral and magnetic data and confirmed by material studio program. DFT calculations suggest the most suitable geometry as well as the stability of the complexes. Some chelates were tested against anti-oxidant.

Keywords: *N,N'*-bis[(ethylcarbamoithiyl)amino]propanediamide, tribochemical, green chemistry

1. Introduction

There is an accretion in examining the synthesis of metal chelates using tribochemical reaction as well as the characterization of the isolated compounds using the various methods including chemical, spectral (IR, UV-Vis., ¹H-NMR, ¹³C-NMR, mass) and magnetic measurements. Also, tribochemical reaction as a type of green chemistry is considered as a brilliant method for synthesis of solid complexes in absence of solvent. The main goal of this method is considered as efficient and economical and declining the pollution. Several Cu²⁺ chelates involving simple ligands have displayed diverse pharmacological activities i.e., copper complexes exhibit anti-inflammatory and cytostatic activities [1]. On the basis of our earlier work [2-9] on the synthesis of complexes by tribochemical reactions as an example of green chemistry [10-11], we embarking on this technique to synthesis and characterizing of new metal complexes derived from *N,N'*-bis[(ethylcarbamoithiyl)amino] propanediamide with some metal ions (Cu²⁺, Co²⁺, Ni²⁺ and Zn²⁺). The detached complexes

were distinctive using different chemical, physical, spectral, magnetic measurements. Material studio program was used for calculating HUMO, LUMO, and DFT parameters on the atoms to confirm the geometry of the isolated compounds. In continuation of our earlier work as well as the work done by my coworker [12-13] concerning synthesis of binary complexes using tribochemical reaction we embarked on this type of reaction to investigate the binary and/or ternary complexes to study the biological significance [14-15]. Biological activities of EAP and some metal chelates were examined.

2. Experimental

2.1. Materials

Diethyl malonate and hydrazine were obtained from Aldrich. The metal chlorides [(CuCl₂.2H₂O, CoCl₂.6H₂O, NiCl₂.6H₂O) and Zn(Ac)₂.2H₂O] were of BDH quality and used in the preparation of the solid metal chelates. The chelates were synthesized by tribochemical reaction and in absence of solvent.

2.2. Instrumentation

The contents of the chelates were carried out at the Microanalytical Unit at Cairo University, Egypt. Metal contents of the chelates have been determined by volumetric methods [16]. IR spectra ($4000\text{--}400\text{ cm}^{-1}$) as KBr discs (in case of the ligand) and/or Nujol mull (metal chelates) were recorded on a Mattson 5000 FTIR spectrophotometer. Electronic spectra were recorded on an ATI Unicomp UV-Vis spectrophotometer version software V3-20. Magnetic measurements were carried out at room temperature (298 K) on a Sherwood magnetic balance. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra of the free ligand in $d_6\text{-DMSO}$ were carried at Mansoura University. The mass spectrum of the ligand was identified using Thermo Scientific ISQ Mass spectrometer model at National center of Research, Dokki, Cairo, Egypt.

2.3. Synthesis of malonic acid dihydrazide

Malonic acid dihydrazide was prepared by adding 9.45 ml (3 mole) of hydrazine hydrate drop by drop and with continuous stirring using magnetic stirrer to 15.2 ml (1mole) of diethyl malonate in the presence of 20 ml of absolute ethanol for 45 mins. The product was filtered, washed continuously with EtOH and diethyl ether, respectively. The isolated white product was crystallized from ethanol; M.p.: $142\text{--}146\text{ }^\circ\text{C}$.

2.4. Synthesis of N, N`-bis[(ethylcarbamothioyl)amino]propanediamide (EAP; L)

EAP was synthesized by dissolving malonic acid dihydrazide (2g; 1mol) in MeOH and then the mixture was refluxed on a hot plate till all the hydrazide was completely dissolved. Ethyl isothiocyanate (3.6 ml, 3mol) was then added drop by drop to the above solution and the reactants were refluxed for 3h on a hot plate. A white precipitate was formed on cooling and scratching in the presence of diethyl ether. The product was filtered off, washed several times using MeOH and diethyl ether M.p.: $198\text{--}200\text{ }^\circ\text{C}$.

2.5. Synthesis of the Cu^{2+} , Co^{2+} , Ni^{2+} and Zn^{2+} chelates using tribochemical reaction

The metal chelates derived from EAP with some metal chlorides (Cu^{2+} , Co^{2+} , Ni^{2+}) and

$\text{Zn}(\text{Ac})_2$ were prepared by grinding one mole of the ligand with the two moles of the metal chlorides and/or $\text{Zn}(\text{Ac})_2$ in agate mortar for 1 h till the color was changed. The grinding process was continued till the reactants became very fine powder. The products were dried in an oven for 1 h at $60\text{ }^\circ\text{C}$. After that the products were transferred in a beaker followed by adding mixture of ethanol (30 ml) and methanol (15 ml) and then the mixture was boiled for 10 mins. The product was filtered off, then washed with EtOH and ether and finally kept in a desiccator over anhydrous CaCl_2 . The results of elemental analyses with some physical data are recorded in Table 1. In all synthesis of the metal chelates (Cu^{2+} , Co^{2+} , Ni^{2+}) the pH of the reaction mixture falls from 6 to 2 after boiling indicating the liberation of H^+ ion in the form of HCl during complex formation (strong acid). On the other hand, the pH of the solution in case of $\text{Zn}(\text{Ac})_2$ falls from 6 to due to 5.5 due to the liberation of acetic acid (weak acid).

2.6. Biological studies

2.6.1. Antioxidant scavenging assays

2.6.1.1. DPPH free radicals assay

The scavenging activity of malonic acid dihydrazide and EAP, L was measured using DPPH (2,3-diphenyl-1-picrylhydrazyl) free radical according to Nazier et al [17]. All the samples were tested in triplicates and the results are presented as the mean \pm . % of DPPH scavenging activity was calculated using the following equation:

$$\% \text{ Scavenging activity} = \left\{ \frac{\text{Blank sample absorbance} - \text{Sample absorbance}}{\text{Blank sample absorbance}} \right\} \times 100$$

2.6.1.2. ABTS free radical assay

ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) free radicals scavenging activity of each of the two compounds was determined according to Nazier et al [18]. % of ABTS scavenging activity was calculated using the same equation used for DPPH.

2.6.1.3. Antimicrobial assay against various bacteria

The pathogenic bacteria are represented by *Staphylococcus aureus* (Sa), *Escherichia coli* (EC), *Pseudomonas aeruginosa* (PA) and *Klebsiella pneumoniae* (Kp). Antimicrobial activity of the various compounds was detected

by the agar well diffusion method Holder and Boyce [19,20].

3. Results and Discussion

The chemical analyses with some physical properties of the metal chelates are listed in Table 1. The results indicate that the chelates have high melting points above $>300\text{ }^{\circ}\text{C}$, except the copper(II) chelate which is decomposed with charring at $290\text{-}292\text{ }^{\circ}\text{C}$. The high melting

Table 1: Elemental analyses of the solid chelates and some physical data

No	Chemical structure	Color	$\mu_{\text{eff.}}$ BM	% Found (Calcd.)				
				C	H	N	M	S
1	$[\text{Co}_3(\text{L}_3\text{-}6\text{H})(\text{H}_2\text{O})_8]\cdot\text{H}_2\text{O}$	Brownish-black	3.8	25.9 (26.3)	5.31 (5.2)	20.1(20.3)	15.36(16.9)	14.12 (14.5)
2	$[\text{Ni}_3(\text{L}_3\text{-}6\text{H})]$	Dirty green	Diam	29.77(30.8)	4.44 (4.55)	23.14(23.9)	17.6 (16.9)	16.16 (16.5)
3	$[\text{Cu}_3(\text{L}_3\text{-}6\text{H})]\cdot 1\frac{1}{2}\text{H}_2\text{O}$	Chocolate brown	1.93	28.67(28.94)	4.5 (3.9)	22.29 (21.8)	17.01 (16.9)	16.7 (16.3)
4	$[\text{Zn}_3(\text{L}\text{-}2\text{H})(\text{H}_2\text{O})_2(\text{Ac})_2]$	Orange	Diam	25.3 (26.8)	3.9 (4.11)	18.17 (16.2)	10.39 (8.9)	31.8 (32.1)

3.1. IR, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ and mass spectra

The IR spectrum of EAP in Nujol mull exhibits three peaks at 3325, 3278 and 3250 cm^{-1} assigned to $\nu(\text{NH})$ (NH-CO), $\nu(\text{NH})$ (NH-CS) and νNH (NH-Et), respectively. Also, the spectrum illustrates four bands at 1695, 1554, 1050 and 803 cm^{-1} . The former bands are attributed to the carbonyl oxygen (C=O) and (CONH), respectively, while the last two bands are assigned to the $\nu(\text{C}=\text{S})$ group. The $^1\text{H-NMR}$ spectrum of the ligand in $d_6\text{-DMSO}$ (Fig. 1) shows three signals at 10.02, 9.345 and 7.92 ppm downfield of TMS assigned to the protons of NH (NH-CO), NH (NH-CS) and NH (NH-Et), respectively. Also the signals observed at 3.42, 3.34 and 1.06 ppm are attributed to the protons of CH_2 (attached to the carbonyl), CH_2 (ethyl) and CH_2 (methyl), respectively. The former three NH signals are disappeared upon adding D_2O . Moreover, the $^{13}\text{C-NMR}$ spectrum of the ligand in $d_6\text{-DMSO}$ (Fig. 2) shows five signals at 180.97, 166.598, 40.277, 38.546 and 14.352 ppm assigned to the carbons of CH_2 (attached to the carbonyl), CH_2 (ethyl) and CH_2 (methyl), respectively. The mass spectrum of the ligand with the general formula, $\text{C}_9\text{H}_{18}\text{N}_6\text{O}_2\text{S}_2$, shows $\text{M}+1$ at 307 which coincide with the theoretical value (306.4).

The comparison of the IR spectra in Nujol of the ligand with the complexes we observed the

points suggest that the bonds between the ligand and the metal ions are very strong and are covalent in nature. The chelates are stable against air and light for more than one year. Also, all the chelates are insoluble in polar and all non-polar solvents suggesting the polymeric nature as well as the presence of strong covalent nature between L and M.

presence of a broad weak band at 3500 cm^{-1} in all metal chelates except the Ni chelate attributed to the presence of H_2O (hydration, coordination or both) which is in accordance with the results of metal analyses. Also, the IR spectra exhibit three bands at 3252, 3210 and 3160 cm^{-1} in all chelates except the Zn complex which shows only one band at 3240 cm^{-1} suggesting the presence of three different NH groups in the former chelates while only one in the latter Zn chelate indicating the disappearance of two NH groups due to the removal of the protons through the carbonyl (C=O) and the thioketo (C=S) groups. In all chelates the carbonyl group disappears either by enolization of this group during coordination. This process proceeds either by removal of the proton of the enolized OH group or the participation without removal of hydrogen atom. In both cases the spectra show new band in the $1621\text{-}1562\text{ cm}^{-1}$ due to the formation of new azomethine group (C=N).

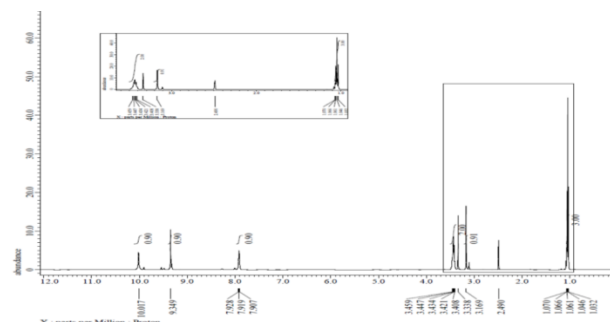


Fig 1

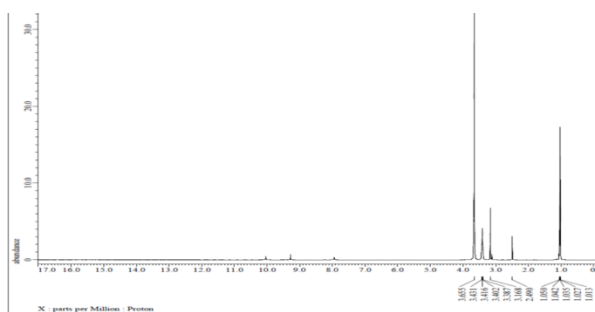


Fig 2

The thioketo group (C=S) in all chelates are obscured either to the participation of this group in bonding or the change of this group to SH with displacement of protons. Finally, the IR spectra of the chelates expose three new peaks in the 635-570, 560-520 and 475-470 cm^{-1} regions assigned to $\nu(\text{M-S})$, $\nu(\text{M-O})$ and $\nu(\text{M-N})$, respectively, [21]. All this observation suggests the participation of these groups in bonding.

3.4. Electronic spectra and magnetic moments

The electronic spectra of the metal chelate (Cu^{2+} , Co^{2+} , Ni^{2+} , Zn^{2+}) in Nujol were carried out in Nujol mull. The electronic spectrum of the brownish black Co(II) complex shows two bands at 20619 and 16770 cm^{-1} assigned to ${}^4\text{T}_{1g} \rightarrow {}^4\text{T}_{2g}(\text{F})$ and ${}^4\text{T}_{1g}(\text{P})$ and ${}^4\text{T}_{1g} \rightarrow {}^4\text{A}_{2g}$ transitions in an octahedral geometry around the Co(II) ions [22]. Also, the values of magnetic (3.8 BM) confirm the existence of octahedral geometry around the Co(II) ions [22].

The electronic spectrum of the chocolate brown copper(II) complex appears two bands at 14903 and 20578 cm^{-1} . The first band is attributed to ${}^2\text{E}_g \rightarrow {}^2\text{T}_{2g}$ transition in a distorted-octahedral geometry around the Cu(II) ion while the second band is mainly due to L \rightarrow M charge-transfer. The magnetic moment value was found to be 1.93 BM.

The electronic spectrum of the deep green Ni(II) chelate shows two bands at 19157 and 25381 cm^{-1} . The first band is attributed to transitions while the band is attributed to charge-transfer. The diamagnetic behavior of the Ni(II) ion is taken as an evidence for the existence of square-planar around the Ni(II) ion. The electronic spectra of the orange Zn(II) chelate shows two bands at 19531 and 25252 cm^{-1} attributable to charge-transfer. The diamagnetic behavior of the Zn(II) ion is taken

as an evidence for the existence d^{10} -configuration around the Zn(II) ion.

3.5. Molecular modeling and DFT calculation studies

The study of molecular modeling was carried out to get insight into the geometries of metal chelates. Also, the computational calculations were performed to illustrate the bonding modes within the isolated chelates, theoretically using DMOL³ program in materials studio package program by density functional theory (DFT). The most important orbitals in the compound are the frontier molecular orbitals, HOMO and LUMO. The HOMO energy shows the ability of electron donating, while LUMO energy indicates the capacity of electron acceptance for the compound. The HOMO used to describe the chemical reactivity and kinetic stability of the molecule. Moreover, it is an important parameter to calculate the molecular electrical transport properties. It is easier for electrons to move from the ground to the excited state with such a small energy gap. So a molecule contains small energy gap means a high chemical reactivity, low chemical stability and named as soft molecule. The energy gap (ΔE) of HOMO and LUMO for both EAP and its chelates are shown in Table 2.

The energies of frontier molecular orbitals (E_{HOMO} , E_{LUMO} and band gap energy) result from DFT calculations indicate the reactivity, molecular stability and site selectivity beside to the ability of donating. Koopman's theorem used the closed shell components as electronegativity (χ), chemical potential (μ), global hardness (η), global softness (S) and global electrophilicity index (ω) [23-24] are calculated using equations (1-6) shown in Table 2, HOMO and LUMO orbitals of EAP and its metal chelates complexes are illustrated in Fig. 3. The ability of donating molecule can predict over the value of HOMO energy in which the high energy values indicating the tendency of donating electrons. The energy gap between HOMO- LUMO is concerned to hardness (η) and softness (S) related to reactivity where high E_{Gap} meaning high stability and smaller hardness more reactive molecule. When the values of hardness are small indicating high reactivity and suggest that

the molecule with a small HOMO- LUMO gap is more reactive and is a softer molecule. The soft molecules undergo changes in electron density more easily and are more reactive than hard molecules. The hardness value for complex is near or lowers than the ligand and hence its reactivity increases.

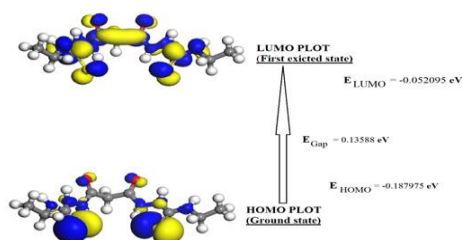


Fig. 3. HOMO and LUMO of EAP (L)

Frontier molecular orbital analysis

$$\chi = -\frac{1}{2} (E_{HOMO} + E_{LUMO}) \quad (1)$$

$$\mu = -\chi = \frac{1}{2} (E_{HOMO} + E_{LUMO}) \quad (2)$$

$$\eta = \frac{1}{2} (E_{LUMO} - E_{HOMO}) \quad (3)$$

$$S = \frac{1}{2} \eta \quad (4)$$

$$\omega = \frac{\mu^2}{2\eta} \quad (5)$$

$$\sigma = \frac{1}{\eta} \quad (6)$$

The results of frontier molecular orbital analysis for all complexes are shown in

Table 2.

Table 2. Calculated total energy, E_{HOMO} , E_{LUMO} , E_{gap} ($E_H - E_L$), hardness (η), softness (σ) and dipole moment (μ) for the isolated chelates

Compound	E_{HOMO}	E_{LUMO}	E_{Gap}	E_H+E_L	$1/2E_H+E_L$	χ	μ
EAP, L	-0.1880	-0.0520	-0.1358	-0.2401	-0.1200	0.1200	-0.1200
$[Co_3(L3-6H)(H_2O)_8] \cdot H_2O$	-0.1376	-0.0803	-0.0572	-0.2179	-0.1089	0.1089	-0.1089
$Ni_3(L3-6H)$	-0.1466	-0.0801	-0.0665	-0.2267	-0.1133	0.1133	-0.1133
$Cu_3(L3-6H)1\frac{1}{2}H_2O$	-0.1559	-0.1455	-0.0104	-0.3014	-0.1507	0.1507	-0.1507
$[Zn_3(L-2H)(Ac)_2(H_2O)_2]$	-0.1896	-0.0766	-0.1130	-0.2662	-0.1331	0.1331	-0.1331

Compound	E_L-E_H	$1/2E_L-E_H$	η	S	μ^2	2η	ω	σ
EAP, L	0.1359	0.0679	0.0679	0.0340	0.0144	0.1359	0.1060	14.719
$[Co_3(L3-6H)(H_2O)_8] \cdot H_2O$	0.0572	0.0286	0.0286	0.0143	0.0119	0.0572	0.2073	34.944
$Ni_3(L3-6H)$	0.0666	0.0333	0.0333	0.0166	0.0128	0.0665	0.1932	30.071
$Cu_3(L3-6H)1\frac{1}{2}H_2O$	0.0104	0.0052	0.0052	0.0026	0.0227	0.0103	2.1892	192.808
$[Zn_3(L-2H)(Ac)_2(H_2O)_2]$	0.1130	0.0565	0.0565	0.0283	0.0177	0.1130	0.1568	1798.

Biological studies

The compounds 1 and EAP, L and malonic acid dihydrazide were tested for their antioxidant activity using DPPH and ABTS methods. The results in Figs 4 and 5 indicated that these compounds expressed antioxidant activities, which were recorded to be concentration-dependent. The efficiency of the various tested compounds as antioxidants varied depending on the structure of the compounds.

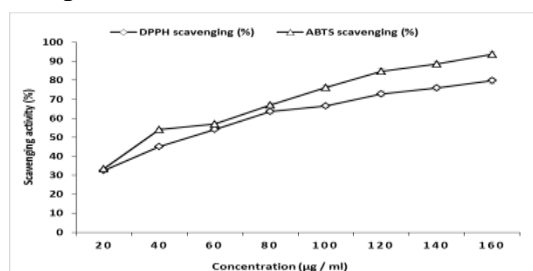


Fig. 4. Scavenging activity of malonic acid dihydrazides

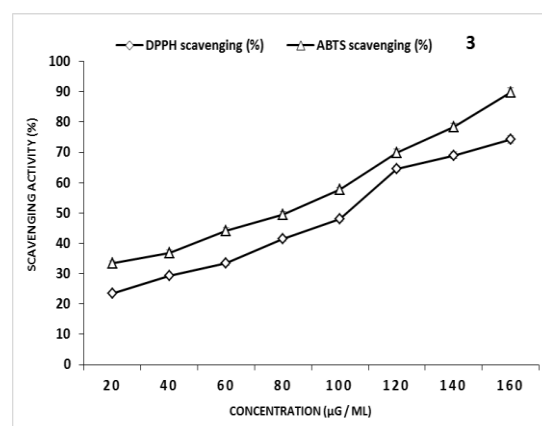


Fig. 5. Scavenging activity of EAP (L)

The results in the figures of $[Zn_3(L-2H)(Ac)_2(H_2O)_2]$, $[Ni_3(L3-6H)]$ and $[Co_3(L3-6H)(H_2O)_8] \cdot H_2O$ revealed that the various tested compounds which contain heavy metals inhibited the growth of the various bacteria and this was apparent from the measured inhibition zone produced by each compound.

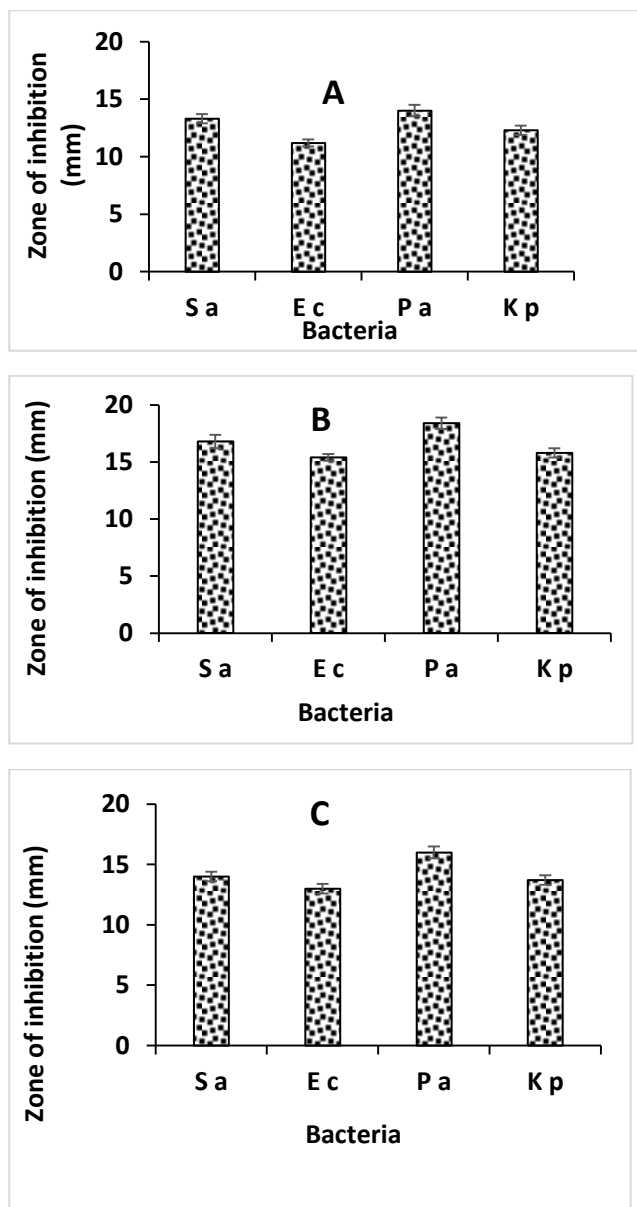


Fig. 6. Effect of compounds $[Zn_3(L-2H)(Ac)_2(H_2O)_2]$ (A), $Ni_3(L3-6H)$ (B) and $[Co_3(L3-6H)(H_2O)_8]H_2O$ (C) on the bacterial growth

According to the World Health Organization (WHO), cobalt (Co), nickel (Ni) and zinc (Zn) are among the most dangerous metals as mentioned by Pandiyan *et al.* [25]. It has been reported that heavy metals inhibited the bacterial growth, denatured the nucleic acids and proteins, and disrupted the enzymes and caused damage to the membrane as described by Syed *et al.* [26]. Disrupting or inhibiting the bacterial cells can be caused by cell damage/injury, which leads to intracellular content leakage/flow, thereby preventing the production of additional polymeric substances and others causing pathogenicity as reported by Bouhdid *et al.* [27].

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