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Mechanochemical Synthesis, Characterization and *In-vitro* Anti-Microbial Studies of Binuclear Cu(II) and Zn(II) Complexes with Schiff Base Derived from Phenylalanine and Vanillin

[1*] Sulaiman, Z., [2]Na'aliya, J. and [2]Usman, B.

[1]Department of Science Laboratory Technology, College of Science and Technology, Hussaini Adamu Federal Polytechnic Kazaure, P. M. B. 5004, Jigawa, Nigeria.

[2]Department of Pure and Industrial Chemistry, Bayero University, P. M. B. 3011, BUK, Kano, Nigeria

***Correspondence Email:** zsjahun@yahoo.com

ABSTRACT

Schiff base derived from phenylalanine and vanillin and its corresponding binuclear Cu(II) and Zn(II) complexes have been synthesized by mechanochemical synthesis. The composition and structure of the complexes were characterized by elemental analysis, Infrared spectroscopy, ESI-mass spectrometry, thermogravimetric analysis, conductivity, and magnetic susceptibility measurements. The presence of a new band at 1660 cm^{-1} in the IR spectrum of the ligand due to υ(C=N) co nfirms the formation of the Schiff base and the shifts of this band (1660 cm^{-1}) to higher frequencies in the spectra of the metal(II) complexes indicate complexation. The experimental results showed that the Schiff base binds to the metal ion in a tridentate (O, N, O) manner, resulting in the formation of binuclear metal(II) complexes. The copper and zinc ions are all six coordinated and coordinates through the azomethine nitrogen, carboxylic and phenolic oxygen in addition to oxygen from three coordinated water molecules respectively. The copper(II) and zinc(II) complexes have the general formula$[M_2L_3(H_2O)_6]$ xH_2O (where x = 3 or 0 and M = Cu or Zn). ESI-MS analysis showed m/z =376.03, 1166.77 and 1171.44 correspond to singly charged ions which confirms the molecular weight of the Schiff base and the binuclear Cu(II) and Zn(II) complexes respectively. The molar conductance of the complexes measured is low in the range 20.01-23.68 Ω^{-1} cm^2 mol^{-1}, indicating their non-electrolytic nature. TGA analysis showed the gradual loss of water of hydration, coordinated water molecules, followed by the step-by-step decomposition of the Schiff base into its metal oxide residue. The magnetic susceptibility measurements indicate a diamagnetic binuclear Zn(II) complex, while Cu(II) complex was found to be paramagnetic. The percentage yield of the synthesized compounds was found to be greater than 90%. The anti-fungal and antibacterial activities of the Schiff base and their corresponding metal complexes were investigated *in vitro* using the disc diffusion method against *Staphylococcus aureus, Streptococcus pneumoniae, Salmonella typhi, Escherichia coli, Tinea capitis*, and *Trichophyton rubrum*. Both Schiff base and metal complexes were found to be active against all selected bacterial and fungal species but a much enhanced activity was observed for the complexes.

Keywords: Binuclear metal complex, ESI-Mass spectrometry, Phenylalanine, Thermogravimetric, Vanillin

INTRODUCTION

Schiff bases are one of the important classes of organic compounds synthesized by the condensation of an amine and carbonyl compound. They are nitrogen analogues of an aldehyde or ketone in which the C=O group is replaced by a C=NR group, where R= alkyl, aryl, or H (Loudon and Parise, 2016). The number of amines and keto or aldehyde precursors available for condensation reactions leading to Schiff base compounds is practically unlimited (Matius, 2015). Reports have shown that Schiff bases are good antimicrobial agents and their biological and chemical activities have been attributed to the imine or azomethine functional group (C=N) (Kundu *et al.*, 2009). Among the significant applications of Schiff base include their use as chelating ligands in coordination chemistry (Pervaiz *et al.*, 2019; Bhowmick *et al.*, 2019).

Schiff base metal complexes are prepared via the addition of the Schiff-base ligand to a metal precursor in an appropriate ratio under suitable experimental conditions (Tunde *et al.*, 2021). Schiff base metal complexes find versatile use some of which include catalytic activity, (Tumer *et al.*, 2008: Liu *et al.*, 2018) and luminescent compounds (Kawamoto *et al.*, 2008). Biologically, they have been tested as antibacterial, (Yousif *et al.*, 2017; Al Zoubi *et al.*, 2018) antifungal, (Iftikhar *et al.*, 2018) antitumor (Palanimurugan and Kulandaisamy, 2018), and antiviral agents (Kumar *et al.*, 2010) including insecticides (Zhu *et al.*, 2000).

The conventional method for preparing Schiff bases is by refluxing the carbonyl compounds and amines in organic solvents (Jarrahpour and Zarei, 2011). However, this method has the disadvantage of long response time and provides a low yield (Zhong and Qin, 2014). Furthermore, the large amount of waste created by the generalized use of solvents throughout synthetic processes negatively affects both the environment and public health (Tan and Garcıa 2019). Mechanochemical synthesis on the other hand provides not only a possibility to eliminate the need for bulk solvent use and reduce the generation of waste, but it also unlocks the door to a different reaction environment in which new synthetic strategies, reactions and molecules previously not accessible in solution, can be achieved (Frisˇcˇic´ *et al.*, 2019).

Vanillin is a phenolic aldehyde organic compound with the molecular formula $C_8H_8O_3$. The functional groups include aldehyde, ether, and phenol. It is the primary component of the extract of the vanilla bean. Condensation of vanillin with amino acids offers a binucleating Schiff base categorized by the bridging group that can span two metals having good complexation ability with metal ions (Al-Shaheen, and Al-Mula, 2015; Gavrilova and Bosnich, 2004). In view of this, we report here mechanochemical synthesis and characterization of copper(II) and zinc(II) binuclear complexes with O, N, O donor Schiff base derived from vanillin with phenylalanine, and characterized by different chemical and analytical techniques.

MATERIALS AND METHODS

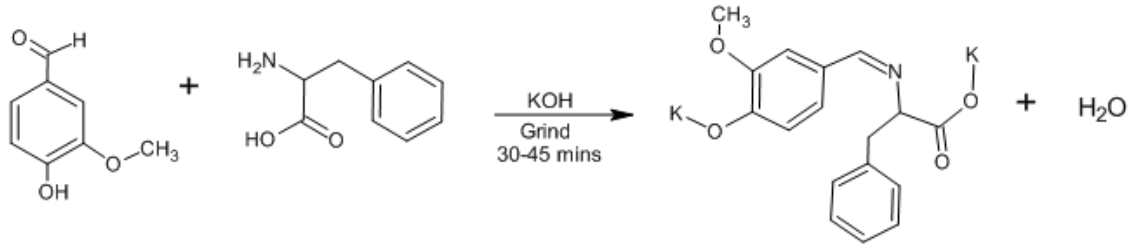
In the synthesis of the Schiff base and their corresponding metal complexes, chemicals (analytical grade) were purchased from Sigma Aldrich and Alfa Aesar and were used without further purification. L–phenylalanine, vanillin, and potassium hydroxide were used as starting materials for the preparation of the Schiff base. The metal salts used were anhydrous $CuCl_2$ and $ZnCl_2$. All glassware 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, *Tinea capitis and Trichophyton rubrum* were obtained from Aminu Kano Teaching Hospital, Kano and identified at the Department of Microbiology, Bayero University Kano. Nutrient agar and potatoes 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 0-1000 ^{o}C. 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

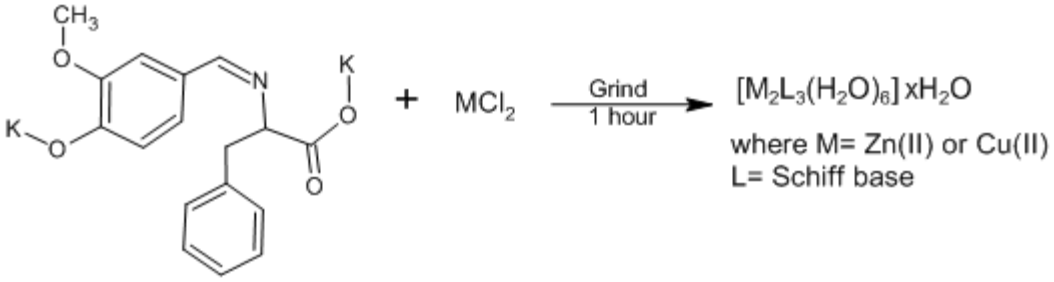
The Schiff base was synthesized using a modified procedure reported by Zhong and Qin (2014), in which phenylalanine (5 mmol) and potassium hydroxide (5 mmol) were weighed and placed in an agate mortar, and continually grinded until the mixture became sticky. Then, vanillin (5 mmol) was added and grinded continuously, until the color of the reactants turned yellow, and a loose solid powder was obtained after about 30-45 minutes. The reaction was carried out at room temperature. The products were washed with cold ethanol and filtered, which was dried for about 30 minutes in a vacuum drying oven at 40 °C.



Scheme 1: Synthesis of the Schiff base

Synthesis of the Metal(II) Complexes

The corresponding metal(II) complexes were obtained using a procedure reported by Zhong and Qin (2014). The metal complexes were synthesized by the addition of each metal(II) salt (5 mmol) and the respective Schiff base (5 mmol) into agate mortar and grinded continuously for about 1 hour. The reaction was carried out at room temperature. The products were washed with cold ethanol and filtered, which was dried for about 30 minutes in a vacuum drying oven at 40 °C.



Scheme 2: Synthesis of the Metal Complexes

Anti-Microbial Activity Screening of Schiff base and Metal complexes

The synthesized Schiff base and the corresponding metal complexes were screened for antibacterial activity against *Stapylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae* and *Selmonella typhi* and antifungal activity against *T. Capitis and T. Rubrum* by disc diffusion method. Ciprofloxacine was used as the standard for anti-bacterial activity, whereas ketoconazole was used as the standard for anti-fungal activity. The Schiff base and each of the two complexes were dissolved in Dimethylsulfoxide (DMSO) to produce four different concentrations of 40, 20, 10, and 5µg per disc. Using 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). A sterilized forceps was used to place the prepared disc of the Schiff base and the complexes on the already inoculated agar plates at various intervals and then incubated at 37^0C for 24hrs (Yusha'u and Salisu, 2011). For the fungal activity, it was left for 3days at room temperature (Hassan *et al.*, 2006). The inhibition zone of the Schiff base and the corresponding metal complexes were then measured (in diameter) around the disc (NCCS, 2008) and were compared with the zone of inhibition due to reference drugs.

RESULTS AND DISCUSSION

The neat griding mechanochemical technique adopted for the synthesis of the compounds produced a yellow, non-hygroscopic crystalline solid Schiff base with a melting point of 118^oC. The complexes were quantitatively obtained as a polycrystalline material by manual grinding of solid metal salts and Schiff base at room temperature having a good percentage yield of 94.30 and 98.13 % for Cu(II) and Zn(II) complexes respectively (Table 1). The composition assigned to the Schiff base and its metal complexes is formulated as presented in Table 1. The solubility screening carried out on the Schiff base and its corresponding metal(II) complexes showed that they are soluble in methanol, ethanol, DMSO, and DMF but insoluble in non-polar solvents such as hexane. The solubility of the synthesized compounds in some common polar solvents was due to the polar nature of the compounds. The values for the elemental analysis obtained were in good agreement with the theoretical values (Table 2).

Table 1: Physical Properties of the Compounds

Compound	Molecular Formula	Molecular Weight	Colour	m.p (^{o}C)	Yield (%)
Ligand	$C_{17}H_{15}O_4NK_2$	376.03	Yellow	118	96.45
$[Cu_2(L)_3(H_2O)_6]3H_2O$	$C_{51}H_{47}O_{23}N_3Cu_2$	1295.00	Blue	-	94.30
$[Zn_2(L)_3(H_2O)_6]$	$C_{51}H_{67}O_{22}N_3Zn_2$	1242.97	White	-	98.13

L= $C_{17}H_{15}O_4NK_2$

Table 2: Elemental analysis of the Compounds

Compound	% Elemental Analyses Observed (Calc)		
	C	**H**	**N**
Ligand	54.25(54.38)	4.25(4.03)	3.72(3.73)
$[Cu_2(L)_3(H_2O)_6]3H_2O$	51.71(51.85)	5.62(5.55)	3.62(3.56)
$[Zn_2(L)_3(H_2O)_6]$	54.01(54.24)	5.31(5.27)	3.80(3.72)

Note: Calculated values are in the bracket

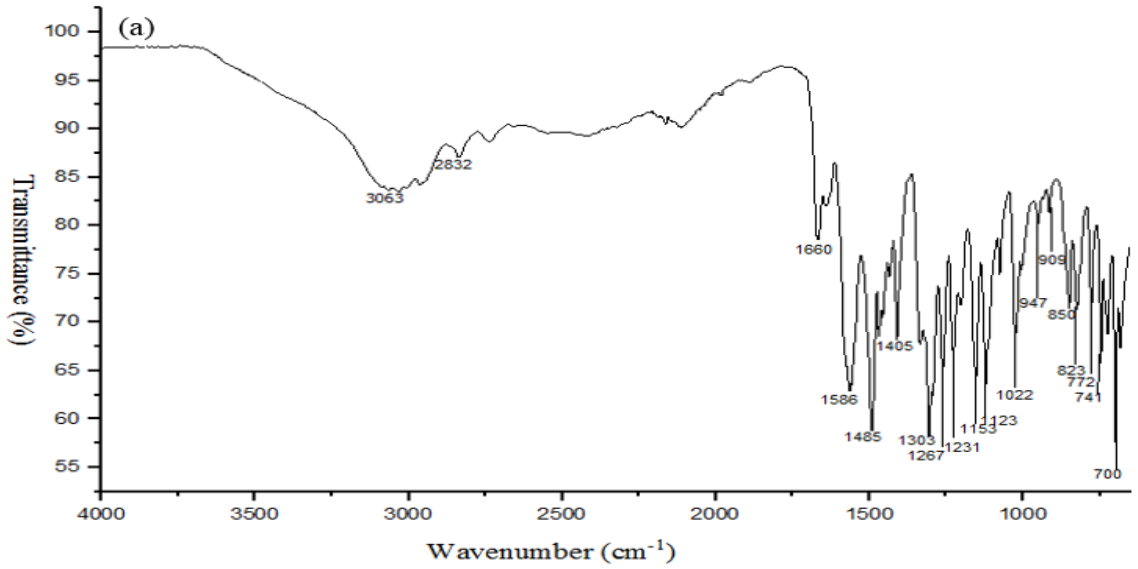
L= $C_{17}H_{15}O_4NK_2$

The important Infrared data of the Schiff base as well as those of the metal complexes are listed in Table 3. The infrared spectrum of the Schiff base (Figure 1a) shows a strong band at 1660 cm^{-1}, which is within the range 1520-1690 cm^{-1} assignment for *v*(C=N) stretching vibrations (Nakamoto, 2009 and Zhong and Qin, 2014) providing evidence of condensation between the aldehyde (CHO) moiety in vanillin and amino group (NH_2) of phenylalanine. On complexation, a shift to a higher wavenumber in the range 1655-1663 $cm^{-1}$was observed in the IR spectra of the complexes, suggesting that the lone pair on nitrogen is involved in the formation of a bond with the metal ion (Figure 1b and 1c). The bands for symmetric and asymmetric carboxyl stretching for the Schiff base were assigned to 1405 cm^{1} and 1586 cm^{-1} respectively (Pretsch *et al.*, 2000). The band for asymmetric stretching in the spectra of the complexes was shifted to lower wavenumber in the range 1559-1565 cm^{-1} while the symmetric carboxyl stretching of *vs*$(COO)^{-}$was shifted to a higher frequency in the range 1428-1441 cm^{-1}. This indicates the formation of linkage between the metal ion and the carboxylate oxygen atom and is also in line with the monodentate type of coordination (Nakamoto, 2009). The spectra of the complexes show a phenolic *v*(C-O) stretching band in the range 1264-1277 cm^{-1} (Pretsch *et al.*, 2000). When compared with that of the ligand (1231cm^{-1}), the band in the complexes shifted towards higher wavenumbers, indicating coordination with the metal ion. Absorption frequencies in the range 3263-3310 cm^{-1} in the infrared spectrum of the metal complexes were assigned to the *v*(O–H) in a water molecule, and the absorption peaks within the range 826-862 cm^{-1} and 739-742 cm^{-1} arise from the rocking and wagging vibrations of the coordinated water molecules (Nakamoto 2009). The bands in the range 443-488 cm^{-1} and 522-527 cm^{-1} were assigned to *v*(M-O) and *v*(M-N) bands respectively.

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	1660	1231	1586	1405	-	-	-	-	
$[Cu_2(L)_3(H_2O)_6]3H_2O$	1663	1264	1559	1428	3263	862	739	443	527
$[Zn_2(L)_3(H_2O)_6]$	1655	1277	1565	1441	3310	826	742	488	522

L=$C_{17}H_{15}O_4NK_2$



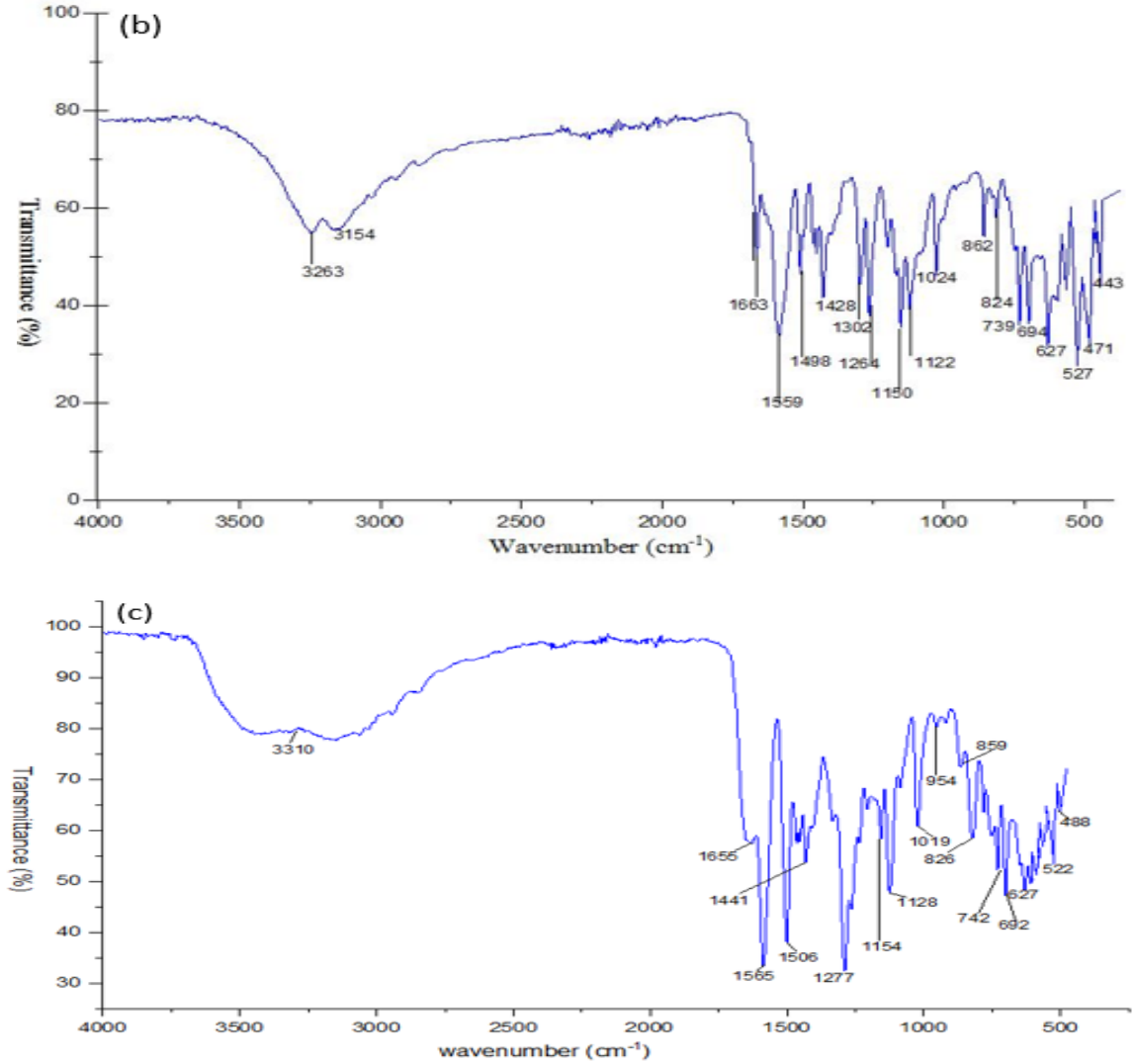
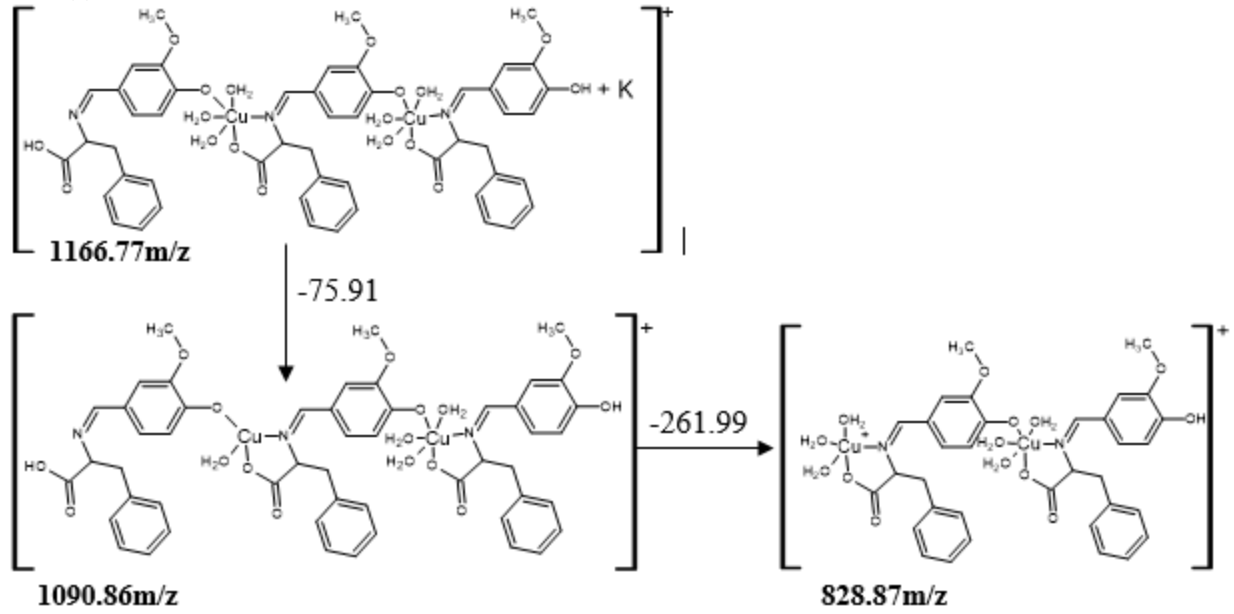
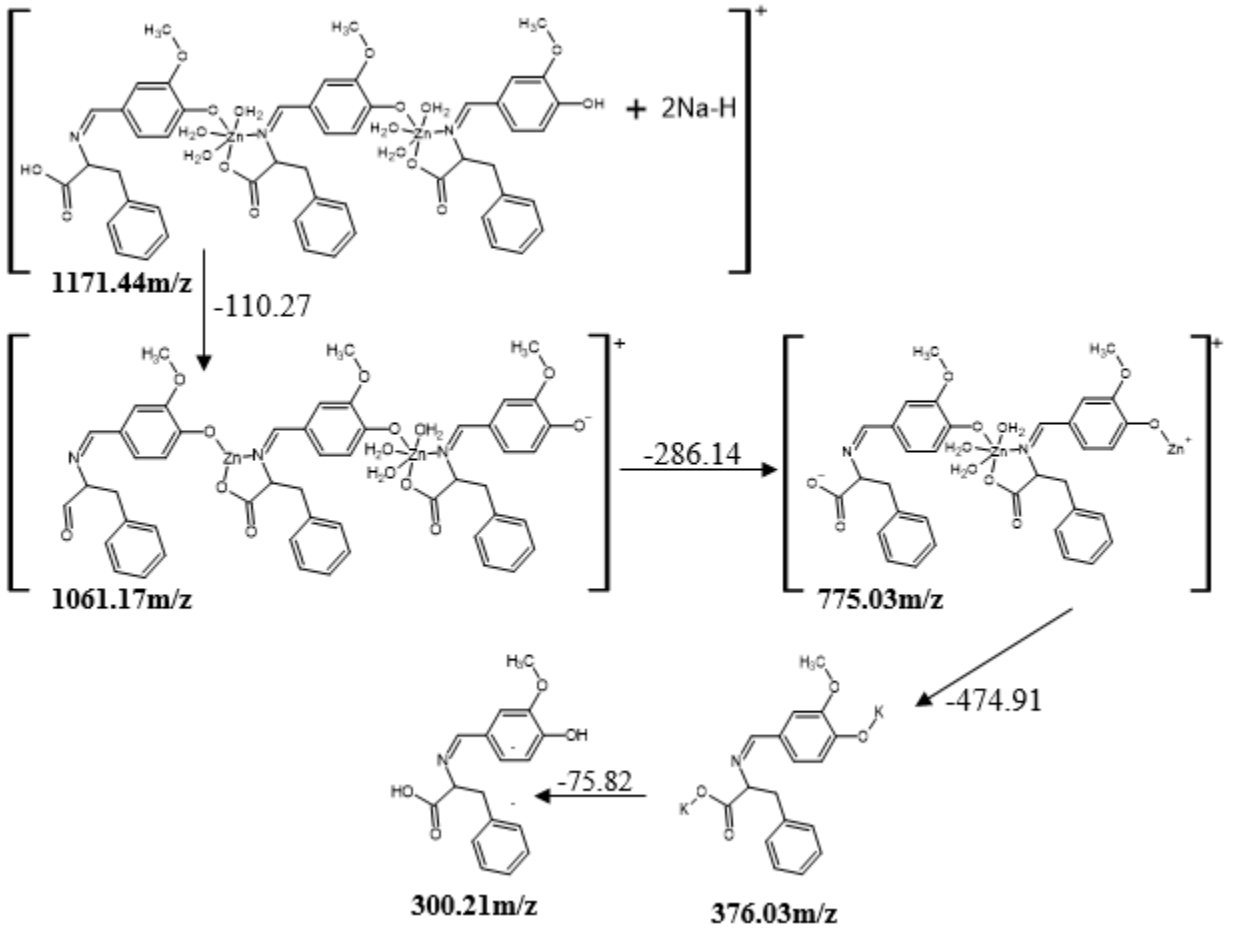


Figure 1: Infrared spectra of (a) the Schiff base (b) $[Cu_2(L)_3(H_2O)_6]3H_2O$ and (c) $[Zn_2(L)_3(H_2O)_6]$

Mass spectrometry performed on the compounds was used as a tool to determine their molecular weight and structure. The mass spectrum of the Schiff base (Fig.2a), showed a peak at 376.03 m/z which corresponds to the molecular weight of the ligand (Table 4). For Cu(II) and Zn(II) complexes, their ESI mass spectra (fig. 2b and 2c) show a base peak at 1166.77 m/z and 1171.44 m/z which corresponds to singly charged potassium and sodium adducts respectively, represented as $[Cu_2L_3(H_2O)_6 +K]^+$ and $[Zn_2L_3(H_2O)_6 +2Na\text{-}H]^+$. This shows their binuclear nature and confirms the presence of coordinated water. Other important peaks include 1090.86m/z, 828.87 m/z and 372.86 m/z corresponding to $[Cu_2L_3(H_2O)_4]^+$, $[Cu_2L_2(H_2O)_6]^+$and $[K_2L]^+$ ion for Cu(II) complex and 1062.17 m/z, 376.03 m/z and 300.12 m/z corresponding to $[Zn_2L_3(H_2O)_2]^+$, $[K_2L]^+$ and $[H_2L]^+$, for Zn(II) complex, showing gradual loss of the Schiff base, coordinated water molecules and the metal ion as seen in Scheme 3 and 4 respectively, further supporting the structure of the compounds. The results of ms-esi are consistent with the proposed formula of the metal(II) complexes as the peaks observed in these spectra support the structure of the complexes and confirm the stoichiometry of the chelates as M_2L_3 type.



Scheme 3: ESI-MS Fragmentation Pattern of $[Cu_2(L)_3(H_2O)_6]3H_2O$



Scheme 4: ESI-MS Fragmentation Pattern of $[Zn_2(L)_3(H_2O)_6]$

Table 4: Mass Spectrometry-ESI for the Compounds

Compound	Found m/z	Expected m/z	Fragment Assigned
Ligand	376.03	375.50	$[L+H]^+$
$[Cu_2(L)_3(H_2O)_6]3H_2O$	1166.77	1168.00	$[Cu_2L_3(H_2O)_6+K]^+$
	1090.86	1091.23	$[Cu_2L_3(H_2O)_4]^+$
	826.87	828.16	$[Cu_2L_2(H_2O)_6]^+$
	376.86	376.13	$[K_2L]^+$
$[Zn_2(L)_3(H_2O)_6]$	1171.44	1171.25	$[Zn_2L_3(H_2O)_6+2Na-2H]^+$
	1062.17	1061.78	$[Zn_2L_3(H_2O)_3]^+$
	775.03	776.12	$[Zn_2L_2(H_2O)_3]^+$
	376.03	376.13	$[K_2L]^+$
	300.12	300.15	$[H_2L]^+$

$L=C_{17}H_{15}O_4NK_2$

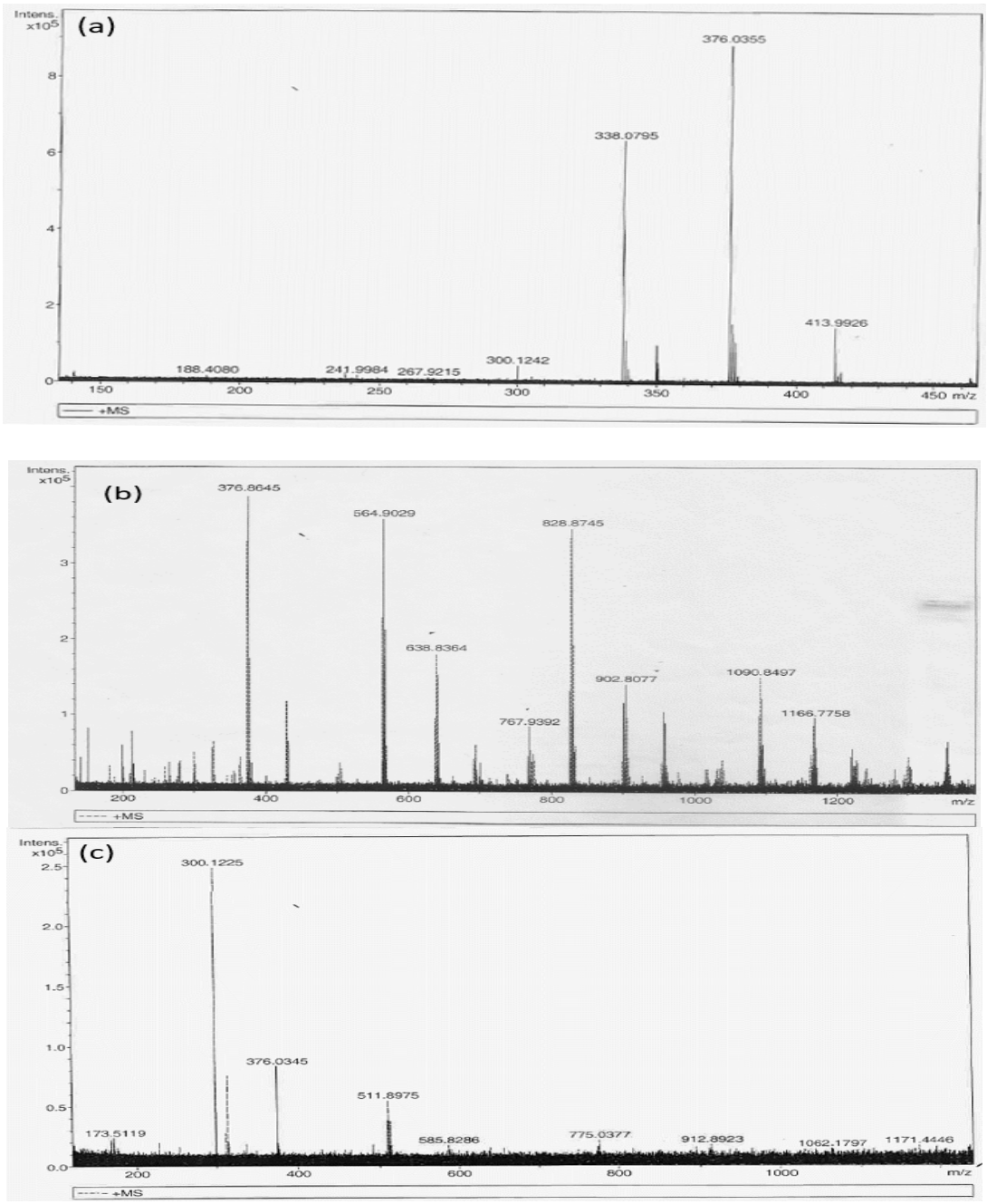


Figure 2: MS-ESI spectra of (a) the Schiff base (b) $[Cu_2(L)_3(H_2O)_6]3H_2O$] and (c) $[Zn_2(L)_3(H_2O)_6]$

The thermogram of Cu(II) complex indicated a total weight loss of 86.61 % up to 1000 ^{o}C, which is observed in various steps (fig.3a). The water of crystallization was lost within the temperature range of 50-101 ^{o}C, with a percentage mass of 4.61%, followed by the removal of coordinated water molecules at a temperature range of 101-149 ^{o}C with a total percent mass loss of 9.81% (Table 5), while the second stage involves the decomposition of the Schiff base having a percentage mass loss of 72.82 % which was found to be very close to with the theoretical mass loss of 72.92%. The final residue was identified to be 2CuO residue and was found to be 13.39 % which is in agreement with the theoretical value (13.36 %).

The decomposition pattern obtained for Zn(II) complex has two distinguishable stages (fig. 3b). The first decomposition represents the loss of water molecules equivalent to 9.55% (calc. 9.57%) in the temperature range 50-165 ^{o}C (Table 5). The second weight-loss represents the decomposition of the Schiff base within a temperature range of 165-898 ^{o}C. The final residue was identified to be 2ZnO and was found to be 14.21 % which is in agreement with the theoretical value (14.16%). In general, metal complexes decompose by losing their water of crystallization first, at a lower temperature as reported by Liao, *et al.*, (2016), as volatilization of lattice water molecules occur mostly in the range 50-110 ^{o}C, while coordinated water is usually eliminated at higher temperatures above 100 ^{o}C up to 315 °C (Mounika *et al.*, 2016).

Table 5: Thermal Decomposition Data of the Complexes

Compound	Temp range (^{o}C)	Mass loss from TG (%)	Theoretical mass loss (%)	Weight loss from TG	Theoretical weight loss		Probable assignment
$[Cu_2(L)_3(H_2O)_6]3H_2O$	50-101	4.61	4.57	54.46	54.03	$3H_2O$	Loss of water of crystallization
	101-149	9.18	9.15	108.44	108.06	$6H_2O$	Loss of coordinated water
	149-800	72.82	72.92	860.18	861.31	$\downarrow 2CO$ $\downarrow 2C_6H_3OCH_3CNCH_2CO_3$ $\downarrow C_6H_3CH_3NCH_2$	Decomposition of the Schiff base
	800-1000	13.39	13.36	158.17	157.85	2CuO	Metal oxide residue
		100	**100**	**1181.25**	**1181.25**	**$[Cu_2L_3(H_2O)_6]$ $3H_2O$**	
$[Zn_2(L)_3(H_2O)_6]$	50-165	9.55	9.57	107.84	108.06	$6H_2O$	Loss of coordinated water
	165-898	76.24	76.27	860.92	861.31	$\downarrow 2OC_6H_3OCH_3CHNCH\ CH_2$ $2C_6H_3CO$ $\downarrow (OC_6H_3OCH_3CNCH$ $CH_2C_6H_3C)$	Decomposition of the Schiff base
	898-1000	14.21	14.16	160.46	159.85	2ZnO	Residue as metal oxide
		100	**100**	**1129.22**	**1129.22**	**$[Zn_2L_3(H_2O)_6]$**	

L= $C_{17}H_{15}O_4NK_2$

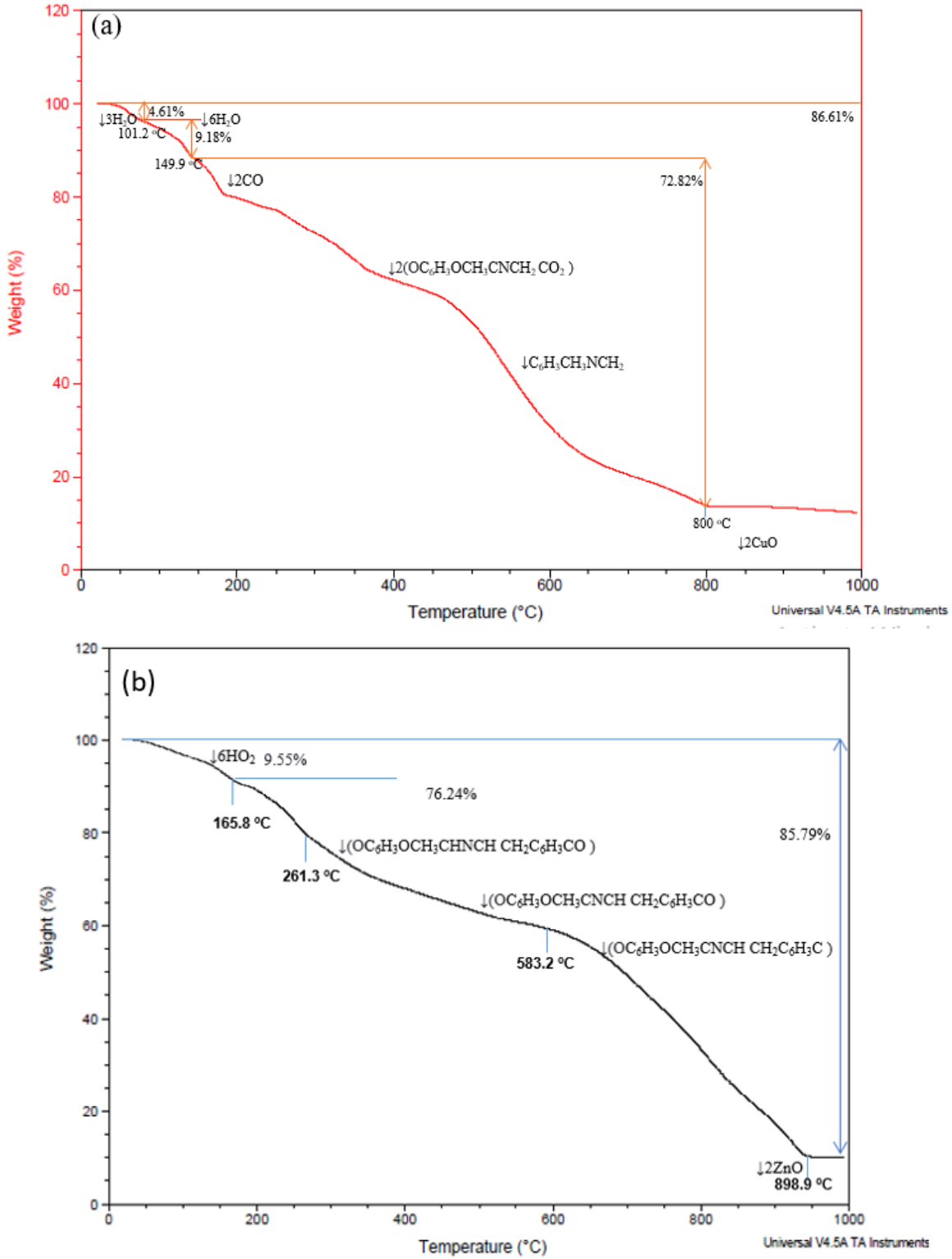


Figure 3: TG curve for (a) $[Cu_2(L)_3(H_2O)_6]3H_2O$ and (b) $[Zn_2(L)_3(H_2O)_6]$

The electrical behavior of the complexes was investigated in 10^{-3} M DMSO solution and the molar conductance data were exploited to ascertain the electrolytic or non-electrolytic nature of metal complexes. The molar conductance data also provides a clue of the number of ions present in a particular solution responsible for the conduction of electric currents (Ali *et al.*, 2013). The molar conductivity values for the binuclear Cu(II) and Zn(II) Schiff base complexes were found in the range 20.01-23.68 $ohm^{-1}cm^2$ mol^{-1} (Table 6) which fall within the limit <50 $ohm^{-1}cm^2$ mol^{-1} for non-electrolytic metal complexes in DMSO. These low molar conductance values indicate non-electrolytic complexes and confirm the absence of counter ions (Ali *et al.*, 2013; Geary, 1971).

Table 6: Conductivity Measurements for the Metal(II) Complexes.

Compounds	Electrical Conductivity ($ohm^{-1}cm^{-1}$)	Molar conductivity ($ohm^{-1}cm^{2}mol^{-1}$)
$[Cu_2(L)_3(H_2O)_6]3H_2O$	$20.01x10^{-6}$	20.01
$[Zn_2(L)_3(H_2O)_6]$	$23.68x10^{-6}$	23.68

L=$C_{17}H_{15}O_4NK_2$

The geometry of the complexes around the Cu(II) and Zn(II) ions was further supported by the values of their magnetic moments as presented in Table 7. Zn(II) complex is diamagnetic, while Cu(II) complex has an effective magnetic moment of 1.97 M.B, this value is very close to the theoretical value expected for two independent copper(II) ions. Thus, these complexes have a non-interacting metal centre with magnetic properties that are similar to the literature reported by Anacona *et al* (2013).

Table 7: Magnetic Measurements for the Metal(II) Complexes

Compound	Xg ($emu\ g^{-1}$)	Xm ($emu\ mol^{-1}$)	Magnetic Moment μeff (BM)
$[Cu_2(L)_3(H_2O)_6]3H_2O$	$8.407x\ 10^{-7}$	$9.931x\ 10^{-4}$	1.97
$[Zn_2(L)_3(H_2O)_6]$	$-5.806x\ 10^{-7}$	$-6.556\ x10^{-4}$	Dia

L= $C_{17}H_{15}O_4NK_2$ Xg=Mass susceptibility Xm=Molar susceptibility

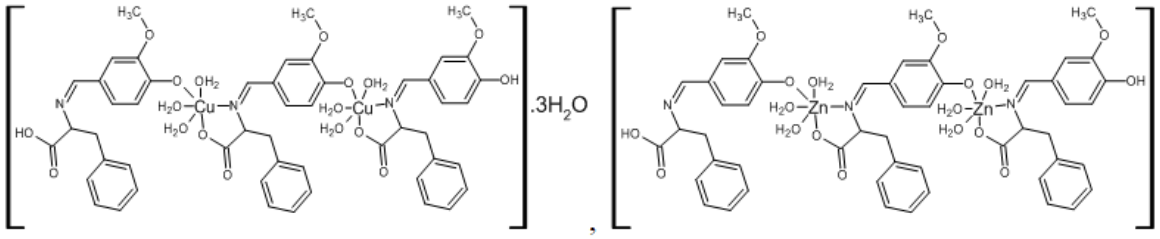


Figure 4: Proposed Structure of binuclear $[Cu_2(L)_3(H_2O)_6]3H_2O$ and $[Zn_2(L)_3(H_2O)_6]$

The Schiff base was found to be active against all the tested bacterial strains, with a zone of inhibition ranging from 11 mm for *S. aureus* and *S. pneumoniae* and 10 mm to 9 mm for *S. typhi* and *E.coli* respectively at 40 µg/disc as presented in table 8. It was also observed that the metal complexes showed an increased zone of inhibition against the bacterial strains compared to the Schiff base. Cu(II) complex was found to be the most effective against all the selected bacterial species, having 18 mm, 16 mm, 14 mm, and 12 mm, respectively (Table 8) for *S. aureus, S. pneumoniae, E. coli,* and *S. tyhpi* respectively at 40µg/disc. Cu(II) complex show intermediate inhibition zones while Zn(II) complex shows the least inhibition zone against all the tested bacterial species. In general, the antibacterial activity data revealed that the Schiff base and their corresponding metal complexes show weak to good activity when compared to the positive control (Ciprofloxacin) and negative control (DMSO). The results showed that the activity of the Schiff bases is more pronounced when coordinated to the metal ions and their activity increases with an increase in concentrations. It was also seen that most of the compounds are most active against *S. typhi* and *S. pneumonia* bacterial strains which are the gram-positive type. This may be because gram-positive bacteria are more susceptible to antibiotics due to the lack of an outer membrane (Lakna, 2017). The weak antibacterial activity against gram-negative bacteria can be ascribed to the presence of an outer membrane that possesses hydrophilic polysaccharides chains as a barrier to the amino acid complexes (Stanila, *et al.,* 2011). However, for anti-fungal assay vanillin-phenylalanine, Schiff base was found to be inactive against *T. capitis* in all concentrations. The metal complexes of the Schiff base showed much-enhanced activity when compared to the uncoordinated ligand, 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 solubility, conductivity and cell permeability mechanism (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

Compound	Concentration (µg/disc)	Inhibition Zone (mm)					
		Anti-bacteria				*Anti-fungal*	
		S.aureus	***S.pneumoniae***	***S. typhi***	***E.coli***	***T.capitis***	***T.rubrum***
Ligand	40	11	11	10	9	6	13
	20	10	10	8	8	6	11
	10	8	9	7	7	6	10
	5	7	7	6	6	6	8
$[Cu_2L_3(H_2O)_6]3H_2O$	40	18	16	14	12	13	14
	20	16	15	11	10	11	12
	10	13	13	9	9	9	10
	5	10	10	8	8	7	9
$[Zn_2L_3(H_2O)_6]$	40	16	15	11	11	12	16
	20	13	11	10	10	13	14
	10	11	9	8	9	11	12
	5	9	8	7	7	9	8
Ciprofloxacin	10	25	27	25	29	-	-
Ketoconazole	10	-	-	-	-	34	44
DMSO	-	6	6	6	6	6	6

L= $C_{17}H_{15}O_4NK_2$

CONCLUSION

The copper(II) and zinc(II) complexes of phenylalanine-vanillin Schiff base have been synthesized by mechanochemical grinding at room temperature. The complexes were characterized by elemental analysis, IR, MS-ESI and TGA and were found to be binuclear, coordinating in a tridentate manner through carboxylic and phenolic oxygen in addition to the imino nitrogen (Fig. 4). The analytical data showed that the metal-ligand stoichiometry in the complexes is 2:3. The complexes are non-electrolytic in DMSO as established from their molar conductivities values. The thermal decomposition processes of the complexes include dehydration and pyrolysis of the Schiff base and the final residue was identified to be metal(II) oxide (2CuO or 2ZnO). The Schiff base and its corresponding metal complexes were found to be biologically active against the selected bacteria and fungi isolates with exception of the Schiff base being inactive against *S. typhi* and *E. coli* at 5 µg concentration and *T. capitis* in all concentrations.

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