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# Mechanochemical Synthesis, Structural Characterization, and Antimicrobial Evaluation of Tri-Nuclear Co(II), Ni(II), and Cu(II) Complexes with Vanillin-L-Leucine Schiff Base

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## ABSTRACT

In this paper, a binucleating Schiff base derived from vanillin and L-Leucine and its Co(II), Ni(II), and Cu(II) tri-nuclear metal complexes were synthesized via a mechanochemical route as an alternative to solution-based methods. The compounds were characterized by elemental analysis, ESI-mass spectrometry, infrared spectroscopy, thermogravimetric analysis (TGA), solubility screening, conductivity and magnetic measurements. The FT-IR spectroscopic data provides evidence for the presence of imine linkage between L-leucine and vanillin resulting in the formation of the Schiff base. In addition, the IR spectral data indicate that the C=N stretching band of the Schiff base shifts from 1673 $cm^{-1}$ to lower frequencies in the range of 1668 to 1629 $cm^{-1}$ upon coordination with Co(II), Ni(II), and Cu(II) ions. This shift confirms the formation of metal-ligand bonds. The Schiff base behaves as a tridentate dibasic ligand and binds to metal ions via the phenolic oxygen, imine nitrogen and carboxylic oxygen (O,N,O) resulting in trinuclear complexes. ESI-MS spectra showed m/z = 342.02, 1208.16, 1131.21 and 1412.87 corresponding to singly charged adduct ions, which confirms the molecular weight of the Schiff base and the trinuclear Co(II), Ni(II) and Cu(II) complexes respectively. Data from thermogravimetric analysis (TGA) confirms the presence of both coordinated water and water of crystallization in the metal complexes. The molar conductivity values ranging from 12.04 to 21.68 $\Omega^{-1}\cdot cm^2\cdot mol^{-1}$ for the tri-nuclear Co(II), Ni(II), and Cu(II) complexes suggest that the tri-nuclear metal complexes are non-electrolytes. The magnetic moments for the tri-nuclear Co(II), Ni(II), and Cu(II) complexes were found to be 4.51, 3.67, and 1.99 BM, respectively, which suggests paramagnetic metal centers. The percentage yield of the synthesized compounds was found to be in the range of 88.61-94.66%. Anti-bacterial and anti-fungal activities of the Schiff base and its corresponding metal complexes were investigated *in vitro* using the disc diffusion method against *Staphylococcus aureus, Streptococcus pneumoniae*, *Salmonella typhi, Escherichia coli, Trichophyton tonsurans* and *Trichophyton rubrum*. The results of the anti-microbial assay showed that the metal complexes are more active compared to the Schiff base.

**Keywords**: Antimicrobial activity, ESI-Mass spectrometry, Mechanochemical, Schiff base, Tri-nuclear complex

## INTRODUCTION

The development of new coordination compounds continues to be a vibrant area of research within inorganic chemistry, driven by the potential applications of these compounds in various fields, including catalysis (Liu *et al.*, 2018) and medicine (Kumar *et al.*, 2003). The number of amines and carbonyl precursors available for condensation reactions leading to Schiff base compounds is practically unlimited (Matius, 2015). In particular, Schiff bases, formed by the condensation reaction between primary amines and aldehydes provide a rich scaffold for the construction of metal complexes with excellent coordination capabilities and biological activities (Al Zoubi *et al.*, 2018; Sulaiman *et al.*, 2023). They can have additional donor groups like oxygen, sulphur, phosphorous etc which makes them good ligands for metal ion complexation and for mimicking biological systems. They can also be functionalized by the insertion of appropriate groups in the aliphatic or aromatic chains (Varsha, 2011). The formation of Schiff base intermediates in reactions of biological importance is well documented. Al-Salami *et al.*, (2014) reported new Schiff bases derived from 2-hydroxy benzaldehyde and amino acid (glycine; D,L-alanine; D,L-phenylalanine, D,L-valine or D,L-threonine) prepared via condensation reactions and were characterized by FT-IR, $^1$H NMR, mass spectrometry and CHN elemental analysis, the Schiff bases act as tridentate ligand through the N (azomethine), O (phenolic) and O (carboxylate) atoms. In 2016, Al-Masoudi reported the condensation of L-arginine with 4-hydroxy-3-methoxy benzaldehyde (vanillin) yielding a novel Schiff base derivative of arginine in good yield. The IR spectra of the synthesized compound display band at 1666 cm$^{-1}$ which was due to C=N group stretching vibration confirming the formation of C=N linkage between L-arginine and 4-hydroxy-3-methoxy benzaldehyde. The toxicity of the compound was also assayed via the determination of their $LD_{50}$ value by using Dixon method. The studied compound was found to have an $LD_{50}$ of 718.6 mg/kg of body weight.

Most of the synthesis of Schiff bases and their transition metal complexes have been carried out in some solvents where ethanol or other solvents were used, which have disadvantages such as long response time, the use of large amounts of solvents that are not environmentally friendly, and low yields (Zhong and Qin, 2014). Mechanochemical synthesis offers an environmentally benign and efficient alternative to traditional solution-based methods for the preparation of metal complexes. Mechanochemical synthesis consists of grinding together two or more compounds to promote a reaction, by inducing the breaking (or forming) of covalent or supramolecular bonds (Chadwick *et al.,* 2007; Vânia *et al.,* 2017). This technique minimizes the use of solvents, reduces reaction times, and often results in products with unique properties not attainable through conventional routes. Mechanochemical synthesis has been successfully applied to synthesize a wide array of metal-organic frameworks, coordination polymers, and discrete metal complexes, highlighting its potential for green chemistry applications.

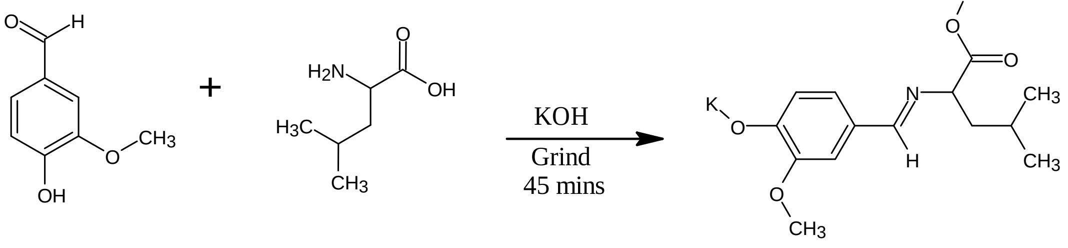
This study focuses on the mechanochemical synthesis, and characterization of tri-nuclear Co(II), Ni(II), and Cu(II) complexes with a Schiff base derived from vanillin (4-hydroxy-3-methoxy benzaldehyde) and the amino acid L-leucine. The choice of vanillin as the aldehyde component is motivated by its structure and biological relevance, while L-leucine, a hydrophobic essential amino acid, contributes to the biological activity and stability of the resulting complexes. Furthermore, we evaluate their *in-vitro* antimicrobial activities against a range of pathogenic microorganisms, including both bacteria and fungi.

## MATERIALS AND METHODS

In the synthesis of the Schiff base and their corresponding metal complexes, chemicals (analytical grade) were purchased from Sigma Aldrich and Alfa Aesar and were used without further purification. L– leucine, vanillin (4-hydroxy-3-methoxy benzaldehyde), and potassium hydroxide were used as starting materials for the preparation of the Schiff base. The metal salts used were $CoCl_2.2H_2O$, $NiCl_2.6H_2O$ and $CuCl_2.2H_2O$ All glasswares used were thoroughly washed with detergent, soaked in 5% nitric acid, rinsed with distilled water and dried in an oven. Four pathogenic bacteria viz: *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae* and *Salmonella typhi* and two fungal isolates namely, *Trichophyton tonsurans and Trichophyton rubrum* were obtained from Aminu Kano Teaching Hospital, Kano and identified at the Department of Microbiology, Bayero University Kano. Nutrient agar and potato dextrose agar were used as growth media for bacteria and fungi respectively. All weighings were carried out on electric balance model AB54. Melting points were determined using a digital WRRS-IB Microprocessor melting points apparatus. Mass spectra were recorded by ESI technique on Apex-III mass spectrometer. Infrared spectral analyses were carried out by using KBr pellets in the range (400-4000cm$^{-1}$), using Perkin Infra-red Model 337. TGA studies were carried on Mettler Toledo Star system in the temperature range of 0-1000ºC. Molar conductance of the metal complexes was determined in DMSO using a coronation digital conductivity meter. Sherwood Scientific Magnetic Susceptibility Balance MK 1 was used to measure the magnetic properties of the metal complexes.

### Synthesis of the Schiff Base

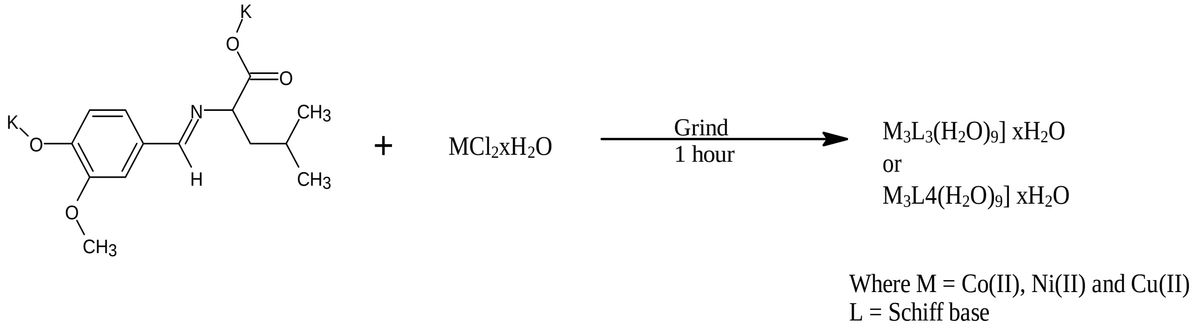
L-leucine (0.005mole) and potassium hydroxide (0.005mole) were weighed and placed in an agate mortar and continually grinded until they became sticky. Then, vanillin (0.005mole) was added and grinded continuously until the color of the reactants turned yellow. A loose yellow solid powder was obtained after about 45 minutes (Sulaiman *et al.*, 2022).



**Scheme 1: Synthesis of the Schiff Base**

### Synthesis of the Metal(II) Complexes

Copper(II) chloride or Nickel(II) chloride (hydrated) (0.005mole) was added into the agate mortar containing the Schiff base and grinded continuously for about one hour (1hr) at room temperature. The products were washed with little cold dehydrated ethanol and filtered, which was dried for about 30 minutes in a vacuum drying oven at 40 °C (Sulaiman *et al.*, 2022; Zhong and Qin, 2014).



**Scheme 2: Synthesis of the Trinuclear Metal Complexes**

**Anti-microbial screening**

The synthesized Schiff base and its corresponding metal complexes were screened for antibacterial activity against bacterial species: *Stapylococcus aureus, Escherichia coli, Streptococcus pneumoniae* and *Salmonella typhi* and antifungal activity against *T. tonsurans and T. rubrum.*

**Anti-bacterial test**

Using an inoculation loop, enough material from an overnight culture of the test organism was transferred into a test tube containing normal saline until the turbidity of the suspension matched the turbidity of the 0.5 Mcfarland standard (as a reference to adjust the turbidity of bacterial suspensions) as described by clinical laboratory standard institute (CLSI), (2020). The standard Inocula of the isolate was swabbed onto the surface area of the prepared agar plates. Nutrient agar was used as the required medium. The Schiff bases were dissolved in dimethyl sulfur oxide (DMSO) to produce four different concentrations 40, 20, 10 and 5µg per disc. A sterilized forceps was used to place the prepared disc of the Schiff bases on the already inoculated agar plates at various intervals and then incubated at 37 °C for 24 hours (Yusha'u and Salisu, 2011).

**Anti-Fungal test**

For anti-fungal activity, potato dextrose agar (PDA) was used as the required medium. A sterilized forceps was used to place the prepared disc of the Schiff bases on the already inoculated agar plates at various intervals and was left for 3 days at room temperature (Hassan *et al.,* 2006). The inhibition zone of the Schiff base and the corresponding metal complexes were measured (in millimeters) around the disc and were compared to ketoconazole as the standard drug.

## RESULTS AND DISCUSSION

The synthesis of the Schiff base was carried out in a solvent-free approach which involved the mechanical grinding of vanillin and L-valine in an equimolar ratio in the presence of potassium hydroxide (as a deprotonating agent). This synthetic approach resulted in high yield (90.70%) yellow powder with high purity as can be seen in the elemental analysis. The complexes were quantitatively obtained as a polycrystalline material by manual grinding of solid metal(II) salts and Schiff base at room temperature having a good percentage yield of 94.66%, 94.30% and 88.61 % for Co(II), Ni(II) and Cu(II) complexes respectively (Table 1). The Schiff base and its metal(II) complexes were found soluble in methanol, ethanol, DMSO, and DMF but insoluble in nonpolar solvents such as hexane and benzene. The polar nature of the synthesized compounds accounts for their solubility in some common polar solvents. The results of the elemental analysis were in good accord with the theoretical values (Table 2).

**Table 1: Some Physical Properties of the Compounds**

| Compound | Molecular Formula | Molecular Weight | Molecular Weight | Colour | m. p. (°C) | Yield (%) |
|---|---|---|---|---|---|---|
| **Ligand** | $C_{14}H_{17}K_2NO_4$ | | 341.48 | Yellow | 104 | 90.70 |
| **$[Co_3(L)_3(H_2O)_9]\ 3H_2O$** | $C_{42}H_{75}Co_3N_3O_{24}$ | | 1182.85 | Dark pink | - | 94.66 |
| **$[Ni_3(L)_3(H_2O)_9]\ 5H_2O$** | $C_{42}H_{79}N_3Ni_3O_{26}$ | | 1218.16 | Pale Green | - | 94.30 |
| **$[Cu_3(L)_4(H_2O)_9]\ 4H_2O$** | $C_{56}H_{96}Cu_3N_4O_{29}$ | | 148.01 | Dark Green | - | 88.61 |

Key: Ligand = $C_{14}H_{17}K_2NO_4$ $L^{-2} = C_{14}H_{17}NO_4^{-2}$

**Table 2: Elemental Analysis of the Compounds**

| **Compound** | **% Elemental Analyses Observed (Calc)** | | |
|---|---|---|---|
| | **C** | **H** | **N** |
| **Ligand** | 49.24(49.12) | 4.97(5.03) | 4.10(4.09) |
| **$[Co_3L_3(H_2O)_9]\ 3H_2O$** | 42.62(42.54) | 6.42(6.37) | 3.49(3.54) |
| **$[Ni_3L_3(H_2O)_9]\ 5H_2O$** | 41.19(41.31) | 6.78(6.52) | 3.51(3.44) |
| **$[Cu_3L_4(H_2O)_9]4H_2O$** | 45.04(45.11) | 6.64(6.70) | 3.79(3.75) |

Key: Ligand = $C_{14}H_{17}K_2NO_4$ $L^{-2} = C_{14}H_{17}NO_4^{-2}$

**Infra-red Spectroscopy**

Comparison of the IR spectra of the Schiff base with those for metal complexes showed *v*(C=N) stretching frequency band of the ligand at 1673 $cm^{-1}$, which undergoes a major shift to lower frequency in the range of 1629-1668 $cm^{-1}$ in the spectra of the metal complexes. This was due to the coordinate bond formed between imine nitrogen and the metal(II) ion. (Nakamoto, 2009). The band for asymmetric carboxyl stretching vibrations *vas*($COO^-$) for the ligand was found to be 1578 $cm^{-1}$, which shifts to a higher frequency in the range of 1588-1596 $cm^{-1}$ in the spectra of the complexes. These values were close to 1596 $cm^{-1}$ reported by Singh and Singh (2014) and Pretsch *et al* (2000) for *vas*($COO^-$) stretching vibrations. In the ligand spectra, the band for symmetric stretching vibration *vs*($COO^-$) at 1425 $cm^{-1}$ shifts to a lower frequency in the range 1394-1415 $cm^{-1}$ for the complexes, which were in agreement with 1390 $cm^{-1}$ reported by Aman and Matela, (2013), and 1393 $cm^{-1}$ and 1392 $cm^{-1}$ for Co(II) complexes with Schiff base derived from thiophehe-2-carbaldeyde and valine or phenylalanine respectively (Sari and Gurkan, (2004) and 1410 $cm^{-1}$ reported by Al-Shaheen and Al-Mula, (2015). This supports a monodentate type of coordination (Nakamoto, 2009: Zhong and Qin 2014) and confirms the coordination of the ligand to the metal ions. IR band in the range 3261-3335 $cm^{-1}$ for *v*(O-H) in spectra of all the metal complexes may be due to coordinated water molecules as well as water of crystallization as seen in ESI-mass spectra and TGA of the compounds. The band at 1229 $cm^{-1}$ in the ligand spectra was assigned to the phenolic *v*(C–O) stretching vibration, which shifts towards a higher wavenumber in the range 1266-1292$cm^{-1}$ in the spectra of the metal complexes. These values were similar to 1242 $cm^{-1}$ and 1291 $cm^{-1}$ reported by Al-Shaheen and Al-Mula, (2015) and Sari and Gurkan, (2004) for *v*(C-O) stretching vibrations due to phenolic oxygen. This confirms phenolic oxygen being coordinated with $M^{2+}$ ion. In the low-frequency region, bands in the range 551-575 $cm^{-1}$ were assigned to *v*(M-N) due to imine nitrogen., while bands in the range 431-476 $cm^{-1}$ were attributed to v(M-O) owing to phenolic, carboxylate and oxygen atoms from $H_2O$ molecules.

**Table 3: Infrared Spectra of the Compounds**

| Compound | *v*(C=N) (cm$^{-1}$) | *v*(C-O) (cm$^{-1}$) | *vas*(COO) (cm$^{-1}$) | *vs*(COO$^-$) (cm$^{-1}$) | *v*(O-H) (cm$^{-1}$) | *Pw*($H_2O$) (cm$^{-1}$) | *Pr*($H_2O$) (cm$^{-1}$) | *v*(M-O) (cm$^{-1}$) | *v*(M-N) (cm$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| **Ligand** | 1673 | 1229 | 1578 | 1425 | - | - | - | - | - |
| **$[Co_3L_3(H_2O)_9]3H_2O$** | 1629 | 1243 | 1588 | 1413 | 3369 | 878 | 742 | 452 | 511 |
| **$[Ni_3L_3(H_2O)_9]5H_2O$** | 1667 | 1292 | 1596 | 1394 | 3261 | 863 | 729 | 476 | 575 |
| **$[Cu_3L_4(H_2O)_9]4H_2O$** | 1668 | 1266 | 1592 | 1415 | 3335 | 858 | 733 | 431 | 551 |

Key: Ligand = $C_{14}H_{17}K_2NO_4$ $L^{-2}$ = $C_{14}H_{17}NO_4^{-2}$

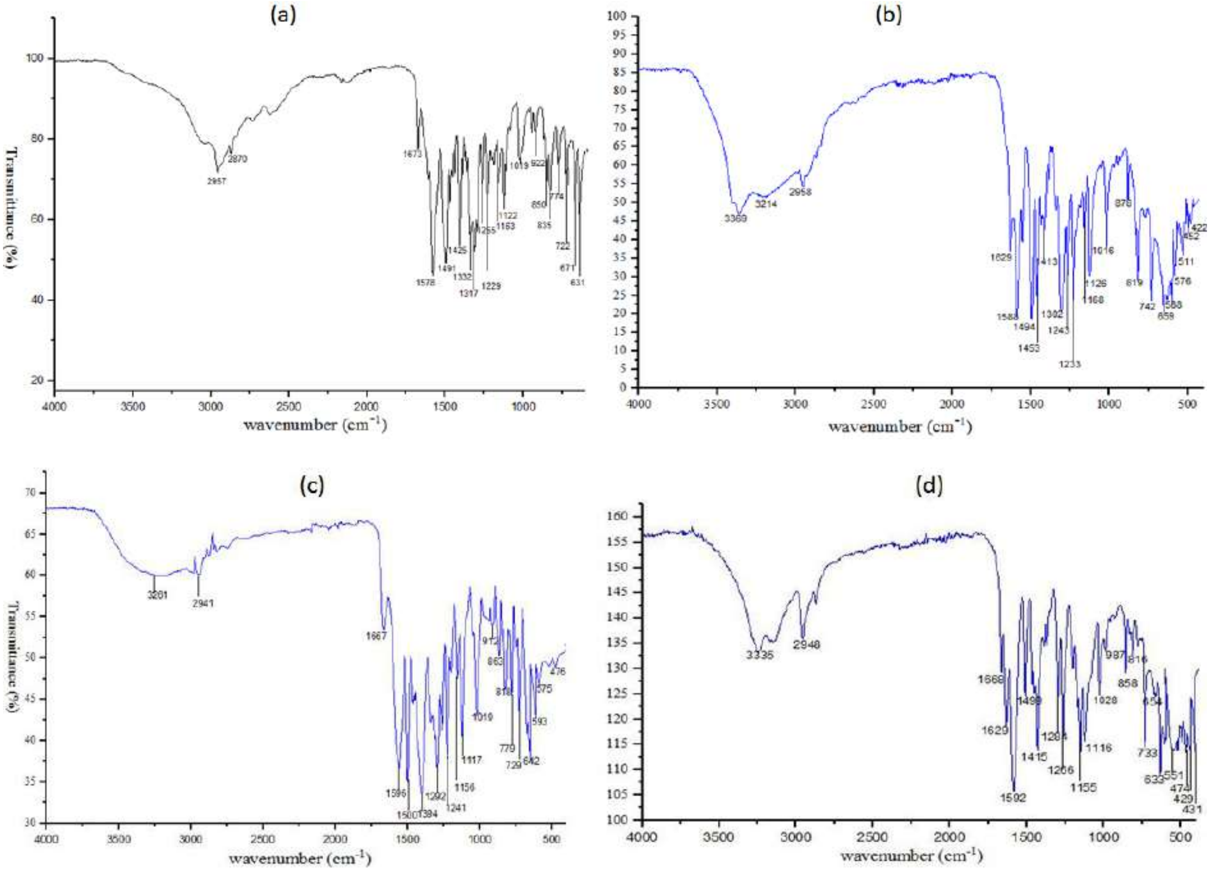
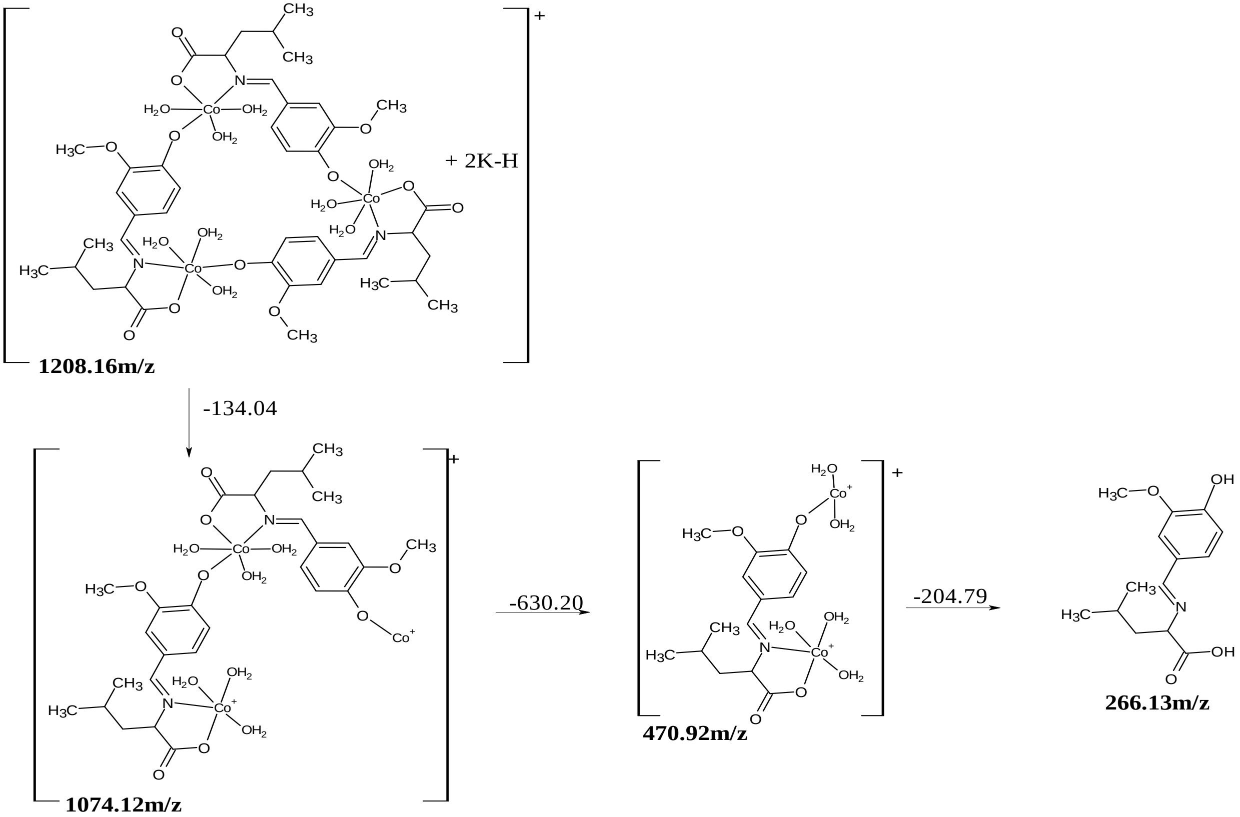


**Figure 1: Infrared spectra of (a) Schiff base (b) $[Co_3(L)_3(H_2O)_9]$ $3H_2O$ (c) $[Ni_3(L)_3(H_2O)_9]$ $5H_2O$ (d) $[Cu_3(L)_4(H_2O)_9]$ $4H_2O$**

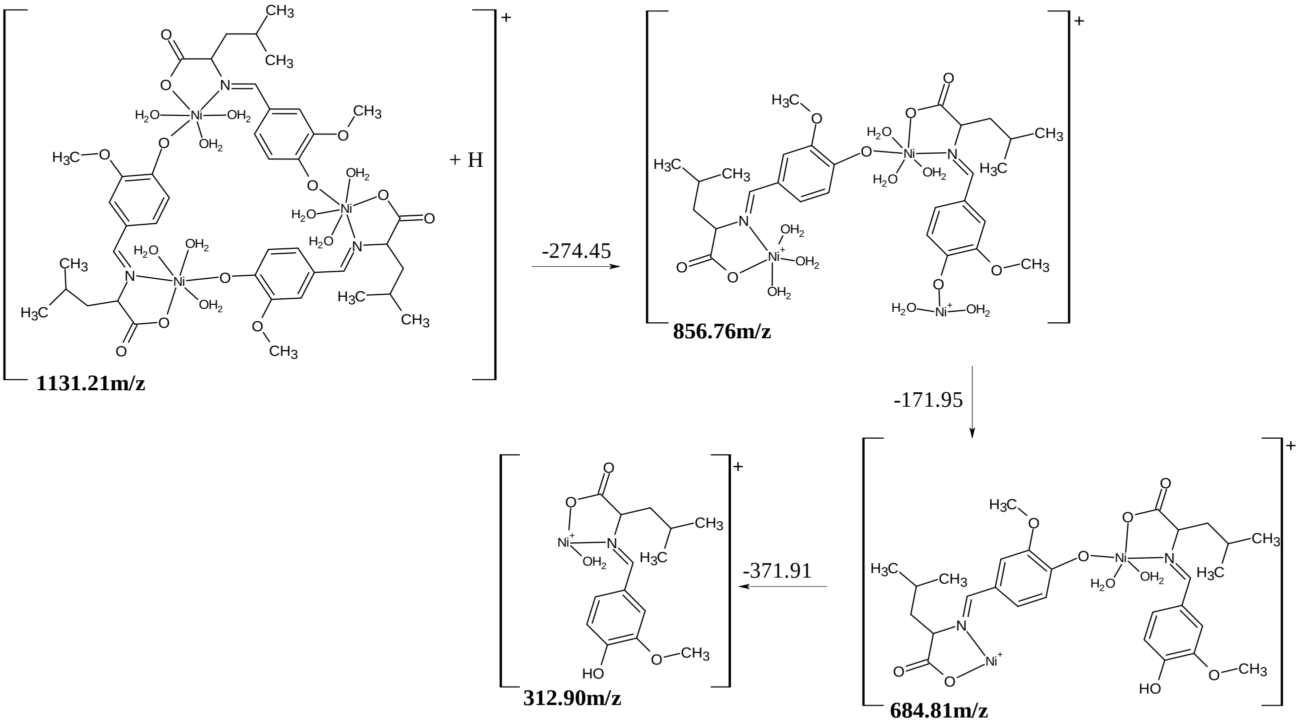
**Mass Spectrometry-ESI**.

In the mass spectrum of the Schiff base (fig.2a), the peak at m/z = 342.05 corresponds to [L+H], as the calculated m/z value was found to be 341.49 (Table 4). ESI mass spectra of tri-nuclear Co(II), Ni(II), and Cu(II) complexes shows peaks at 1208.16 m/z, 1131.21 m/z and 1412.87m/z respectively, corresponding to singly charged potassium adduct ion $[Co_3L_3(H_2O)_9 +2K\text{-}H]^+$, and the protonated $[Ni_3L_3(H_2O)_9 +H]^+$ and $[Cu_3L_4(H_2O)_9 +H]^+$ molecules respectively. Similar adduct ions and protonated molecules were reported by Anneli and Karl (2017), Stricker, *et al* (2021), and Sulaiman *et al* (2023). The formation of these adducts is due to properties such as chelating ability, often linked to oxygen species that can donate electron pairs (Lewis base), as well as the polar surface area of the compounds (Erngren *et al.*, 2019).

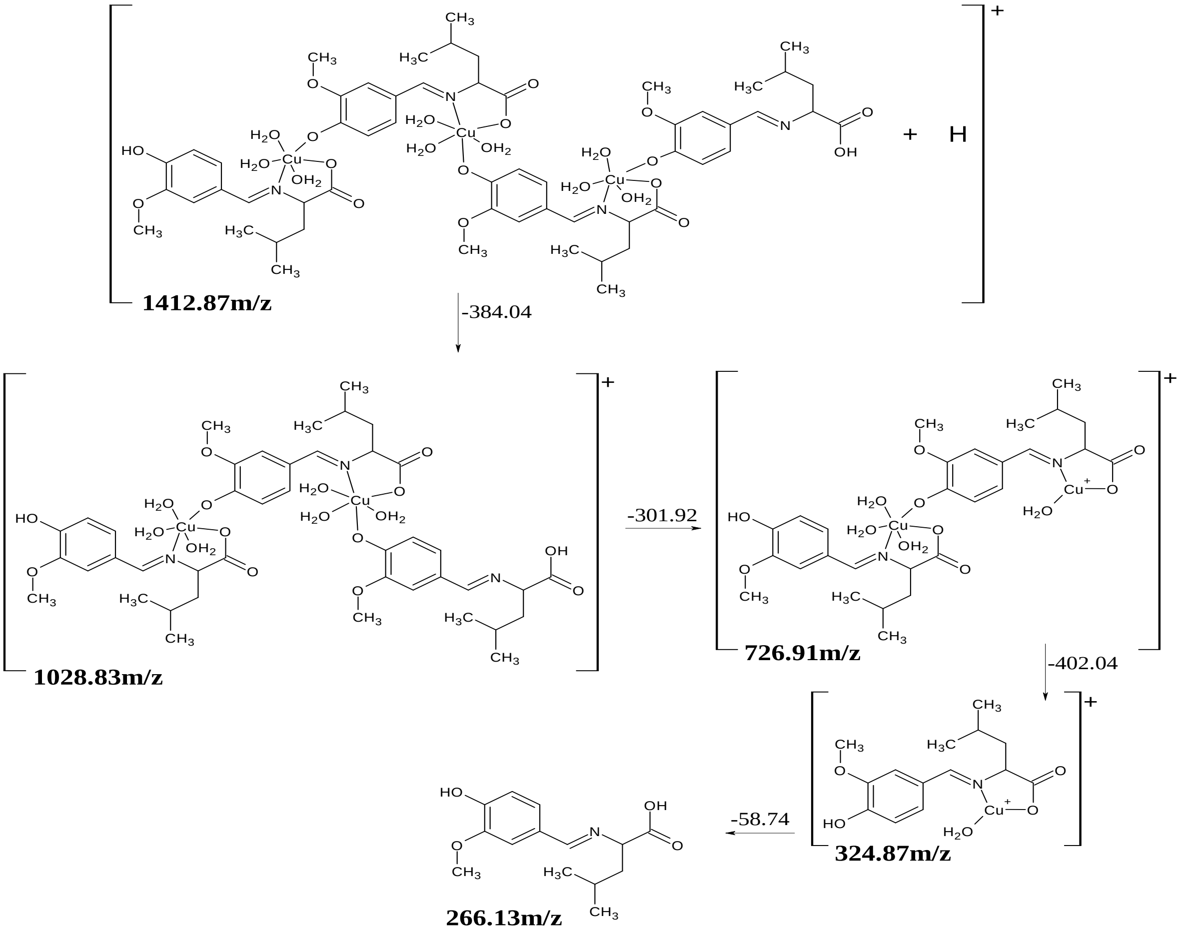
Additionally, well-defined peaks in the spectrum of Co(II) complex at 1074.12 m/z, 470.92 m/z and 266.13 m/z are due to $[Co_3L_3(H_2O)_6]^+$, $[Co_2L(H_2O)_5]^+$ and $[H_2L]^+$ ions respectively (fig.2b). These ions indicate the step by step fragmentation of the tri-nuclear Co(II) complex and are consistent with the proposed structure of the complex (Scheme 3). The mass spectrum recorded for the Ni(II) complex (fig 2c) shows peaks at 856.76 m/z corresponding to $[Ni_3L_2(H_2O)_8]^+$ which show the loss of six coordinated water molecules, Ni(II) ion and one of the Schiff base from the parent ion. Another important peak appeared at 684.81m/z corresponding to $[Ni_2L_2(H_2O)_2]^+$ ion. These fragments are consistent with those expected from the proposed structure of the compound as presented in Scheme 4 below. The mass spectrum of the Cu(II) complex (fig. 2d) exhibits additional peaks at m/z = 1028.83, 726.91, 342.87, and 266.13, corresponding to the ions $[Cu_2L_3(H_2O)_6]^+$, $[Cu_2L_2(H_2O)_4]^+$, $[CuL(H_2O)]^+$, and $[H_2L]^+$, respectively. These peaks align with the expected fragmentation patterns derived from the proposed structure (Scheme 5), thereby supporting the formation of a trinuclear complex. The ESI-mass spectrometry results of the metal(II) complexes, specifically the peaks observed at m/z = 1208.16, 1131.21, and 1412.84 for Co(II), Ni(II), and Cu(II) complexes, respectively, are in accordance with the proposed structure of the tri-nuclear metal(II) complexes. These spectral data provide strong evidence for the proposed structure and confirm the stoichiometry of the chelates as $M_3L_3$ and $M_3L_4$ types, respectively (Table 4).



**Scheme 3: ESI-MS fragmentation pattern of [$Co_3(L)_3(H_2O)_9$] $3H_2O$**



**Scheme 4: ESI-MS fragmentation pattern of [$Ni_3(L)_3(H_2O)_9$] $5H_2O$**



**Scheme 5: ESI-MS fragmentation pattern of $[Cu_3(L)_4(H_2O)_9]$ $4H_2O$**

**Table 4: Mass Spectrometry-ESI for the Compounds**

| Compound | Found m/z | Expected m/z | Fragment Assigned |
|---|---|---|---|
| **Ligand** | 342.05 | 342.21 | $[L+H]^+$ |
| **$[Co_3L_3(H_2O)_9]3H_2O$** | 1208.16 | 1208.86 | $[Co_3L_3(H_2O)_9 +2K-H]^+$ |
| | 1074.12 | 1074.75 | $[Co_3L_3(H_2O)_6]^+$ |
| | 470.92 | 471.03 | $[Co_2L(H_2O)_5]^+$ |
| | 266.13 | 266.13 | $[H_2L]^+$ |
| **$[Ni_3L_3(H_2O)_9]$ $5H_2O$** | 1131.21 | 1131.14 | $[Ni_3L_3(H_2O)_9 +H]^+$ |
| | 856.76 | 856.82 | $[Ni_3L_2(H_2O)_8]$ |
| | 684.81 | 681.00 | $[Ni_2L_2(H_2O)_2]$ |
| | 312.90 | 310.98 | $[NiL(H_2O)]^+$ |
| **$[Cu_3L_4(H_2O)_9]4H_2O$** | 1412.87 | 1413.17 | $[Cu_3L_4(H_2O)_9 +H]^+$ |
| | 1028.83 | 1028.87 | $[Cu_2L_3(H_2O)_4]^+$ |
| | 726.91 | 726.52 | $[Cu_2L_2(H_2O)_4]^+$ |
| | 342.87 | 342.00 | $[CuL(H_2O)]^+$ |
| | 266.13 | 266.13 | $[H_2L]^+$ |

Key: Ligand = $C_{14}H_{17}K_2NO_4$ $L^{-2} = C_{14}H_{17}NO_4^{-2}$

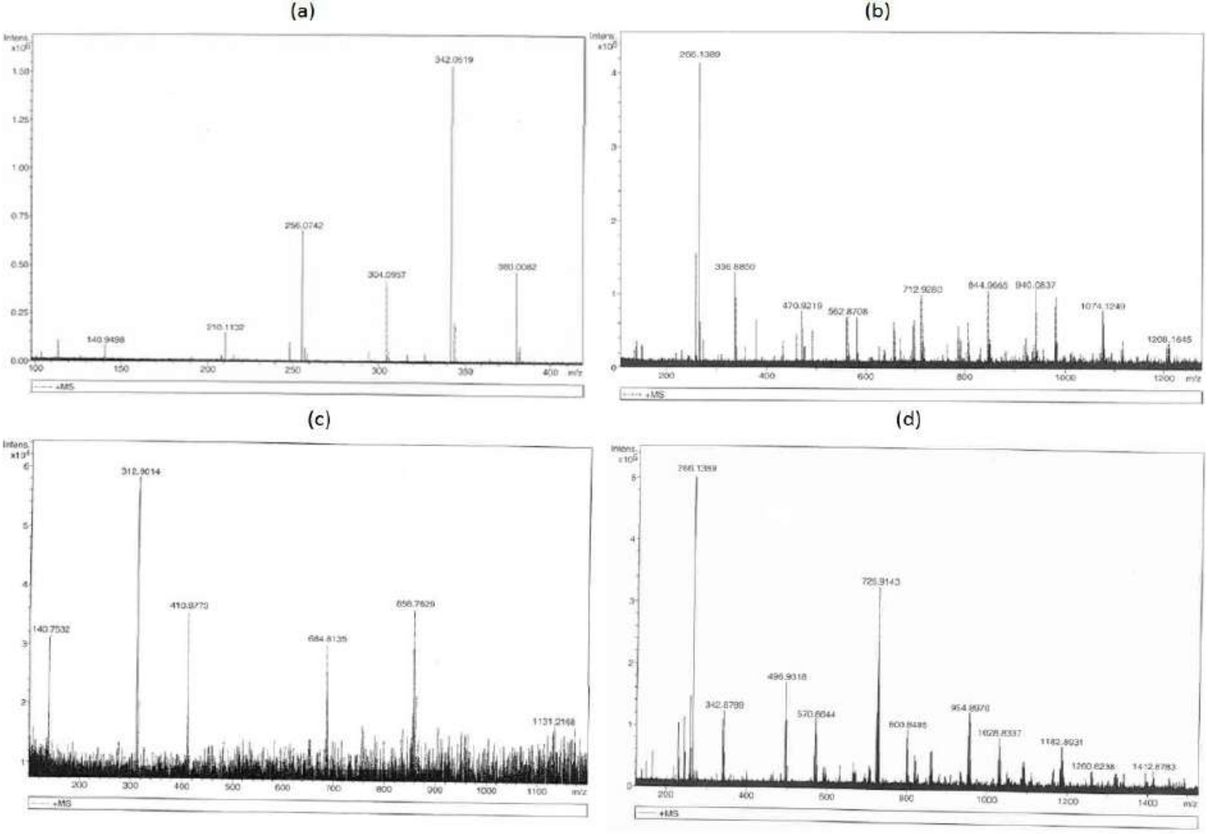


**Figure 2: ESI Mass spectra of (a) Schiff base (b) $[Co_3(L)_3(H_2O)_9]\ 3H_2O$ (c) $[Ni_3(L)_3(H_2O)_9]\ 5H_2O$ (d) $[Cu_3(L)_4(H_2O)_9]\ 4H_2O$**

**Thermogravimetric Analysis**

Thermogravimetric analysis (TGA) was employed to ascertain the thermal properties of the tri-nuclear metal(II) complexes, with the aim of assessing their thermal stability, identifying the presence or absence of coordinated water and water of crystallization, and to confirm their structures. The thermogram of Co(II) complex showed a weight loss of 4.49 % within a temperature range of 50-105 $^oC$ (Fig. 3a), revealing the release of $3H_2O$ as water of crystallization (calc. 4.56 %). The complex further decomposes, showing a mass loss of 13.72% within a temperature range of 105-195 $^oC$. This mass loss indicates the loss of 9 $H_2O$ as coordinated water (calc. 13.67 %) as presented in Table 5 A mass loss of 62.76 % was observed at a temperature range of 195-875 $^oC$, corresponding to the loss of the Schiff base as 3CO, $((CH_3)_2CHCH_2CH_2CN)_3$ and $(C_6H_3O_2CH_3)_3$ (calc. 62.81 %). The remained residue of 19.03 % (calc. 18.96%) was in agreement with the calculated value for 3CoO.

The TGA curve for Ni(II) complex (Fig.3b) displayed a mass loss of 7.73 % within a temperature range of 50-105 $^oC$, corresponding to the loss of water of crystallization (calc. 7.76 %). The second weight loss occurs within the temperature range of 105-240 $^oC$ with a mass loss of 12.86 %, corresponding to the loss of coordinated water molecules (calc. 13.27). The third stage of decomposition occurs at the temperature range of 240-335 $^oC$, with a mass loss of 42.91 % corresponding to the loss of $2CO_2$, $((CH_3)_2CHCH_2CH_2CHN)_2$ and $(C_6H_3O_2CH_3)_2$ (calc. 42.59 %). A mass loss of 18.11 % was observed within a temperature range of 335-456 $^oC$, which corresponds to the loss of $((CH_3)_2CHCH_2CH_2CHN)$ and $(C_6H_3O_2CH_3)$ part of the Schiff base (calc. 17.70%). The final stage exhibits a mass loss of 18.39 % within a temperature range of 456-900 $^oC$ corresponding to a weight of the Ni(II) oxide residue (calc. 18.23 %).

The thermogram of the Cu(II) complex (Fig. 3c) exhibits a multi-step decomposition process, resulting in a total weight loss of 83.93% up to 1000°C. The initial stage involves the removal of water molecules within the temperature range of 50-185°C, resulting in a mass loss of 16.29%. The second stage involves the decomposition of the Schiff base, resulting in the loss of 4CO and $((CH_3)_2CHCH_2CH_2CN)_2$, with a mass loss of 23.17%. This is close to the theoretical mass loss of 23.88%. The third stage involves the further decomposition of the Schiff base within the temperature range of 450-900°C, resulting in the loss of $((CH_3)_2CHCH_2CH_2CN)_2$ and $(C_6H_3O_2CH_3)_4$ groups as part of the ligand, with a mass loss of 44.47%. The final product is identified as a metal oxide residue (3CuO), which corresponds to a mass loss of 16.07%. This is in agreement with the theoretical value of 16.09% (Table 5). These decomposition stages demonstrate the thermal stability and decomposition patterns of the Cu(II) complex.

In general, the tri-nuclear metal(II) complexes exhibit a characteristic thermal decomposition pattern, where they initially lose their water of crystallization at a lower temperature, as observed by Liao et al. (2016). This process typically occurs within the temperature range of 50-110°C, corresponding to the volatilization of lattice water molecules. In contrast, coordinated water molecules are typically eliminated at higher temperatures, ranging from 100°C to 315°C, as reported by Mounika et al. (2010). The final residue (metal oxide) after decomposition for all the metal complexes (3CoO, 3NiO and 3CuO) confirms the tri-nuclear nature of the complexes and agreed with results obtained from ESI-ms and elemental analyses.

**Table 5: Thermal Decomposition Data of the Complexes**

| Compound | Reactions | Mass loss (%) | | Temperature (ºC) |
|---|---|---|---|---|
| | | $M_{exp}$ | $M_{theor}$ | |
| **$[Co_3L_3(H_2O)_9]\ 3H_2O$** | ↓$-3H_2O$ | 4.49 | 4.56 | 50-105 |
| | ↓$-9H_2O$ | 13.72 | 13.67 | 105-195 |
| | ↓$3CO$ | | | |
| | ↓$3(CH_3)_2CHCH_2CH_2CN$ | | | |
| | ↓$3C_6H_3O_2CH_3$ | 62.76 | 62.81 | 195-875 |
| | $3CoO$ | 19.03 | 18.96 | 875-1000 |
| **$[Ni_3L_3(H_2O)_9]\ 5H_2O$** | ↓$-5H_2O$ | 7.73 | 7.76 | 50-105 |
| | ↓$-9H_2O$ | 12.86 | 13.72 | 105-240 |
| | ↓$-2CO_2$ | | | |
| | ↓$2(CH_3)_2CHCH_2CH_2CHN$ | | | |
| | ↓$2C_6H_3O_2CH_3$ | 42.91 | 42.59 | 240-335 |
| | ↓$(CH_3)_2CHCH_2CH_2CHN$ | 18.11 | 17.70 | 335-456 |
| | ↓$C_6H_3O_2CH_3$ | | | |
| | $3NiO$ | 18.39 | 18.23 | 456-900 |
| **$[Cu_3L_4(H_2O)_9]\ 4H_2O$** | ↓$-4H_2O$ | 16.29 | 16.30 | 50-185 |
| | ↓$-9H_2O$ | | | |
| | ↓$4CO$ | | | |
| | ↓$2(CH_3)_2CHCH_2CH_2CHN$ | 23.17 | 23.88 | 185-450 |
| | ↓$2(CH_3)_2CHCH_2CH_2CHN$ | 44.47 | 43.73 | 450-900 |
| | ↓$4C_6H_3O_2CH_3$ | | | |
| | $3CuO$ | 16.07 | 16.09 | 900-1000 |

Key: Ligand = $C_{14}H_{17}K_2NO_4$ $L^{-2} = C_{14}H_{17}NO_4^{-2}$

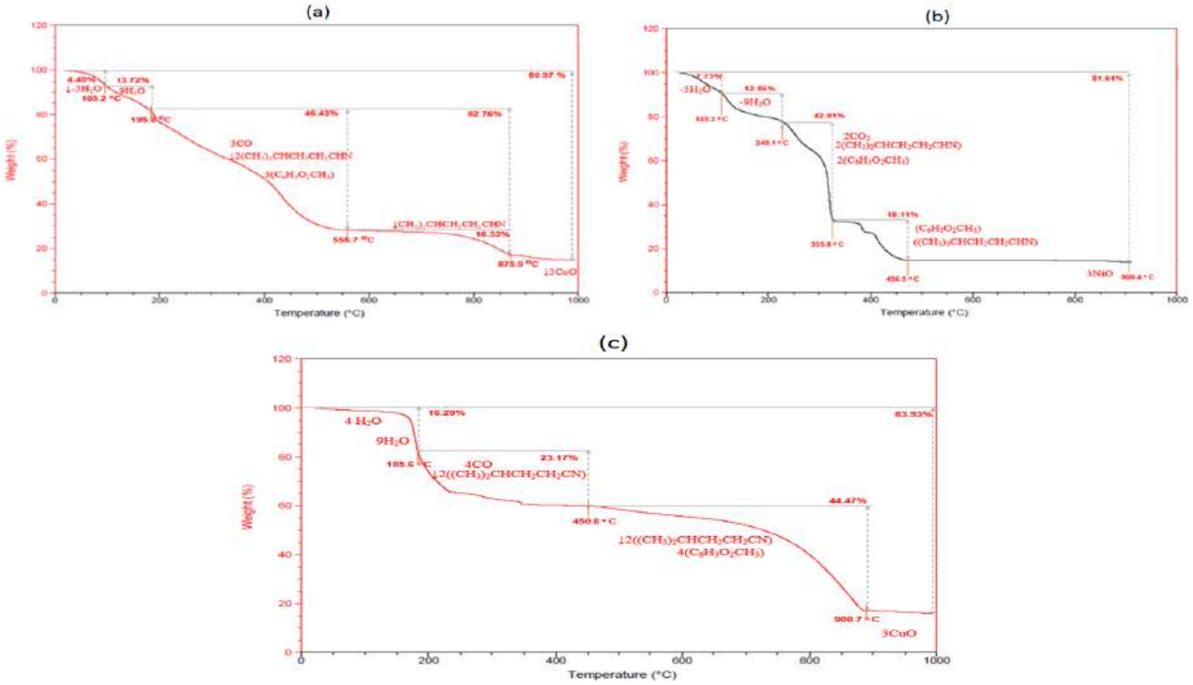


**Figure 3: TGA Curve for (a) $[Co_3(L)_3(H_2O)_9]\ 3H_2O$ (b) $[Ni_3(L)_3(H_2O)_9]\ 5H_2O$ (c) $[Cu_3(L)_4(H_2O)_9]\ 4H_2O$**

**Conductivity**

In this study, the electrical behavior of the complexes was investigated in $10^{-3}$ M DMSO solution. The molar conductivity values for Co(II), Ni(II) and Cu(II) Schiff base complexes were found in the range of 12.04-21.68 $ohm^{-1}$ $cm^2$ $mol^{-1}$ as seen in Table 6. These values were also close to values for non-electrolytic metal complexes reported by Al-Shaheen and Al-Mula, (2015). The low values indicate that the complexes do not dissociate significantly in DMSO, which suggests that they are non-electrolytic in nature (Ali *et al.*, 2013; Geary, 1971). Additionally, the complexes of Co, Ni, and Cu with the Schiff base exhibit a neutral charge due to the balance between the +2 charge (primary valency) of the metal ions and the charge of the ligand. This is reflected in their molar conductivity values, which indicate a zero net charge for the complexes.

**Table 6: Conductivity Measurements of the complexes in $10^{-3}$M DMSO Solution.**

| Compounds | Concentration | Electrical Conductivity ($ohm^{-1}cm^{-1}$) | Molar conductivity ($ohm^{-1}cm^2mol^{-1}$) |
|---|---|---|---|
| **$[Co_3(L)_3(H_2O)_9]3H_2O$** | $1x10^{-3}$ | $13.64x10^{-6}$ | 13.64 |
| **$[Ni_3(L)_3(H_2O)_9]5H_2O$** | $1x10^{-3}$ | $12.04x10^{-6}$ | 12.04 |
| **$[Cu_3(L)_4(H_2O)_9]4H_2O$** | $1x10^{-3}$ | $21.68x10^{-6}$ | 21.68 |

Key: $L^{-2} = C_{14}H_{17}NO_4^{-2}$

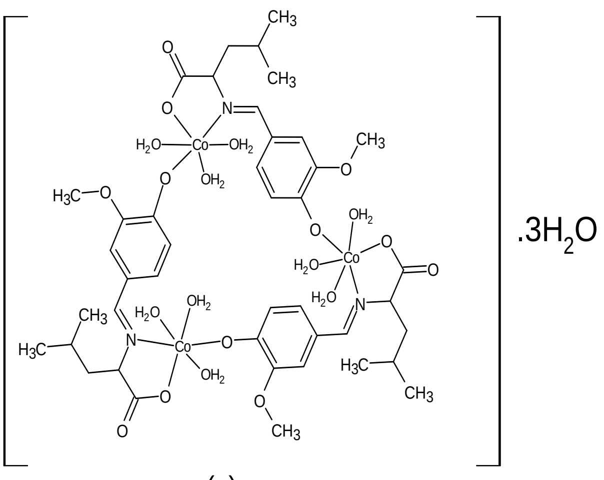
**Magnetic Susceptibility**

The geometry of the complexes around the Co(II), Ni(II) and Cu(II) ions were further supported by the values of their magnetic moments as presented in Table 7. The effective magnetic moment values of Co(II), Ni(II) and Cu(II) complex were found to be 4.51 BM, 3.67 BM, and 1.99 BM respectively. The effective magnetic moment for the complexes was found to be close to the values reported by Housecroft and Sharpe (2008), and Noori *et al* (2013) for paramagnetic metal center resulting in an octahedral geometry. In general, the values of effective magnetic moments are similar to the theoretical value expected for uncoupled $Co^{2+}$, $Ni^{2+}$ or $Cu^{2+}$ ions (Anacona, *et al.*, 2013; El-ghamry *et al.*, 2013) and the paramagnetism observed in the complexes of $Co^{2+}$, $Ni^{2+}$ and $Cu^{2+}$ were due to 3, 2 and 1 unpaired electrons in their d-orbitals respectively, which agreed with the assertion made by Satya *et al* (2006) that weak ligands cannot force electrons of the inner shell to occupy outer d orbitals.

**Table 7: Magnetic Measurements Values of the complexes**

| Compound | Xg (emu $g^{-1}$) | Xm (emu $mol^{-1}$) | Magnetic Moment µeff (BM) |
|---|---|---|---|
| **$[Co_3L_3(H_2O)_9]3H_2O$** | $5.549x10^{-6}$ | $6.579 x 10^{-3}$ | 4.51 |
| **$[Ni_3L_3(H_2O)_9]5H_2O$** | $3.00x10^{-6}$ | $3.663x10^{-3}$ | 3.67 |
| **$[Cu_3L_4(H_2O)_9]4H_2O$** | $5.648 x 10^{-7}$ | $8.422x 10^{-4}$ | 1.99 |

Key: $L^{-2} = C_{14}H_{17}NO_4^{-2}$ Mass susceptibility (Xg) Molar susceptibility (Xm)



(a)

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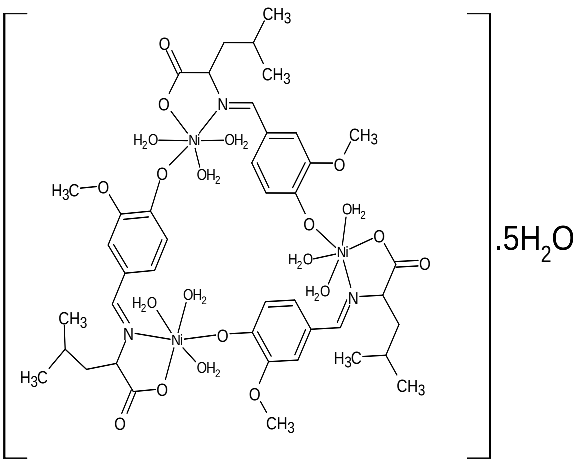
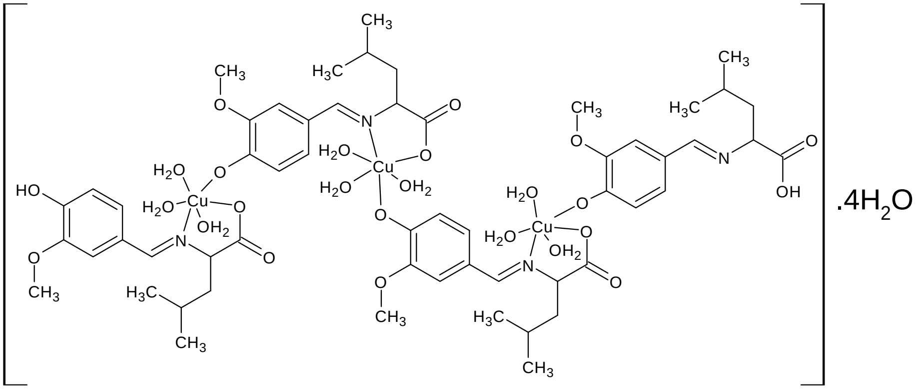


(b)



(c)

**Figure 4: Proposed Structure of trinuclear (a) $[Co_3(L)_3(H_2O)_9]\ 3H_2O$ (b) $[Ni_3(L)_3(H_2O)_9]\ 5H_2O$ (c) $[Cu_3(L)_4(H_2O)_9]\ 4H_2O$**

**Biological Activity**

Bioactivity screening for the anti-bacterial and anti-fungal activities of stock solutions of the ligand and the metal complexes was performed qualitatively using disk diffusion assay (Table 5). Ciprofloxacin and ketoconazole were used as standard drugs. The compounds yielded clear inhibition zones around the disk. Anti-bacterial activities of the ligand and its metal(II) complexes were carried out against two Gram positive bacteria (*Staphyloccus aureus* and *Streptococcus pneumoniae)* and two Gram negative bacteria (*Escherichia coli* and *Salmonella typhi*). The Schiff base was found to be most active against *S. pneumoniae* and *S. aureus* at 40 µ/disc, however, a decrease in activity was observed as the concentration was decreased (Table 8). The ligand was observed to be slightly active against *S. typhi* and in active against *E. coli* bacterial strains. For the metal(II) complexes, the antibacterial assessment revealed greater activity towards *S. aureus* and *S. pneumoniae*, as evident from the inhibition zone (Table 8). The complexes were found to be less active against *S. typhi* and *E. coli* bacterial strains having a zone of inhibition in the range of 6-14 mm at 40 µ/disc, which decreases with a decrease in concentration.

In the case of anti-fungal activity, Ketoconazole was used as a positive control and DMSO as a negative control. The Schiff base was found active against all the fungal species at 40 and 20 µ/disc with a zone of inhibition in the range of 9-11mm. However, the results indicated that the activity of the Schiff base is more pronounced when coordinated with the metal ion which increases with an increase in concentration of the metal(II) complexes. It was evident from the data that, their activity significantly increased upon coordination. The results for anti-fungal activities are presented in Table 8. It is suspected that factors such as cell permeability mechanisms influenced by the presence of metal ions may be the possible reasons for the increase in activity (Akila *et al.,* 2013).

**Table 8: Growth Inhibition Zone of Microbes**

| **ompound** | **Concentration (µg/disc)** | **Inhibition Zone (mm)** | | | | | |
|---|---|---|---|---|---|---|---|
| | | ***Anti-bacterial*** | | | | ***Anti-fungal*** | |
| | | ***.aureus*** | ***.pneumoniae*** | ***. typhi*** | ***.coli*** | ***.tonsurans*** | ***.rubrum*** |
| **igand** | 40 | 11 | 13 | 11 | 6 | 9 | 11 |
| | 20 | 10 | 10 | 9 | 6 | 8 | 8 |
| | 10 | 8 | 8 | 7 | 6 | 6 | 6 |
| **$o_3L_3(H_2O)_9]3H_2O$** | 40 | 15 | 18 | 13 | 11 | 15 | 17 |
| | 20 | 13 | 16 | 14 | 9 | 11 | 14 |
| | 10 | 11 | 14 | 11 | 8 | 9 | 12 |
| **$i_3L_3(H_2O)_9]5H_2O$** | 40 | 155 | 18 | 12 | 14 | 16 | 26 |
| | 20 | 13 | 15 | 11 | 11 | 14 | 22 |
| | 10 | 11 | 13 | 10 | 9 | 12 | 18 |
| **$u_3L_4(H_2O)_9]4H_2O$** | 40 | 14 | 16 | 12 | 10 | 18 | 15 |
| | 20 | 11 | 13 | 10 | 8 | 14 | 12 |
| | 10 | 9 | 10 | 9 | 7 | 9 | 8 |
| **iprofloxacine** | 10 | 25 | 27 | 29 | 25 | - | - |
| **etoconazole** | 10 | - | - | - | - | 34 | 44 |
| **MSO** | - | 6 | 6 | 6 | 6 | 6 | 6 |

Key: Ligand = $C_{14}H_{17}K_2NO_4$ $L^{-2} = C_{14}H_{17}NO_4^{-2}$

## CONCLUSION

This study successfully demonstrates the mechanochemical synthesis, thorough characterization, and notable antimicrobial activity of tri-nuclear Co(II), Ni(II), and Cu(II) complexes with a Schiff base derived from vanillin and L-leucine. The solvent-free synthesis of the Schiff base via mechanical grinding of vanillin and L-valine in an equimolar ratio proved to be highly efficient, yielding a yellow powder with a 90.70% yield. The subsequent formation of metal(II) complexes through manual grinding with solid metal(II) salts at room temperature also resulted in high yields (94.66% for Co(II), 94.30% for Ni(II), and 88.61% for Cu(II)). These compounds exhibited solubility in polar solvents such as methanol, ethanol, DMSO, and DMF, but were insoluble in nonpolar solvents, indicating their polar nature. The infra-red spectroscopic data confirm the formation of the Schiff base via an imine linkage between L-leucine and vanillin. Additionally, the IR spectral data show a shift in the C=N stretching band from 1673 cm$^{-1}$ to lower frequencies (1668-1629 cm$^{-1}$) upon coordination with Co(II), Ni(II), and Cu(II) ions, indicating the formation of metal-ligand bonds. The Schiff base acts as a tridentate dibasic ligand, coordinating with metal ions through phenolic oxygen, imine nitrogen, and carboxylic oxygen (O, N, O), leading to the formation of trinuclear complexes. Elemental analysis, ESI-mass spectrometry, and thermogravimetric analysis results were consistent with the proposed structures and stoichiometries of the complexes. The molar conductivity values fell within the expected range of non-electrolytes, and the effective magnetic moments for the Co(II), Ni(II), and Cu(II) complexes were found to be 4.51 BM, 3.67 BM, and 1.99 BM, respectively, further confirming the geometry of the metal centers. These findings underscore the efficacy of the solvent-free synthetic method and the stability of the resulting complexes. The Schiff base and its corresponding metal complexes exhibited biological activity against various bacteria and fungi strains. Notably, the Schiff base displayed no activity against *E. coli*. Further investigation is needed to determine the minimum inhibitory concentration (MIC) and specific mechanisms of action.

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