# ANTIMICROBIAL AND DNA CLEAVAGE STUDIES OF TRINUCLEAR Cu(II), Ni(II) AND Mn(II) SCHIFF BASE COMPLEXES

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**Abstract**

Complexes of Cu(II), Ni(II) and Mn(II) containing a novel homotrinuclear Schiff base metal complexes were synthesized by using 2,3-pentanedione, p-phenylenediamine and 2-aminobenzaldehyde. The complexes of the type [LM$_3$Cl$_6$]. The characterization and nature of bonding of the trinuclear complexes have been deduced from elemental analysis, molar conductivity, FT-IR, UV-visible, magnetic and EPR studies. The pUC18 DNA cleavage study was monitored by gel electrophoresis method. The results suggest that trinuclear Cu(II), Ni(II) and Mn(II) complexes cleaves pUC18 DNA in presence of the oxidant H$_2$O$_2$. The in vitro antimicrobial activity of the synthesized complexes have been tested against the Gram negative bacteria like *Klebsiella pneumoniae, Escherichia coli* and Gram positive bacteria *Staphylococcus aureus*. The trinuclear Schiff base complexes were found to be more active when compared to Standard Streptomycin.

**Keywords:** Schiff base, Trinuclear, pUC18 DNA cleavage, Antimicrobial and 2-aminobenzaldehyde,

**Introduction**

The polynuclear copper, nickel and manganese complexes containing nitrogen donor atoms are of considerable interest in inorganic and bioinorganic chemistry [1]. These complexes have numerous applications, such as antibacterial agents, antivirus agents, fungicide agents and other biological properties [2]. The metal complexes as pharmaceuticals have gained access over traditional organic dominated drugs, due to their potential use as regulators of gene expression and tools of molecular biology [3]. The major

intracellular target of anticancer metallodrugs is DNA, therefore metal complexes that can bind to specific nucleobases of DNA are of interest in the development of antitumor agents. The binding and cleavage of transition metal complexes with DNA has received a great deal of attention during past decade [4]. The interaction of transition metal complexes with DNA has been extensively studied in the development of new tools for nanotechnology [5,6]. Metal ion coordination to nucleic acids is not only required for charge neutralization, it is also essential for the biological function of nucleic acids, in purifying nucleic acids [7], in probing the structure and biochemistry of nucleic acids.

Herein we have reported the synthesis and characterization of three novel homotrinuclear Cu(II), Ni(II) and Mn(II) complexes by using 2,3-pentanedione, p-phenylenediamine and 2-aminobenzaldehyde. We have also investigated the antimicrobial and cleavage ability of these complexes.

**Experimental**

**Materials and physical measurements**

CuCl$_2$.2H$_2$O, NiCl$_2$.6H$_2$O, MnCl$_2$.4H$_2$O, 2,3-pentanedione and p-phenylenediamine were purchased from Loba chemicals. 2-aminobenzaldehyde was obtained from Aldrich. Ethanol, DMSO and DMF were used as solvents. The solvents and reagents with analytical grade were obtained commercially and used without further purification. The Elemental analysis was performed using Carlo-Eraba 1106 instrument. Molar conductances of the complexes in DMF solution were measured with ELICO CM 185 conductivity Bridge. The Infrared spectra were recorded on the Perkin Elmer FT-IR-8300 model spectrometer using KBr disc. Electronic absorption spectra in the UV-Visible range was recorded on Perkin Elmer Lambda-25 between 200-800 nm by using DMF as the solvent. ESR spectra were recorded on a Varian JEOL-JES-TE100 ESR spectrophotometer at X-band microwave frequencies for powdered samples at room temperature. Magnetic susceptibility data were collected on powdered sample of the compounds at room temperature with PAR155 vibrating sample magnetometer.

**Synthesis of mononuclear Schiff base metal complexes.**

The ethanolic solution (20 ml) of p-phenylenediamine (2 mmol) and ethanolic solution (20 ml) of 2,3-pentanedione (1 mmol) were mixed slowly with constant stirring. This mixture was refluxed at 90°C for 2 hours. Metal chloride (0.5 mmol) in ethanol (20 ml) were then added to the resulting solution and again refluxed for one hour as shown in figure 1. When it was cooled to room temperature a precipitate was obtained. It was filtered, washed several times with ethanol and dried over fused $CaCl_2$.
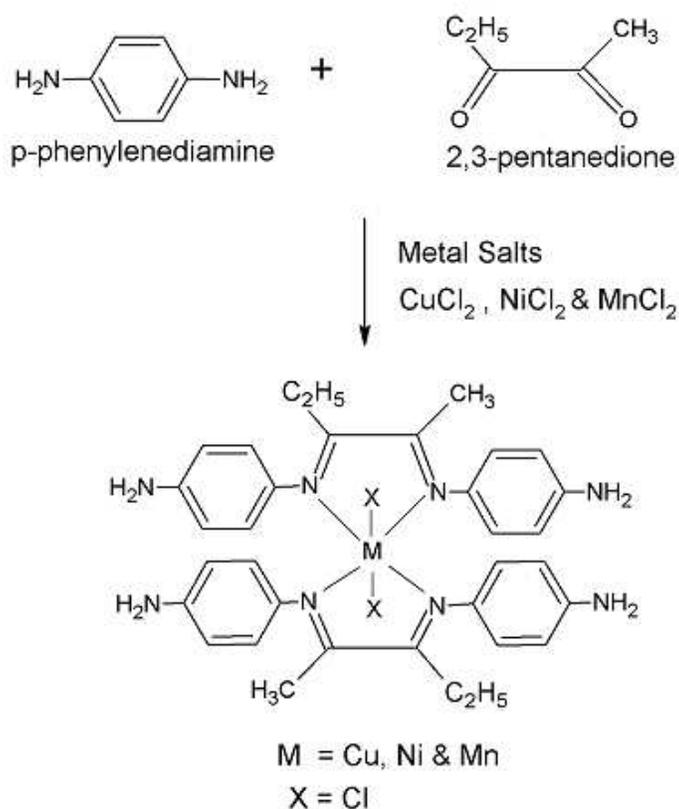


**Figure 1. Synthesis of mononuclear Schiff base metal complexes**

**Synthesis of trinuclear Schiff base metal complexes**

An ethanolic solution (20 ml) of bis-(p-phenylenediamine, 2,3-pentanedione) metal(II) complexes (1 mmol) and 2-aminobenzaldehyde (4 mmol) were refluxed for 2 hours and metal chloride (2 mmol) in ethanol(20 ml) were then added drop wise to the resulting solution. The contents were again refluxed for one

hour as shown in figure 2. It was cooled to room temperature. The precipitate was obtained. It was filtered and
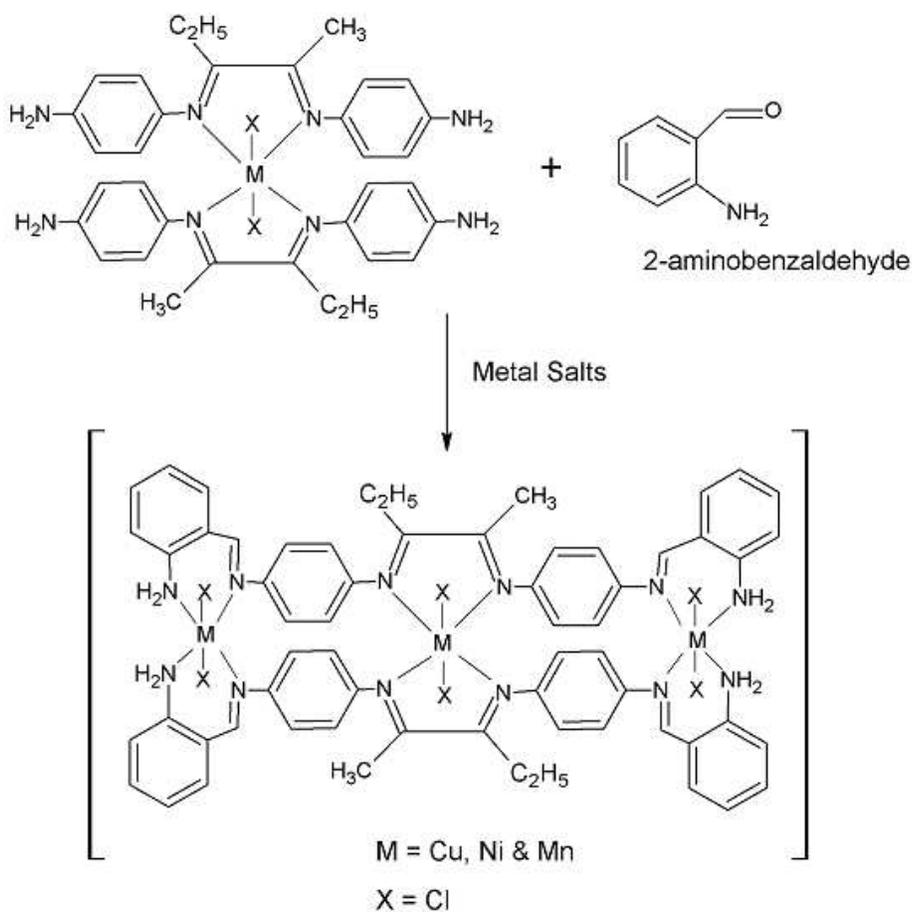
dried over fused $CaCl_2$.



**Figure 2. Synthesis of trinuclear Schiff base metal complexes**

**Antimicrobial activity**

The antibacterial activity of the complexes of Cu(II), Ni(II) and Mn(II) were checked by the disc

diffusion technique [8]. This was done on Gram negative bacteria like *Klebsiella pneumoniae, Escherichia*

*coli and* Gram positive bacteria *Staphylococcus aureus* at 37°C. The disc of Whatmann no.4 filter paper

having the diameter 8.00 mm were soaked in the solution of compounds in DMSO (1.0 mg $cm^{-1}$). After drying

it was placed on nutrient agar plates. The inhibition areas were observed after 36 hours. DMSO was used as a

control and Streptomycin as a standard.

**Gel electrophoresis**

The cleavage of pUC18 DNA was determined by agarose gel electrophoresis [9]. The gel electrophoresis experiments were performed by incubation of the samples containing 40 μM pUC18 DNA, 50 μM metal complexes and 50 μM $H_2O_2$ in Tris-HCl buffer (pH 7.2) at 37°C for 2 h. After incubation, the samples were electrophoresed for 2 h at 50 V on 1% agarose gel using Tris-acetic acid-EDTA buffer (pH 7.2). The gel was then stained using 1 μg $cm^{-3}$ ethidium bromide (EB) and photographed under ultraviolet light at 360nm. All the experiments were performed at room temperature.

**Results and discussion**

**Elemental composition**

The results of elemental analyses were in good agreement with those required by the proposed formulae given in Table 1.

**Table 1.** Analytical and physical data of the trinuclear Schiff base metal complexes.

| Complexes | Calculated (Found) | | | | $\mu_{eff}$ (B.M) | $\Lambda_M$ (Ohm$^{-1}$ cm$^2$ mol$^{-1}$) |
| | C | H | N | Metal | | |
| --- | --- | --- | --- | --- | --- | --- |
| [Cu$_3$C$_{62}$H$_{60}$N$_{12}$Cl$_6$] | 54.14 (54.10) | 04.36 (04.32) | 12.22 (12.20) | 13.97 (13.93) | 1.56 | 12.26 |
| [Ni$_3$ C$_{62}$H$_{60}$N$_{12}$Cl$_6$] | 54.86 (54.72) | 04.42 (04.39) | 12.38 (12.34) | 12.83 (12.85) | 2.64 | 13.42 |
| [Mn$_3$ C$_{62}$H$_{60}$N$_{12}$Cl$_6$] | 55.23 (55.28) | 04.45 (04.42) | 12.47 (12.52) | 12.24 (12.23) | 5.60 | 15.74 |

**Conductivity studies**

The trinuclear complexes are dissolved in DMF and the molar conductivities of $10^{-3}$ M of their solutions at 30°C were measured. Table 1 shows the molar conductance values of the complexes. It is concluded from the results that the Cu(II), Ni(II) and Mn(II) complexes have a molar conductivity in the range of 12.26 – 15.74 Ohm$^{-1}$ cm$^2$ mol$^{-1}$ (Table 1) which indicates the non-ionic nature of these complexes

and they are considered as non-electrolytes, and also indicating that the chloride ions are present inside the coordination sphere. [10, 11].

**Infrared spectra**

The IR spectra of the complexes exhibit an intense band in the 1612-1618 cm$^{-1}$ region may be assigned to azomethine $\upsilon$(C=N) vibrations [12]. The IR spectra of mononuclear complexes the intense band in the ~3400 cm$^{-1}$ region which may be assigned to $\upsilon$(N-H) of the free primary amine group. In Trinuclear complexes the band appears in the region 3320-3333 cm$^{-1}$ its due to the mononuclear complexes of the terminal nitrogen atom of the p-phenylenediamine participate in bond formation with 2-aminobenzaldehyde and coordinate to the metal ions and forms the stable trinuclear Schiff base complexes, and also the primary amine group of the 2-aminobenzaldehyde coordinated to the metal ion. Which has been further confirmed by the appearance of bands in the 450 cm$^{-1}$ region in all the complexes corresponding to $\upsilon$(M-N) vibrations [13] and the appearance of bands in the region of 360-380 were due to $\upsilon$(M-Cl) vibrations[14]

**Table 2.** Infrared spectral data for trinuclear Schiff base metal complexes.

| Complexes | (C=N) (cm$^{-1}$) | NH$_2$ (cm$^{-1}$) | (M-N) (cm$^{-1}$) | (M-Cl) (cm$^{-1}$) |
|---|---|---|---|---|
| [Cu$_3$C$_{62}$H$_{60}$N$_{12}$Cl$_6$] | 1612 | 3333 | 453 | 360 |
| [Ni$_3$ C$_{62}$H$_{60}$N$_{12}$Cl$_6$] | 1616 | 3320 | 457 | 364 |
| [Mn$_3$ C$_{62}$H$_{60}$N$_{12}$Cl$_6$] | 1618 | 3325 | 458 | 375 |

**Electronic spectral studies**

Electronic spectra of all the complexes were recorded in DMF medium. The data are summarized in Table 3 The peaks obtained in the range of 278-285 nm was assigned to the intra ligand charge transfer transition ($\pi \rightarrow \pi^*$). An intense peak in the range of 380-410 nm was due to ligand-to-metal charge transfer

transition. Electronic spectra of the mononuclear nickel(II) complex shows two d-d transitions. The observed transitions are assigned to $^3A_{2g}\rightarrow^3T_{2g}(F)$ (710 nm), $^3A_{2g}\rightarrow^3T_{1g}(F)$ (565 nm) suggesting a distorted octahedral arrangement around the nickel(II) [15]. The mononuclear copper(II) complex shows a broad absorption peak at 545 nm and arises due to the d-d transition $^2E_g\rightarrow^2T_{2g}$ (545 nm) of Cu(II) ion suggest that the copper ion exhibits a distorted octahedral geometry [16]. The mononuclear Mn(II) complex displays four weak absorption bands(530 nm – 750 nm) characteristics of the octahedral geometry [17]. In this complex, these bands may be assigned to the transitions $^6A_{1g}\rightarrow^4T_{1g}(4G)$, $^6A_{1g}\rightarrow^4E_g(4G)$, $^6A_{1g}\rightarrow^4E_g(4D)$, $^6A_{1g}\rightarrow^4T_{1g}(4P)$. The homotrinuclear copper(II), nickel(II) and Mn(II) complexes show an additional band in the region 510-700 nm which may be expected to arise from the terminal Ni(II), Cu(II) and Mn(II) ions.

**Table 3.** UV–Visible data of trinuclear Schiff base metal complexes.

| Complexes | Absorption (nm) | | | |
|---|---|---|---|---|
| | $\pi\rightarrow\pi^*$ | $n\rightarrow\pi^*$ | L→M CT | d-d |
| $[Cu_3C_{62}H_{60}N_{12}Cl_6]$ | 278 | 324 | 410 | 545, 658 |
| $[Ni_3 C_{62}H_{60}N_{12}Cl_6]$ | 280 | 325 | 409 | 565, 657, 710 |
| $[Mn_3 C_{62}H_{60}N_{12}Cl_6]$ | 285 | 310 | 380 | 530, 642, 678, 728 |

**Magnetic properties**

The magnetic moments of the solid state complexes were measured at room temperature. The measured magnetic moments of mononuclear copper(II) complex 1.66B.M. Magnetic susceptibility measurements shows that these complexes are paramagnetic, which corresponds to the +2 oxidation state of copper(II) complexes. The magnetic moment of trinuclear Cu(II) complex 1.56 B.M. It can be observed that the magnetic moment values of trinuclear copper(II) complexes are slightly lower than the mononuclear copper(II) complexes. The strong antiferromagnetic coupling that was found for trinuclear copper(II)

complexes were explained as the good superexchange properties [18]. Similarly, the magnetic moment of

trinuclear Ni(II) complex has 2.64 B.M. indicating a octahedral geometry [19], and the magnetic moment of

trinuclear Mn(II) complex 5.60 B.M. indicating a octahedral geometry [20].

**EPR spectra**

The EPR spectra of the trinuclear Schiff base complexes recorded on powder samples with room

temperature, on X-band at frequency 9.3 GHz under the magnetic field strength 4000G. This band is well

defined for complexes at "g" values of around 2. At room temperature the spectra of complexes are

anisotropic, the fitting results of all the three complexes are the following $g_{iso}$ = 2.1863, 2.1321 and 2.0047

[21]. The spectra of the homotrinuclear complexes, the intensity pattern of the lines is complex. Also, the

resonance line at the higher magnetic field shows an increased broadness than the monomer precursors and

this indicates the presence of copper, nickel and manganese ions was found to be more than one environment

[22]. This feature can be assigned to the complex having trinuclear pattern.

**Cleavage of Plasmid pUC18 DNA**

DNA cleavage is controlled by relaxation of supercoiled circular conformation of pUC18 DNA to

nicked circular conformation and linear conformation. When circular plasmid DNA is conducted by

electrophoresis, the fastest migration will be observed for the supercoiled form (Form I). If one strand is

cleaved, the supercoils will relax to produce a slower-moving open circular form (Form II). If both strands are

cleaved, a linear form (Form III) will be generated that migrates in between.

Figure 3 illustrates the gel electrophoresis experiments showing the cleavage of plasmid pUC18 DNA

induced by the three trinuclear complexes. The control experiments did not show any apparent cleavage of

DNA (lane 1 & 2). Copper trinuclear complex in the presence of $H_2O_2$ (lane 1) at higher concentration

(50μM) shows more cleavage activity compared to trinuclear nickel and manganese complexes. The

supercoiled plasmid DNA was completely degraded. This shows that a slight increase in the concentration

over the optimal value led to extensive degradations, resulting in the disappearance of bands on agarose gel [23]. Nickel trinuclear complex in the presence of $H_2O_2$ resulting the conversion of supercoiled form (Form-I) into linear form (Form-III) (lane 4). Manganese trinuclear complex in the presence of $H_2O_2$ (lane 5) at higher concentration (50µM) shows cleavage activity in which supercoiled DNA (Form-I) cleaved and supercoiled form converted to open circular form (Form-II).
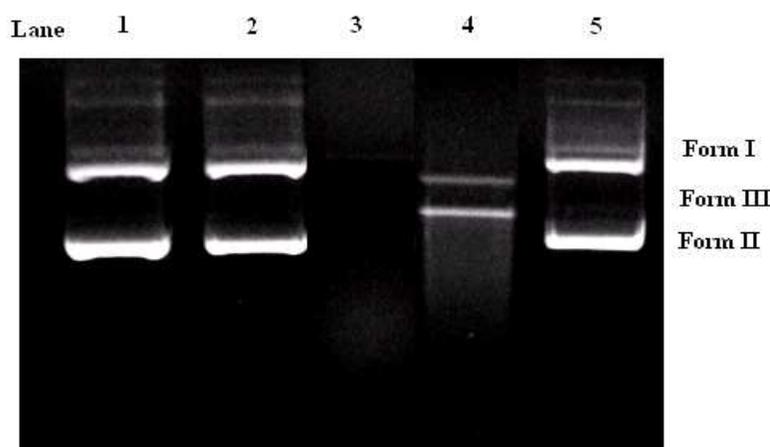


**Figure 3.** Changes in the agarose gel electrophoretic pattern of pUC18DNA induced by $H_2O_2$ and metal complexes: Lane 1, DNA alone; Lane 2, DNA alone + $H_2O_2$; Lane 3, DNA + Cu trinuclear complex + $H_2O_2$; Lane 4, DNA + Ni trinuclear complex + $H_2O_2$; Lane5, DNA + Mn trinuclear complex + $H_2O_2$.
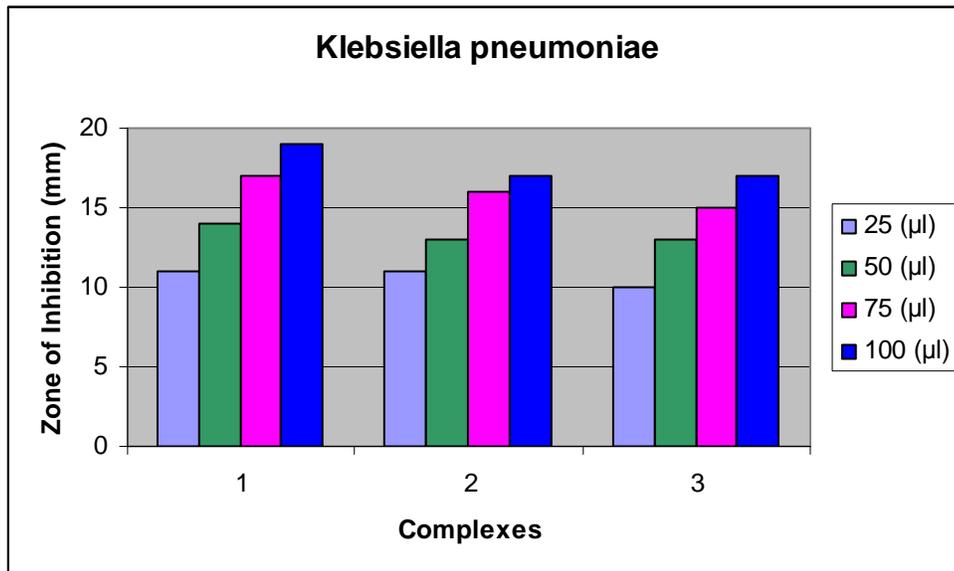
**Antimicrobial assay**

The antibacterial activity of the trinuclear complexes were evaluated by the disc diffusion technique. The antimicrobial activity of the synthesized compounds was tested against the bacterias like *Klebsiella pneumoniae, Escherichia coli and Staphylococcus aureus*. The minimum inhibitory concentration (MIC) values of the proposed compounds are listed in Table 4. From this table, the observed MIC values mentioned that the complexes have higher antimicrobial activity.

**Table 4**. Antibacterial activity of the trinuclear Schiff base metal complexes.

| Compounds | Klebsiella pneumoniae (mm) | | | | Escherichia coli (mm) | | | | Staphylococcus aureus (mm) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 25 (µl) | 50 (µl) | 75 (µl) | 100 (µl) | 25 (µl) | 50 (µl) | 75 (µl) | 100 (µl) | 25 (µl) | 50 (µl) | 75 (µl) | 100 (µl) |
| $[Cu_3C_{62}H_{60}N_{12}Cl_6]$ | 11 | 14 | 17 | 19 | 12 | 13 | 15 | 17 | 11 | 15 | 16 | 18 |
| $[Ni_3 C_{62}H_{60}N_{12}Cl_6]$ | 11 | 13 | 16 | 17 | 10 | 12 | 14 | 16 | 11 | 13 | 16 | 17 |
| $[Mn_3 C_{62}H_{60}N_{12}Cl_6]$ | 10 | 13 | 15 | 17 | 10 | 12 | 13 | 16 | 11 | 12 | 15 | 17 |

Figure 2 suggest that increase in the concentration of metal complexes with increase in inhibition efficiency also. The bacterial growth inhibition capacity of the trinuclear complexes follow the order Cu(II)>Ni(II) >Mn(II). The MIC value against the growth of microorganisms was much larger for homotrinuclear complexes when compared to standard Streptomycin.

To improve the activity of copper complex can be explained on the basis of chelation theory [24, 25]. On chelation, the polarity of the metal ion will subside greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further, it increases the delocalization of π-electrons around the whole chelate ring and inhibits the penetration of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organisms.

(A)
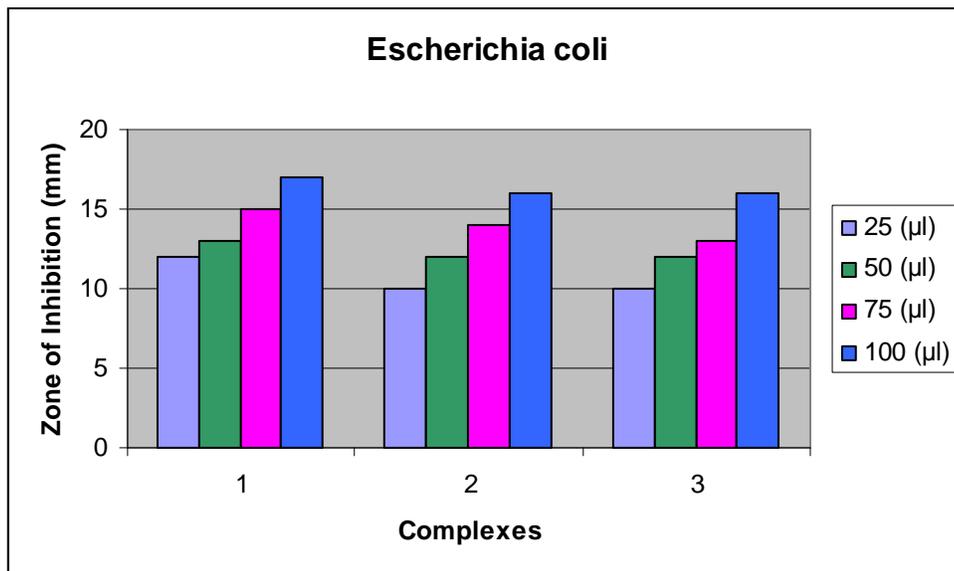


(B)
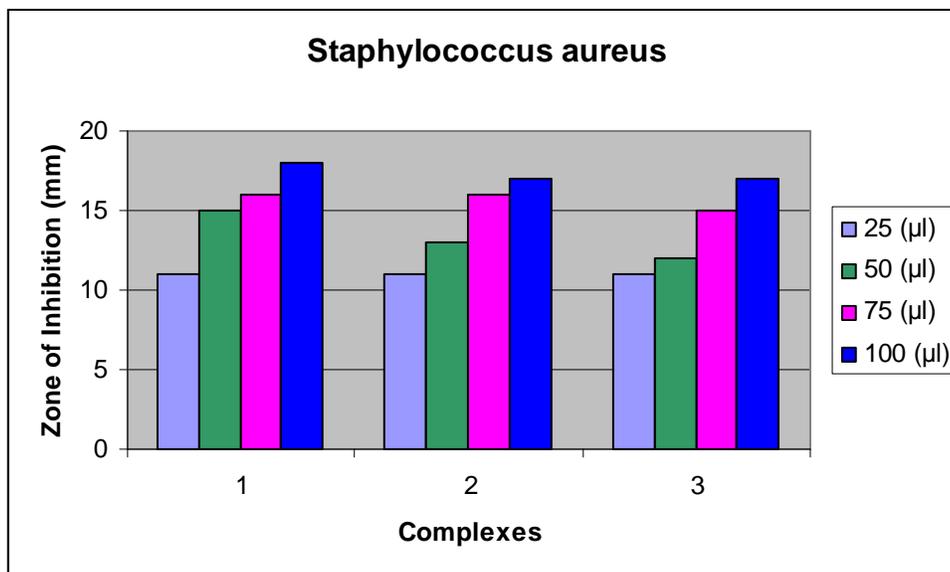
(C)

**Figure 4.** Difference between the antimicrobial activities of the trinuclear metal complexes 1.$[Cu_3C_{62}H_{60}N_{12}Cl_6]$, 2. $[Ni_3\ C_{62}H_{60}N_{12}Cl_6]$, 3. $[Mn_3\ C_{62}H_{60}N_{12}Cl_6]$,

**Conclusion**

The novel trinuclear Cu(II), Ni(II) and Mn(II) complexes were synthesized from p-phenylenediamine, 2,3-pentanedione and 2-aminobenzaldehyde containing $N_4$ donors set in different environments. The mononuclear complexes have been synthesised and are assembled in the step wise to form homo-metallic trinuclear complexes. This methodology can be employed to build complexes with more metal centers. Octahetral structures were assigned to the trinuclear complexes based on elemental and spectral data (molar conductivity, IR, electronic, ESR and magnetic studies). All the trinuclear complexes along with existing antibacterial drugs were screened for antibacterial activity against Gram(–ve)(E.coli, K.pneumonia) and Gram(+ve) (S.aureus) bacteria. The DNA cleaving activities of metal complexes with pUC18 DNA show more pronounced activity of Cu(II), Ni(II) and Mn(II) complexes in presence of the oxidant. In trinuclear Cu(II) complex has more cleavage activity when compared to other trinuclear Ni(II) and Mn(II) complexes.

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