# Biological investigation including *in vivo* wound healing studies of Schiff base transition metal complexes

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## Abstract

Amino acid based Schiff bases are excellent biological agents. During the coordination with the metal ions the activity gets enhanced. The current study focuses on the synthesis and characterization of L-Leucine based Schiff base metal complexes containing TMPDA (N, N, N', N'-tetramethyl-1, 3-propanedimaine) as a co-ligand. Various physicochemical and spectroscopic techniques like UV-Vis., FTIR and EPR were used to study the coordination nature of the metal complexes. FTIR studies confirmed that the Schiff base ligand acted as an ONO donor. In order to evaluate the biological properties of the synthesized complexes antimicrobial, antioxidant and larvicidal activities were investigated.

Among the synthesized compounds, the copper complex effectively inhibits the growth of microorganisms and both copper and cobalt complexes exhibit significant radical scavenging activities. The results of antioxidant activities, assessed using four different methods (DPPH, $H_2O_2$, FRAP, CUPRAC) demonstrated that cobalt (II) and copper (II) Schiff base complexes possess notable antioxidant properties. Schiff base cobalt (II) and copper (II) complexes were subjected to in vivo wound healing studies on Sprague Dawley rats. The results revealed that the wound healing process was faster in animals treated with the synthesized complexes than the control.

Key words: Schiff base, antimicrobial, larvicidal and in vivo wound healing.

## 1. Introduction

In coordination chemistry, Schiff bases are often used as ligands to form metal complexes. The versatility of Schiff base metal complexes makes them valuable in many scientific and technological areas and ongoing research continues to explore new applications for these compounds. It has to be noted that the compounds produced from Schiff base amino acids efficiently coordinate with a variety of metal ions and enhance the pharmacological activities [1]. The chemistry of Schiff bases is diverse and their properties can vary based on the nature of the constituents involved in their formation. Schiff bases derived from amino acids have widespread applications in various fields, including organic chemistry, biochemistry and coordination chemistry. They can serve as intermediates in the synthesis of a variety of compounds, including pharmaceuticals. Therefore, many researchers are interested in the discovery of novel synthesized Schiff base complexes with medicinal efficacy.

Resistance to therapeutics is a major problem in medical treatment since most pathogenic organisms periodically improve their capacity to breakdown drugs. Researchers have been developing novel drug materials as a result of realizing the threat that multidrug resistance poses. Interestingly, the medicinal properties of Schiff base derivatives and their metal complexes have been thought to be the most efficient way to treat many of these problems [2].

Research on the wound healing properties using Schiff base complexes is an area of interest in the field of medicinal chemistry and biochemistry. Wound healing is a complex and dynamic process involving interactions between cells, growth factors, extracellular matrix components, and other factors. However, there could be significant challenges with wound healing, resulting in significant therapy costs and it is therefore vital to develop more effective approaches to disclose the problem of treating wounds [3-5].

Therefore, this issue stresses on implying the available requirement of preparing new compounds which possess the therapeutic efficacy towards the wound healing properties along with the antioxidant and biological activities [6, 7]. In order to explore the biological properties of Schiff base metal complexes obtained from amino acids, an attempt was made to examine the antioxidant, antimicrobial, larvicidal and wound healing properties of Schiff base metal complexes derived from L-Leucine.

## 2. Materials and Methods

All chemicals and reagents were provided by Sigma Aldrich and were used without further purification. Using a digital conductivity meter, the molar conductivities of the synthesized Schiff base metal complexes were measured at room temperature in a freshly prepared DMSO solution ($1.0 \times 10^{-3}$ M). Using a SYSTRONICS 2201 spectrophotometer and a sample concentration of
$10^{-5}$ M in DMSO UV-Vis., spectra were plotted at room temperature. A Perkin - Elmer FTIR spectrometer model 1600 was used to record FTIR spectra in the 4000-400 $cm^{-1}$ range on KBr discs. The EPR spectra were recorded in solid state at room temperature using Bruker EMX -10/2.7 spectrometer using DPPH as g marker.

### 2.1 Synthesis of Schiff base ligand ($L^1$) and metal (II) complexes

Schiff base was synthesized by taking L-leucine (2 mmol), KOH (2 mmol) and o-vanillin (2 mmol) in 25 mL of ethanol. It was stirred in a magnetic stirrer at 60$^o$ C for two hours. To the yellow colored Schiff base, hydrated metal (II) acetate (1 mmol) was added to a hot ethanolic solution. Stirring was continued for two hours by maintaining the same temperature. Finally, TMPDA (1 mmol) was added, and the mixture was stirred for two more hours. Then the solution was evaporated at room temperature. The resultant product was washed with ethanol and dried.

### 2.2 Antioxidant activity

Antioxidant activities of the Schiff base complexes were investigated by four different methods using a standard procedure. Without the synthesized complexes a blank was also run for each case. α-tocopherol was employed as a positive control for DPPH and $H_2O_2$ methods. Similarly, butylated hydroxyl anisole was employed as a positive control for the FRAP and CUPRAC methods.

Following equation was used to determine the scavenging activity.

$$\text{Percentage of radical scavenging activity} = \frac{(\text{absorbance of Control} - \text{absorbance of Sample})}{\text{Control absorbance}} \times 100$$

In hydrogen peroxide scavenging method, to the Schiff base complex (2 mg/2 mL in DMSO), phosphate buffer (50 mM, pH 7.4) was added during the preparation of Hydrogen peroxide solution (40 mM) and the concentration of $H_2O_2$ was calculated by using a UV-Vis., spectrophotometer based on adsorption at 230 nm after 10 minutes of adding hydrogen peroxide [8].

In DPPH scavenging method, 2 mg/2mL of the synthesized compounds in DMSO were added to 2 mL of a DPPH 0.05 M methanol solution and it was incubated for 30 minutes, at room temperature in the dark. The decrease in DPPH absorbance at 517 nm was measured [9-12].

In CUPRAC method, 30 mL of the synthesized complex (2 mg/2 mL in ethanol) was mixed with a solution containing 40 μL of copper chloride (10 mM), 40 μL of neocuproine in ethanol (7.5 mM), and 50 μL of ammonium acetate buffer (1 M, pH 7) solution. After 45 min of incubation the absorbance was measured at 450 nm [13, 14].

Finally, in FRAP method, 2mg/2mL of ethanolic solution of the complexes 0.5 mL of 1% potassium ferricyanide and 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1 mL of a 10% trichloroacetic acid solution was added. After 30 minutes of incubation at 50° C absorbance was measured at 700 nm [15-17].

### 2.3 Antimicrobial activity

Antimicrobial studies were executed based on the guidelines of NCCLS [18, 19]. Using *in vitro* method, antibacterial activity was performed against Gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus faecalis* and Gram-negative bacteria such as *Salmonella typhimurium* and *Escherichia coli* using the Disc Diffusion method and Ciprofloxacin was used as the positive control.

Antifungal activity was performed against the fungal strain like *Candida albicans,*

*Aspergillus niger*, *Aspergillus flavus*, and *Penicillium sps.*, with a reference drug (Ketoconazole). $10^{-6}$ M concentration of synthesized compounds in DMSO were used for antimicrobial studies. The value of the zone of inhibition was measured in millimeters [20].

### 2.4 Larvicidal bioassay

The larvae of *Culex quinquefasciatus* eggs and egg rafts obtained from the Zonal Entomological Unit in Vellore, Tamil Nadu. The larvicidal activity of all the synthesized complexes were studied by following a standard procedure suggested by WHO [21].

### 2.5 *In vivo* studies for Schiff base copper and cobalt complexes

#### 2.5.1 Animals, housing, diet, and water

The *Sprague Dawley* rats (weighing 150-250 g) were procured from the DKM College for Women (Autonomous) in Vellore, Tamil Nadu, following approval from the Institutional Animal Ethics Committee. The rats were accustomed to conventional cages containing sawdust bedding and were subjected to a lighting regimen approximating a 12 hour cycle of light and dark. The temperature was maintained at 23±2˚C and the rats had unrestricted access to a standardized feed and drinking water. The experiments were conducted in compliance with the guidelines outlined by the OECD [22].

#### 2.5.2 Evaluation of Wound-Healing activity

The study (DKM/IAEC/VI/19/2022) involved 21 male Sprague Dawley rats, which were divided into seven groups of three each. They were anaesthetised with an open mask and 10% of their hair was shaved prior to wounding. A surgical blade was used to create a dorsal excisional wound of 1±0.06 cm along the rat's midline, reaching the deep fascia. The wound was left bare and exposed to the environment for regular observation of wound healing. The animals were assigned to three groups and received 250 mg/kg, 500 mg/kg or 1000 mg/kg body weight of the sample, respectively. The animals that did not receive any treatment acted as controls. The wound-healing experiment lasted for 15 days, starting from the day of wounding. A small amount of drugs was applied to the entire wound using a metal spatula every day.

#### 2.5.3 Visual examination of wound-healing activity

On days 1, 5, 10, and 15 the signs of inflammation and wound healing were visually assessed, and images of the wounds were taken. On the first, fifth, tenth, and fifteenth days, the wound size was measured with Vernier callipers.

The size reduction rate (mm/day) is given by the following equation.

Size reduction rate (mm/day) = $\frac{L_a - L_t}{T}$

where, $L_a$ represents wound length (mm) on day one, $L_t$ represents wound length (mm) at the defined time (days) and T represents time (days).

### 2.6 Results and discussion

At room temperature, all the synthesized complexes are readily soluble in common solvents such as DMSO, DMF and ethanol. Table 1 shows the analytical results for the synthesized compounds. All of the Schiff base complexes are stable at room temperature. The complexes possess higher melting temperatures than the Schiff base ligand, suggesting their stability compared with the Schiff base ligand. The molar conductivity measurements of the prepared Schiff base complexes ($10^{-3}$ M) in DMSO indicated the non-electrolytic nature [23].

**Table 1: Analytical data of the synthesized compounds**

| Compound | Molecular Formula | Molecular Weight | Decomposition Point | Molar conductance ($\Omega^{-1}$ cm$^2$ mol$^{-1}$) |
|---|---|---|---|---|
| $L^1$ | $C_{21}H_{36}O_4N_3$ | 394 | >240 $^0$C | - |
| $[CoL^1L^2]$ | $C_{21}H_{36}O_4N_3Co$ | 452 | >300 $^0$C | 28 |
| $[NiL^1L^2]$ | $C_{21}H_{36}O_4N_3$ Ni | 452 | >300 $^0$C | 18 |
| $[CuL^1L^2]$ | $C_{21}H_{36}O_4N_3Cu$ | 457 | >300 $^0$C | 15 |
| $[ZnL^1L^2]$ | $C_{21}H_{36}O_4N_3Zn$ | 459 | >300 $^0$C | 20 |

**$L^1$ - Schiff base ligand derived from L-leucine and o-vanillin**
**$L^2$ - N, N, N', N'-tetramethyl-1, 3-diaminopropane**

#### 2.6.1 Electronic Absorption Spectra

The absorption maxima (Table 2) at 278 nm in the Schiff base ligand is caused by $\pi \rightarrow \pi^*$ non-bonding electron transitions on the nitrogen of the azomethine group. All of the metal complexes produced $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions around 275 nm and 376 nm respectively. The metal complexes showed a board band ranging from 638 nm to 712 nm, which corresponds to a d-d transition. Due to lack of unpaired electrons d-d transition was not observed in the case of Schiff base zinc (II) complex.

**Table 2: UV-Visible spectral data of the synthesized compounds**

| Compound | Absorption ($\lambda_{max}$ nm) | | |
|---|---|---|---|
| | $\pi$-$\pi^*$ | n-$\pi^*$ | d-d |
| $L^1$ | 278 | 410 | - |
| $[CoL^1L^2]$ | 279 | 379 | 638 |
| $[NiL^1L^2]$ | 274 | 405 | 712 |
| $[CuL^1L^2]$ | 279 | 410 | 638 |
| $[ZnL^1L^2]$ | 277 | 376 | - |

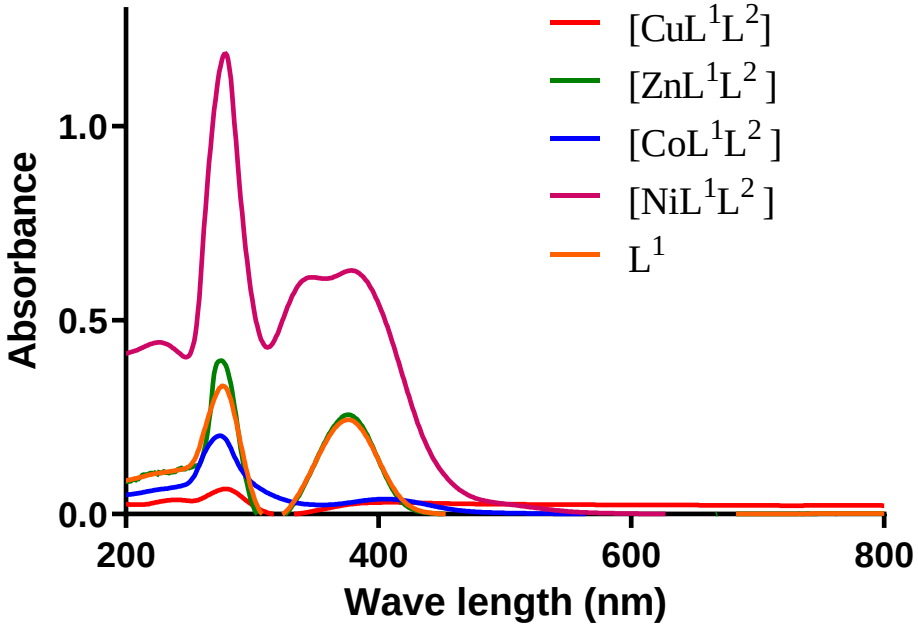


**Figure 1: UV-Vis spectra for Schiff base ligand and its metal complexes**

### 2.6.2 FTIR Spectra

**Table 3: FT-Infrared absorption frequencies ($cm^{-1}$) of compounds**

| Compound | υ(OH) $cm^{-1}$ | υ(C=N) $cm^{-1}$ | $COO^-$ $cm^{-1}$ | | Δυ=[$υ_{as}$ - $υ_s$] $cm^{-1}$ | M-N $cm^{-1}$ | M-O $cm^{-1}$ |
|---|---|---|---|---|---|---|---|
| | | | $υ_{as}$ | $υ_s$ | | | |
| $L^1$ | 3335 | 1541 | 1431 | 1217 | 214 | - | - |
| $[CoL^1L^2]$ | 3645 | 1664 | 1415 | 1211 | 204 | 524 | 443 |
| $[NiL^1L^2]$ | 3635 | 1641 | 1431 | 1217 | 210 | 524 | 416 |
| $[CuL^1L^2]$ | 3429 | 1637 | 1452 | 1228 | 224 | 590 | 408 |
| $[ZnL^1L^2]$ | 3182 | 1631 | 1446 | 1217 | 229 | 542 | 430 |

**Figure 2: FTIR spectrum of (a) Schiff Base ligand [$L^1$]; (b) [$CoL^1L^2$] complex; (c) [$NiL^1L^2$] complex; (d) [$CuL^1L^2$] complex; (e) [$ZnL^1L^2$] complex**

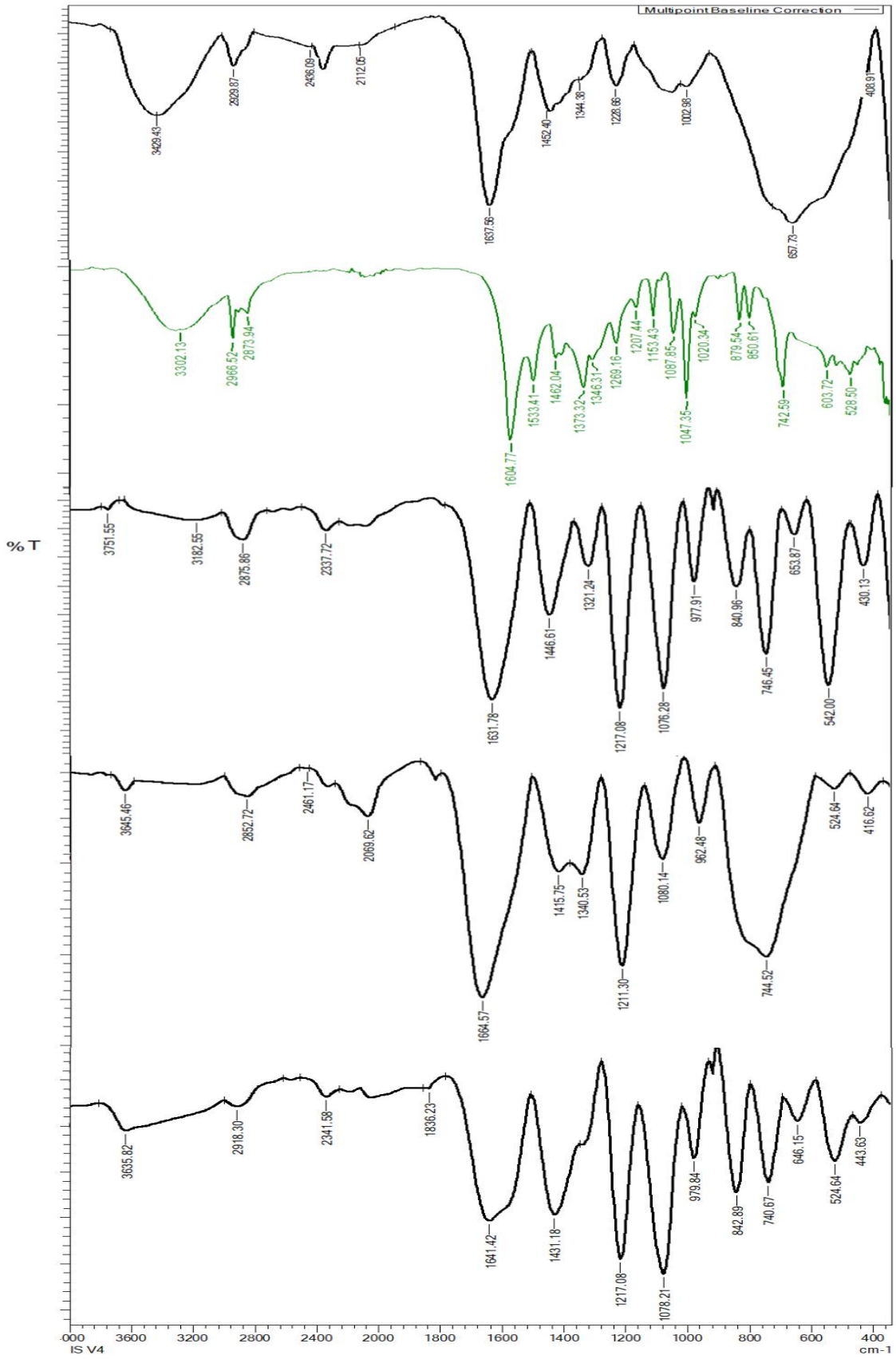


**Figure 2: FTIR spectrum of (a) Schiff Base ligand [$L^1$]; (b) [$CoL^1L^2$] complex; (c) [$NiL^1L^2$] complex; (d) [$CuL^1L^2$] complex; (e) [$ZnL^1L^2$] complex**

Medium to strong peaks obtained around 1200 $cm^{-1}$ are related to the coordination of phenolate oxygen atoms to metal ions. A strong peak observed at 1541-1631 $cm^{-1}$ in all the complexes are due to the conjugated C=N stretching of the Schiff base moiety [24]. The Schiff base ligand and its metal complexes exhibited asymmetric stretching ($\upsilon_{as}\, COO^-$) of carboxylate group at 1430 $cm^{-1}$. The symmetric stretching of the carboxylate group of Schiff base ligand and its complexes ($\upsilon_s\, COO^-$) appeared around 1200 $cm^{-1}$ respectively. It became apparent that the asymmetric and symmetric stretching frequency difference ($\Delta\upsilon = [\upsilon_{as}\, COO^- - \upsilon_s\, COO^-]$) is larger than the comparable free carboxylate anion. Hence proved the monodentate coordination of the carboxylate anion of the Schiff base ligand. However, new bands with medium to weak intensities developed in the complexes in the ranges of 408–443 $cm^{-1}$ and 590–525 $cm^{-1}$, which are assigned to M-O and M-N (metal-ligand) modes, respectively. The Schiff base ligand functions as a tridentate ligand and is coordinated through the phenolic oxygen, azomethine nitrogen and oxygen atom of the carboxylate anion.

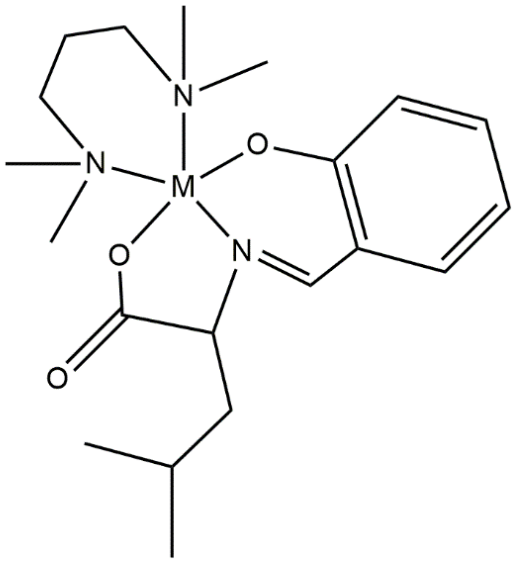


**Figure 3: Proposed structure of the metal complexes**

*Where, M is Cobalt (II), Nickel (II) Copper (II) and Zinc (II) metal ions*

### 2.6.3 EPR Spectrum

ESR spectrum (Fig. 4) of the Schiff base copper (II) complex showed an isotropic peak with a $g_{iso}$ value of 2.01 and confirmed the paramagnetic character of the Schiff base Cu(II) complex. This shows axial symmetry or principal axes are parallel to one another i.e $g_{xx} = g_{yy} = g_{zz}$. Such an isotropic peak would be observed with the symmetrical systems such as square planar, square pyramidal and octahedral.

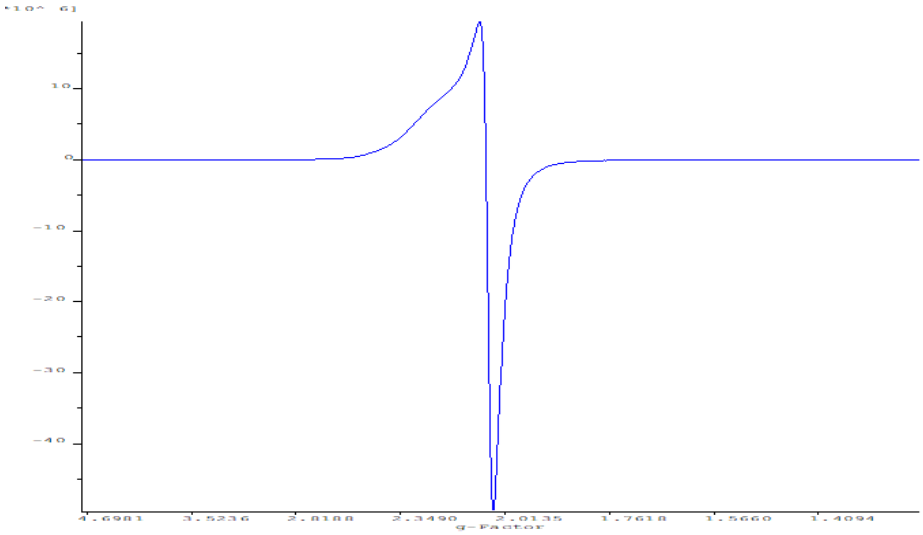


**Figure 4: EPR spectrum for [CuL$^1$L$^2$] complex**

### 2.6.4 Antioxidant activity

The antioxidants scavenging properties are potent in DPPH and $H_2O_2$ which has a capacity to donate the hydrogen atoms readily [25]. Free radical activities are crucial for preventing various harmful diseases. The Schiff base ligand and their complexes show higher scavenging activity than the control. Copper (II) complexes have the highest scavenging ability than the other prepared Schiff base metal complexes.

Compared to the Schiff base ligand, the other Schiff base metal complexes (figures 5 and 6) exhibit greater scavenging activity for all methods. When compared to all other methods in DPPH and $H_2O_2$ method, the prepared Schiff base metal complex of copper have been revealed to exhibit significant free radical scavenging activity. According to the Gur'eva A. et al., the chelation of ligands to the copper (II) ion increases further due to the radical scavenging activity [26]. This may occur due to the imine moiety contains a nitrogen atom, which could contribute an electron to create a stable free radical. The results of the investigation showed that prepared substances had the ability to give up an electron or a hydrogen atom, which can then react with free radicals or stop chain reactions in a dosage-dependent way [27].

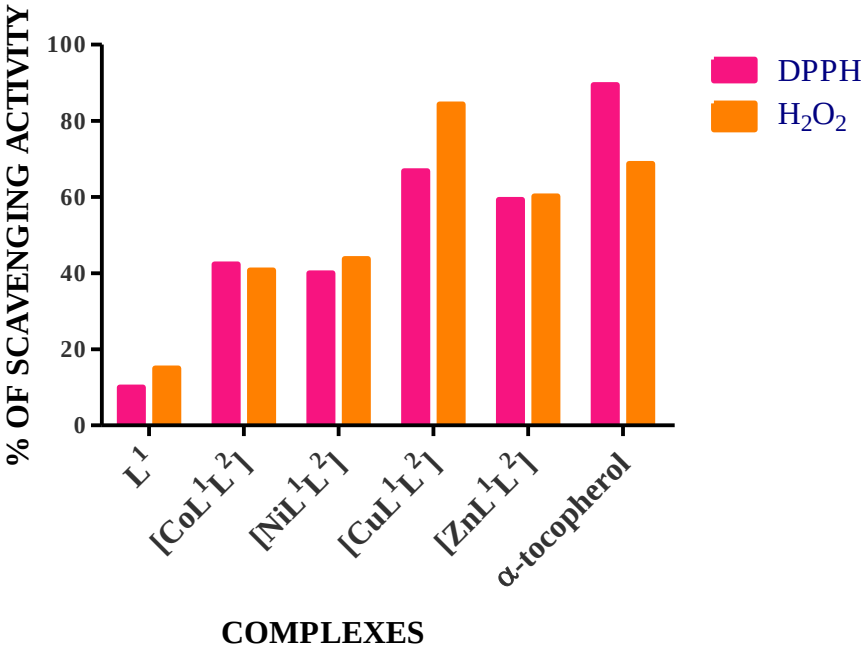


**Figure 5: Antioxidant scavenging activity of DPPH and $H_2O_2$**

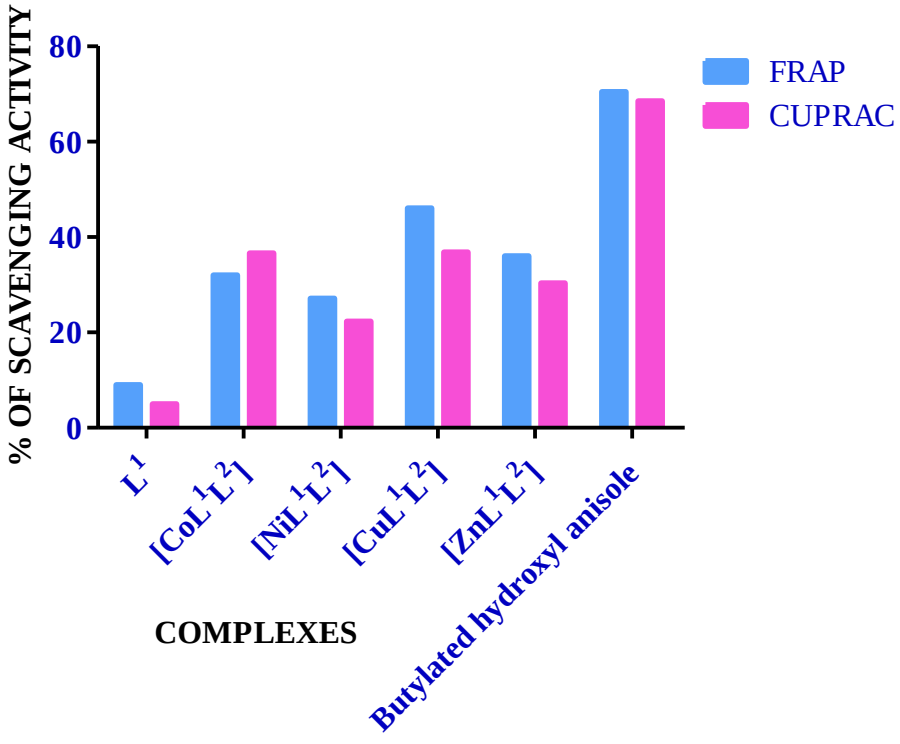


**Figure 6: Antioxidant scavenging activity of FRAP and CUPRAC**

### 2.6.5 Antibacterial Activity

This study reports the antibacterial activity of the synthesized metal complexes (Figure 7) against gram-positive and gram-negative organisms. Table 4 shows the zone of inhibition (in mm) of the metal complexes against the tested bacteria. All of the metal complexes had extremely strong antibacterial action against *Salmonella typhimurium and Escherichia coli*. The complexes' antibacterial activity was greater than the corresponding Schiff base ligand ($L^1$). The Schiff base Cu(II) complex, one of the synthetic compounds, had remarkable antibacterial activity against the research bacterial strain. *Staphylococcus aureus* was resistant to all synthetic compounds, similar to Tabrizi et al [28]. The main reason for the high antibacterial activity of the compounds is the release of metal ions into the bacterial cells. When the metal ions enter the cell, they exchange with the enzyme's prosthetic groups. The copper (II) complexes showed higher activity than other Schiff base metal complexes. This may be due to the copper compound's chelating action to the ligand and the change in the compounds' nature due to the change in polarity of the metal ions. This leads to the formation of a zone due to the death of microorganisms.

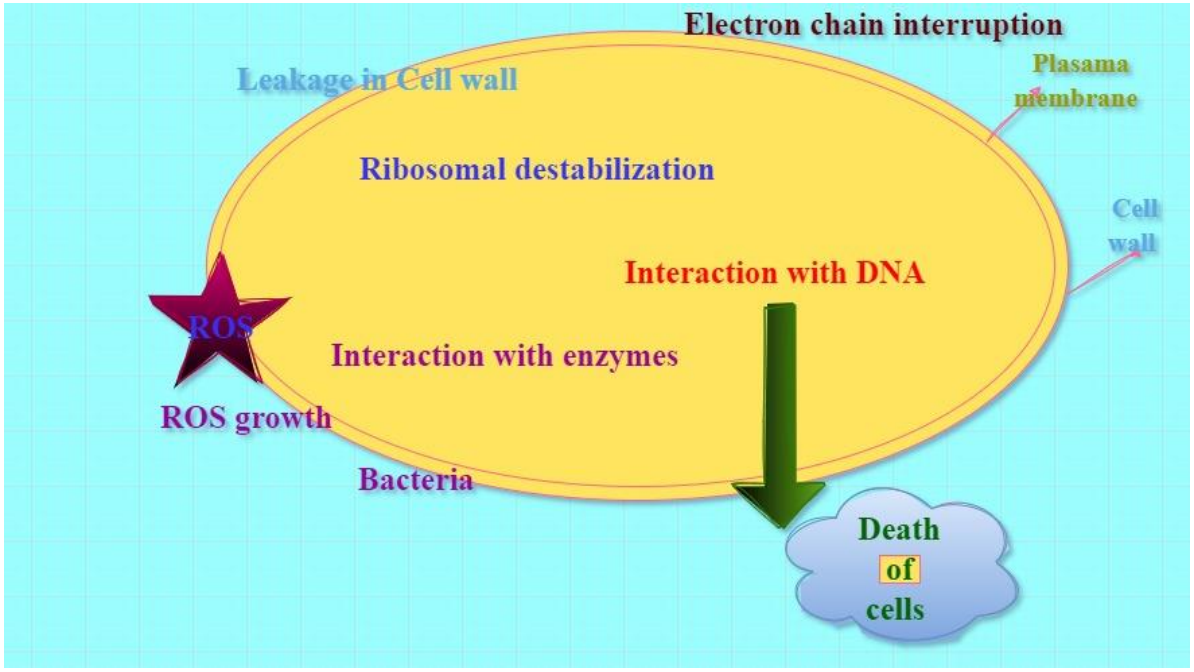


**Table 4: *In vitro* data of antibacterial activity of the prepared compounds**

| Microorganism | Zone of inhibition in mm | | | | | |
|---|---|---|---|---|---|---|
| | $L^1$ | $[CuL^1L^2]$ | $[ZnL^1L^2]$ | $[NiL^1L^2]$ | $[CoL^1L^2]$ | Ciprofloxacin |
| ***Staphylococcus aureus*** | 07 | 23 | 22 | 28 | 20 | 20 |
| ***Streptococcus faecalis*** | 05 | 22 | 18 | 15 | 17 | 21 |
| ***Escherichia coli*** | 15 | 24 | 18 | 15 | 16 | 10 |
| ***Salmonella typhimurium*** | 09 | 23 | 10 | 09 | 12 | 28 |

**Figure 7: Zone of inhibition for antibacterial activities for the prepared compounds**

### 2.6.6 Antifungal Activity

The investigation into the antifungal properties of the synthesized metal complexes against *Candida albicans* revealed remarkable findings. The maximum observed zone of inhibition for *C. albicans* antifungal strains was 29 mm for Schiff base Cu(II) complex compared to the other complexes (Table 5). The antifungal efficacy of the Schiff base metal complexes was particularly significant than the reference drug, Ketoconazole. The complexes of Co(II), Ni(II), and Zn(II) also demonstrated effective inhibition of *Candida albicans*, each exhibiting zones of inhibition exceeding 20 mm. Beyond *Candida albicans*, the antifungal activity of the synthesized complexes was further explored against *Aspergillus Niger, Aspergillus flavus, and Penicillium sps*. The results indicated a spectrum of antifungal activity ranging from mild to high, underscoring the versatility of the synthesized metal complexes (figure 8) against a variety of fungal strains. This comprehensive assessment provides valuable insights into the potential applications of these complexes as effective antifungal agents, paving the way for further exploration in the field of antimicrobial research.

**Table 5: *In vitro* data of Antifungal activity of the prepared compounds**

| **Microorganism** | **Zone of inhibition in mm** | | | | | |
|---|---|---|---|---|---|---|
| | **$L^1$** | **$[CuL^1L^2]$** | **$[ZnL^1L^2]$** | **$[NiL^1L^2]$** | **$[CoL^1L^2]$** | **Ketoconazole** |
| ***Candida albicans*** | **06** | **29** | **23** | **25** | **25** | **24** |
| ***Aspergillus niger*** | **06** | **20** | **20** | **21** | **19** | **22** |
| ***Aspergillus flavus*** | **05** | **28** | **22** | **20** | **20** | **16** |
| ***Penicilliumsps*** | **07** | **26** | **20** | **22** | **24** | **20** |

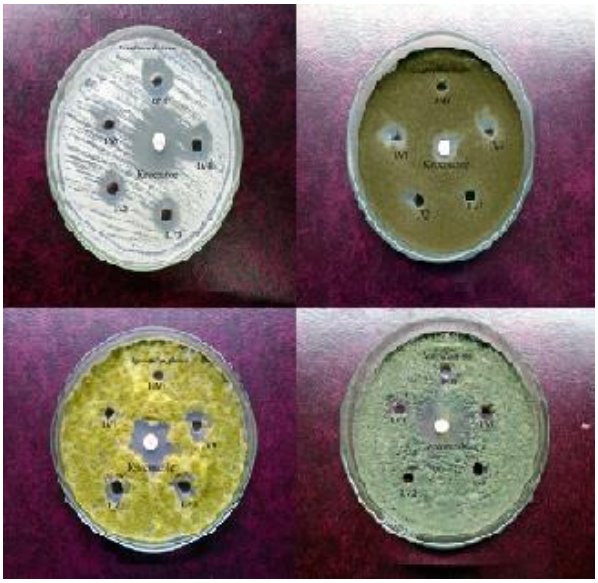

**Figure 8: A region of inhibition for antifungal activities for prepared compounds**

### 2.6.7 Larvicidal activity

Tables 6 and 7 provide insights into the larvicidal potential of the synthesized compounds against *C. quinquefasciatus* larvae. Statistical parameters, including Standard Deviation (SD), chi-square values ($\chi^2$), and toxicity values such as $LC_{50}$ and $LC_{90}$, were calculated based on average larval mortality data to assess the efficacy of the synthesized metal complexes. The minimum lethal concentration of the metal complexes serves as an indicator of their toxicity towards the larvae. Observations of *C. quinquefasciatus* activity in control solutions revealed robust and active movements, contrasting with the continuous movements observed in the presence of Schiff base metal complexes. Notably, as the duration increased, the motion of larvae decreased, indicating a time-dependent impact on their activity. The ionization of metals facilitates interaction with the larvae, resulting in denaturation and subsequent suppression of cellular functions, ultimately leading to larval mortality. This establishes the ability of Schiff base metal complexes to effectively control larval populations. A comparative analysis underscores the highest larvicidal activity of copper complexes compared to other metal complexes against *Culex quinquefasciatus*, emphasizing the potential of these compounds for effective mosquito larval control measures.

**Table 6: A data of larvicidal activity of Schiff base metal complexes**

| **Complex** | **Concentration / mortality** | | | |
|---|---|---|---|---|
| | **4 mg/100 mL** | **2 mg/100 mL** | **1 mg/100 mL** | **0.5 mg/100 mL** |
| $L^1$ | - | - | - | - |
| $[Co\ L^1L^2]$ | 18 | 15 | 12 | 10 |
| $[Ni\ L^1L^2]$ | 16 | 12 | 09 | 07 |
| $[CuL^1L^2]$ | 20 | 18 | 14 | 12 |
| $[ZnL^1L^2]$ | 16 | 12 | 10 | 09 |

**Table 7: Statistical investigation of Larvicidal activity of Schiff base metal complexes**

| Complex | Concentration / %Mortality±SD | | | | $LC_{50}$ | $LC_{90}$ | $\chi^2$ | df |
|---|---|---|---|---|---|---|---|---|
| | 4 mg/100 mL | 2 mg/100 mL | 1 mg/100 mL | 0.5 mg/100 mL | | | | |
| $L^1$ | - | - | - | - | - | - | - | 3 |
| [Co $L^1L^2$] | 70 ± 7.16 | 60 ± 6.11 | 40 ± 3.89 | 30 ± 3.87 | 1.6 | 3.3 | 13.76 | |
| [Ni $L^1L^2$] | 80 ± 7.36 | 80 ± 6.78 | 50 ± 5.65 | 30 ± 2.84 | 1.1 | 2.3 | 23.75 | |
| [Cu$L^1L^2$] | 90 ± 6.26 | 90 ± 8.12 | 80 ± 7.28 | 70 ± 6.51 | 1.1 | 2.0 | 18.17 | |
| [Zn$L^1L^2$] | 60 ± 7.64 | 40 ± 6.32 | 40 ± 3.89 | 20 ± 1.65 | 3.0 | 6.5 | 21.66 | |

### 2.6.8 Wound Healing studies

It has been identified that the generation of reactive oxygen species (ROSs) at the site of a wound can hinder the natural healing process, indicating the importance of regulating oxidative stress for successful wound healing [29]. Wound healing is a dynamic and intricate process involving multiple pathways, including fibroblast collision, collagen formation, angiogenesis in granulation tissue, scar development, injury contraction and epithelialization at the wounded sites [30]. Recognizing the role of antioxidants, antioxidative enzyme systems and free radical scavenging in the body's healing process, past observations have highlighted the enhanced effectiveness of Schiff base compounds, particularly when coordinated with metal ions such as copper, against various harmful bacteria [31]. Consequently, the antimicrobial activity of the copper (II) Schiff base complex was investigated, revealing its superior efficacy against a range of microorganisms. This compound was deemed suitable for inclusion in a wound healing formulation due to its evident antibacterial action, coupled with copper's potent ability to stimulate angiogenesis and collagen deposition [32]. These findings align with studies on copper dressing-treated mice, where substantial upregulation (32-fold increase) of transforming growth factor-beta (TGF-β) gene expression was observed. Copper, either directly or indirectly, influences keratinocyte and fibroblast proliferation, epithelialization, collagen synthesis, extracellular matrix remodeling, and angiogenesis, collectively expediting the wound healing process [33].

The biological activity of the prepared Schiff base metal complexes proves to be significantly relevant. The antioxidant properties of these synthesized complexes, attributed to their hydroxyl scavenging properties, play a crucial role in the mechanism enhancing wound healing properties. In light of antioxidant study results, *in vivo* experiments were conducted on healing wounds in *Sprague Dawley* rats. Wound healing involves the restoration of cellular and anatomical tissue formation, encompassing the reduction of wound area due to chemical, physical, thermal agents and microbial infections. During the study period, both Schiff base copper and cobalt complexes exhibited a preliminary effect on wound healing activity from day 1 to day 15. Visual inspections of the wounds assessed signs of inflammation and tracked the healing process. Wound length, calculated using the size rate reduction method (mm/day) on every fifth day, gradually decreased after the application of copper and cobalt complexes. A noticeable difference was observed between the control group and the copper and cobalt complex groups. The correlation between wound healing and antioxidant activity was further explored in diabetic mice [34, 35].

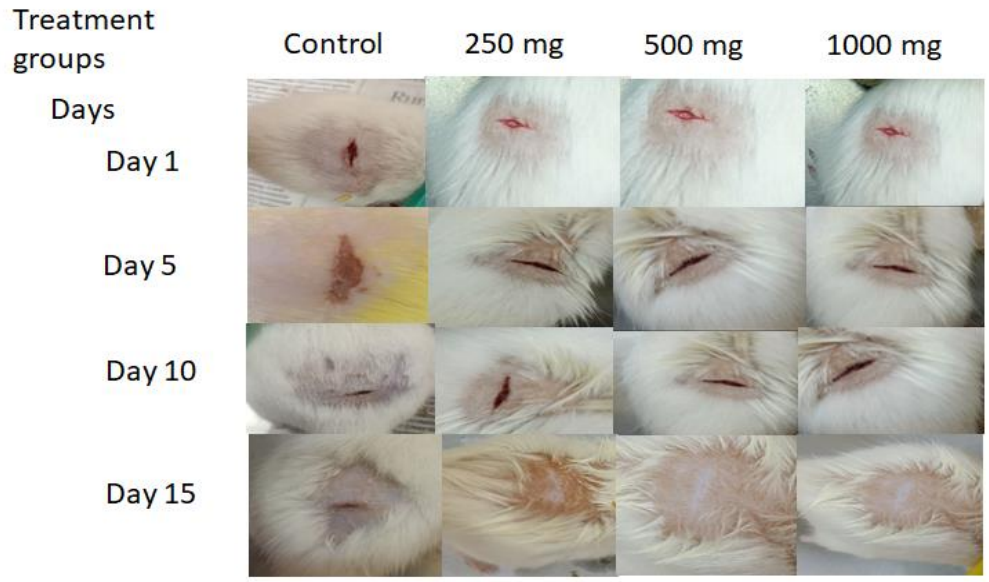


**Figure 9: The gross look of wound healing for cobalt complex**

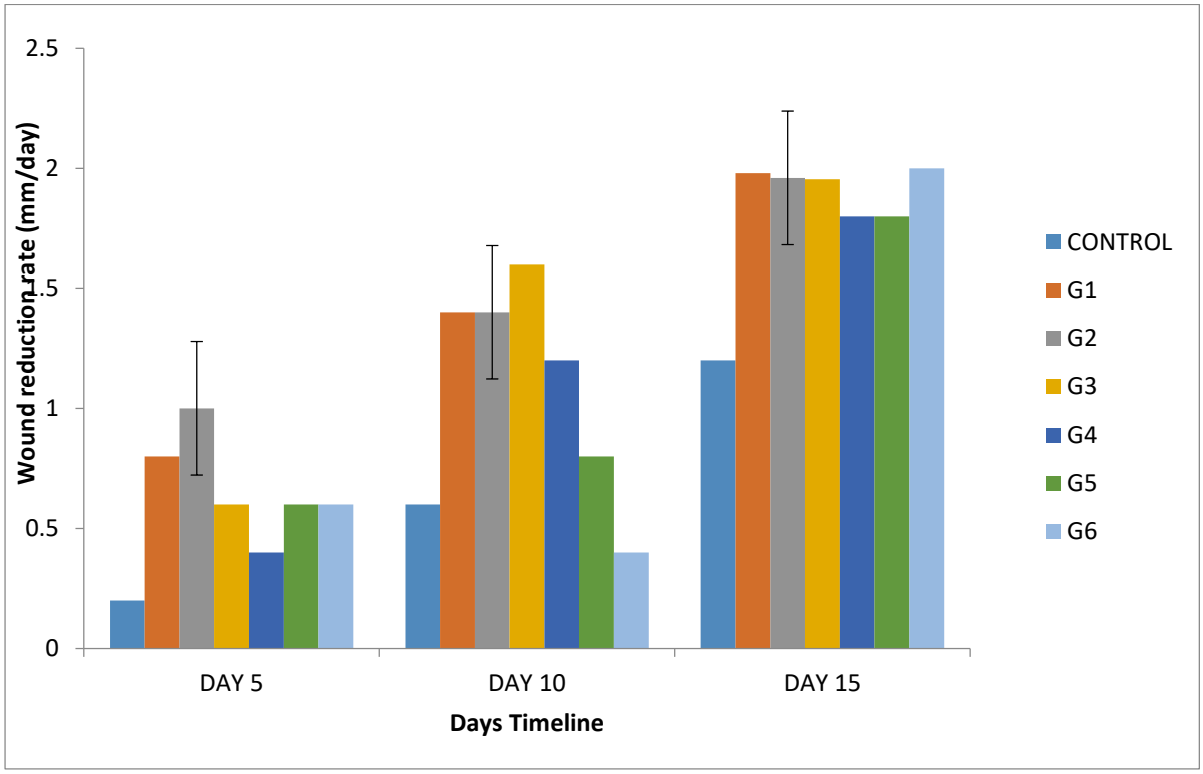


**Figure 10: Graphical representation of Wound-length reduction rate (mm/day) at day 5, 10, and 15 (***Where G1, G2, G3 is 250 mg/kg, 500 mg/kg, 1000 mg/kg body weight for the complex [Co $L^1$ $L^2$] and G4, G5, G6 is 250 mg/kg, 500 mg/kg, 1000 mg/kg body weight for the complex [Cu $L^1$ $L^2$])*

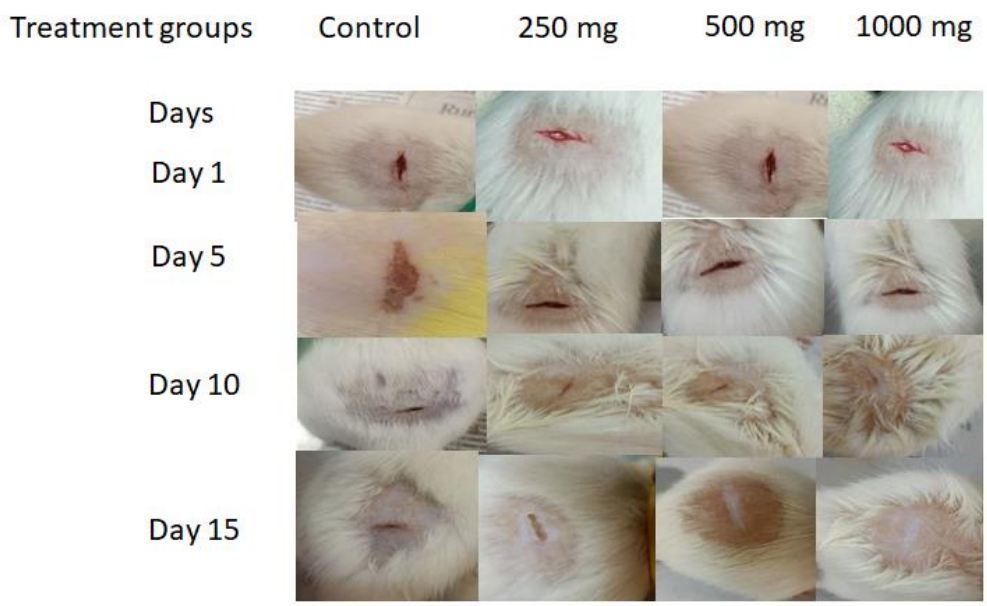


**Figure 11: The gross look of wound healing for copper complex**

## 2.7 Conclusions

Four metal complexes were prepared in the current investigation using N, N, N', and N'-tetramethyl-1, 3-diaminopropane and tridentate Schiff base. The antimicrobial screening of the synthesized compounds showed that the synthesised metal complexes have better activity than the Schiff base ligand. The prepared Schiff base metal complexes exhibited good antioxidant properties. The larvicidal effects of the prepared Schiff base metal complexes against *C. quinquefasciatus* are similarly quite good. The wound area had reduced scarring, according to the histology findings on $15^{th}$ day after the wound first appeared. Because of the good ability to destroy germs, these complexes hence could be used as a promising agent in pharmaceutical sector.

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