

## Synthesis, characterization and antimicrobial studies of *o*-phenylenediamine Schiff base, Ni(II), Cu(II), Zn(II) complexes and Cu(II) adduct

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### Abstract

The Schiff base ligand, *N, N'*-bis(salicylidene)-1,2-phenylenediamine (H<sub>2</sub>PSA), was derived by the reaction of *o*-phenylenediamine and salicylaldehyde under reflux. Ni(II), Cu(II) and Zn(II) complexes of H<sub>2</sub>PSA were also obtained. The Cu(II) adduct was derived by the equimolar addition of 1,10-phenanthroline to the precipitated Cu(II) complex. H<sub>2</sub>PSA was characterized by melting point, <sup>1</sup>H and <sup>13</sup>C NMR, infrared and UV/Vis spectroscopies; and the metal compounds by magnetic susceptibility measurements, percentage metal and elemental analyses. Percentage metal and elemental analysis showed the stoichiometry of the complexes as [Ni(PSA)], [Cu(PSA).H<sub>2</sub>O], [Zn(H<sub>2</sub>PSA)(SO<sub>4</sub>).ZnSO<sub>4</sub>], and the copper(II) adduct as [Cu(PSA)(Phen)]. The infrared (IR) spectrum of the ligand showed the bands due to ν(C=N) and ν(C–O) at 1613 cm<sup>-1</sup> and 1316 cm<sup>-1</sup> respectively. These bands were shifted in the IR spectra of the metal complexes to 1616-1607 cm<sup>-1</sup> and 1340-1324 cm<sup>-1</sup> respectively, indicating the participation of imine nitrogen and phenolic oxygen in the chelate formation. Both nickel(II) and zinc(II) complexes were diamagnetic with magnetic moments of zero while the copper(II) complex and adduct had magnetic moments of 1.70 and 2.20 B.M respectively. The electronic spectra of nickel(II) and Cu(II) complexes demonstrated band absorptions between 450 nm and 490 nm, which were assigned to transitions in the square planar geometry. The antimicrobial activity of the compounds against four bacterial and two fungal strains showed moderate activity at inhibition diameters of 14–18 mm.

Keywords: *o*-phenylenediamine; Schiff base; metal complexes; copper(II) adduct; antimicrobial activity

### Introduction

Schiff base transition metal complexes are one of the most interesting and thoroughly studied systems. Schiff base of transition metals and adducts have received a lot of attention due to their unique coordination, catalytic, clinical, photochemical, electrochemical, analytical and biological activities [1–4]. Metal(II) complexes of Schiff bases derived from salicylaldehyde and *o*-phenylenediamine are of particular interest due to their synthetic flexibility, rich coordination chemistry, stability and application in catalysis [3, 5-7]. They form symmetrical tetradentate Schiff base metal complexes where the coordination takes place through the N<sub>2</sub>O<sub>2</sub> donor set [1, 8-10]. This study reports the findings on the spectral and antimicrobial assay of Ni(II), Cu(II) and Zn(II) complexes, and the adduct with 1,10-

phenanthroline. The ligand used in this study was derived from condensation of salicylaldehyde and *o*-phenylenediamine and synthesized with Ni(II), Cu(II) and Zn(II) metal salts. The derived metal complexes were reacted with 1,10-phenanthroline in order to form the adduct compounds.

### Materials and methods

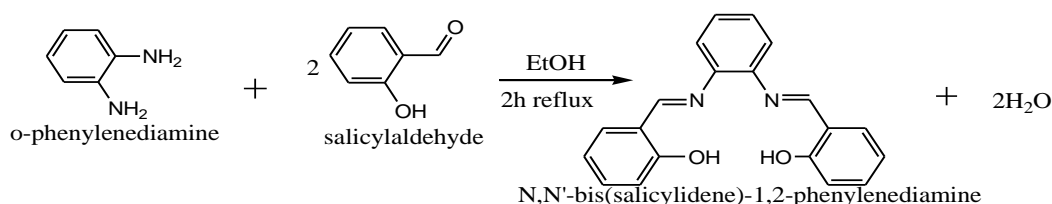
All the reagents and solvents were of analytical grade and used without further purification. The uncorrected melting/decomposition points were determined on Gallenkamp melting point apparatus. The percentage metal content was determined by EDTA complexometric titration. Elemental analyses (CHNS) were carried out on Elementar Analysensysteme vario MICRO 1.6.2 GmbH. <sup>1</sup>H and <sup>13</sup>C



NMR spectra of the ligand were obtained in DMSO- $d_6$  relative to the residual proton in the solvent on Bruker Avance 400 MHz NMR spectrometer. The infrared spectra were obtained as KBr discs on a Perkin-Elmer FTIR spectrophotometer within the range of 4000–400  $\text{cm}^{-1}$ . Room temperature magnetic susceptibilities were measured on a Sherwood scientific magnetic susceptibility balance and diamagnetic corrections were calculated using Pascal's constants.

### Synthesis

The ligand, H<sub>2</sub>PSA, was synthesized following the described method [1]: *o*-phenylenediamine (0.4144g, 3.8321 mmol) was dissolved in 10 mL EtOH, and salicylaldehyde (0.8 mL, 7.6641 mmol) was added in drops while stirring on a magnetic hot plate stirrer. On completion of the addition, the mixture was heated to reflux. The reaction was monitored by thin layer chromatography, after 2 h, the precipitate formed was filtered, washed several times using ethanol and allowed to dry. The golden yellow precipitate gradually changed to orange colour and the mass obtained was 1.1786 g. The reaction Scheme was presented in Scheme 1.



**Scheme 1:** The reaction scheme for the synthesis of H<sub>2</sub>PSA

### Antimicrobial studies: Procedure for antimicrobial susceptibility test

The antimicrobial studies of the synthesized compounds were carried out using the surface spread method. The microorganisms used for this work were gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia Coli*) and fungi (*Candida albicans*, *Aspergillus niger*). Gentamicin (10  $\mu\text{g/mL}$ ) was used as a standard drug for bacteria (positive control) and Ketoconazole for fungi.

Using agar well diffusion method, 20 mL Muller Hinton Agar medium was prepared and allowed to cool to room temp 20–30°C. The agar medium was poured into different Petri dishes and allowed to set. The surface of the agar medium was dried. Cells from overnight broth cultures were suspended in normal saline until it produces turbidity equal to 0.5 McFarland from which inoculation was surface

[Ni(PSA)] was prepared as follows: H<sub>2</sub>PSA (0.3000 g, 0.9483 mmol) was dissolved in warm ethanol, 3 drops of triethylamine were added and NiCl<sub>2</sub>·6H<sub>2</sub>O (0.2253 g, 0.9483 mmol) in 3 mL ethanol was added while stirring. The mixture was left to reflux for 2 h. The red coloured precipitate was left to cool and was obtained by filtration. The mass of the product was 0.2010 g. [Cu(PSA)] and [Zn(H<sub>2</sub>PSA)(SO<sub>4</sub>)]·ZnSO<sub>4</sub>, were similarly prepared using H<sub>2</sub>PSA (0.3000 g, 0.9483 mmol), CuCl<sub>2</sub>·2H<sub>2</sub>O (0.1617 g, 0.9483 mmol), ZnSO<sub>4</sub>·7H<sub>2</sub>O (0.2727g, 0.9483 mmol). The masses of the products obtained were 0.1178 g and 0.2870 g respectively.

To prepare the adducts, each metal complex [Ni(PSA)] (0.0710 g, 0.1903 mmol); [Cu(PSA)] (0.0300 g, 0.0794 mmol) or [Zn(H<sub>2</sub>PSA)(SO<sub>4</sub>)]·ZnSO<sub>4</sub> (0.1000 g, 0.2634 mmol) was dissolved in 3 mL ethanol, while heating and stirring, an equimolar amount of 1,10-phenanthroline (Phen) was added in bits. The mixture was refluxed for 3 h, cooled and the precipitates obtained by filtration. The analytical data for the metal complexes and copper adduct have been presented in Table 1.

spread on Agar medium plate with the aid of swab sticks. A sterile cork borer (8 mm) was used to create equidistance well on each of the set and inoculated agar plate. The test compounds were prepared by dissolving 10 mg of a compound in 1 mL of DMF and 9 mL of water, giving a concentration of 1000  $\mu\text{g/mL}$ . A serial dilution with water was done to obtain two other concentrations of 100  $\mu\text{g/mL}$  and 10  $\mu\text{g/mL}$ . Each well was filled with 4 drops of the different concentrations of the compounds and allowed to diffuse for 1 h before inoculation. The 10% DMF used in dissolving the extract was used as negative control to see if it has antimicrobial effect. Gentamicin and Ketoconazole were used as the standard drugs (positive controls). The plates were then incubated at 37°C for 18–24 h and 25°C for 48 h for bacteria and fungi respectively. All the plates were done in duplicate.

The plates were then examined for zone of inhibition measured in duplicates.

#### *Minimum Inhibition Concentration (MIC) Procedure.*

The MIC of the ligands and metal complexes were determined using Broth Dilution method. Different concentrations (1000, 750, 500, 250 and 125  $\mu\text{g/mL}$ ) of the ligand, metal complexes and adducts were prepared. The test compounds were prepared by dissolving 10 mg of a compound in 1 mL of DMF and 9 mL of nutrient broth. A serial dilution was done by adding 7.5 mL of the previous mixture to 2.5 mL of broth to obtain 1000, 750 and 500  $\mu\text{g/mL}$ . 5 mL of the previous mixture was then added to 5 mL of nutrient broth to obtain 250 and 125  $\mu\text{g/mL}$ . All broth used were double strength. Standard volume of each isolate i.e. 0.1 mL was aseptically inoculated into different concentrations of the test samples. All tubes were incubated at 37°C for 24 h. MIC was determined using visual method i.e. the lowest concentration of the test sample that showed no turbidity.

### **Results and discussion**

The melting points, percentage metal, elemental analysis, and magnetic moments were used to characterize the synthesized compounds. The melting points of the complexes were different from the Schiff base. The elemental analysis for the metal compounds and the percentage metal analysis were in good agreement with the proposed formulas. The analysed also showed the adducts of Ni(II) and Zn(II) complexes with phen were not formed, only the Cu(II) adduct was obtained. The analytical data of the ligand H<sub>2</sub>PSA, metal complexes and adduct are shown in Table 1. The ligand was soluble in dichloromethane, acetone, ethyl acetate and DMF while the metal compounds were only soluble in DMF.

#### *Infrared spectra*

The important stretching modes in the infrared spectra of the synthesized compounds were identified as  $\nu\text{O-H}$ ,  $\nu\text{C=N}$ ,  $\nu\text{C-O}$ ,  $\nu\text{C-N}$  and  $\nu\text{C=C}$  (Table 2). The band at 3430  $\text{cm}^{-1}$  was assigned to O-H in the ligand. The band was absent in the spectra of the complexes and adduct, indicating the

deprotonation and involvement of the enol oxygen in chelation. The broad band at 3399  $\text{cm}^{-1}$  in the spectrum of [Cu(PSA)].2H<sub>2</sub>O and 3416  $\text{cm}^{-1}$  in the spectrum of [Zn(PSA)SO<sub>4</sub>].ZnSO<sub>4</sub> are assigned to the O-H frequency of the coordinated water [2]. The Schiff base ligand showed a strong IR band at 1613  $\text{cm}^{-1}$  region characteristic of azomethine absorption (C=N) [9]. This band shifted to lower frequencies in the metal complexes and adduct, indicating coordination of the nitrogen atom of Schiff base to the metal. The Schiff base, H<sub>2</sub>PSA, showed bands at 1585-1480  $\text{cm}^{-1}$  which were assigned to C=C, these were also observed in the spectra of the metal complexes in the range 1585-1487  $\text{cm}^{-1}$  [8]. Similarly, the phenolic C-O stretching vibrations, which appeared at 1316  $\text{cm}^{-1}$  in the Schiff base underwent a shift towards higher frequency (8-24  $\text{cm}^{-1}$ ) in the complexes. This shift confirmed the participation of oxygen in the C-O-M bond [1] and the involvement of O donor atoms in coordination to the metal ions [2]. The C-N band at 1277  $\text{cm}^{-1}$  in H<sub>2</sub>PSA was lowered by 13-32  $\text{cm}^{-1}$  in the metal complexes and adduct. In the low frequency region, the bands observed in the metal complexes in the regions 437-459  $\text{cm}^{-1}$  and 544-561  $\text{cm}^{-1}$  were attributed to M→O (phenolic) and M→N respectively. The IR data suggested that the metals were bonded to the Schiff bases through the phenolic oxygen and the imine nitrogen [1]. The strong bands, which are very prominent in the ranges 387-370  $\text{cm}^{-1}$  and 600-630  $\text{cm}^{-1}$  were attributed to N-H and C-H band of 1,10-phenanthroline respectively [2].

#### *Nuclear magnetic resonance (NMR)*

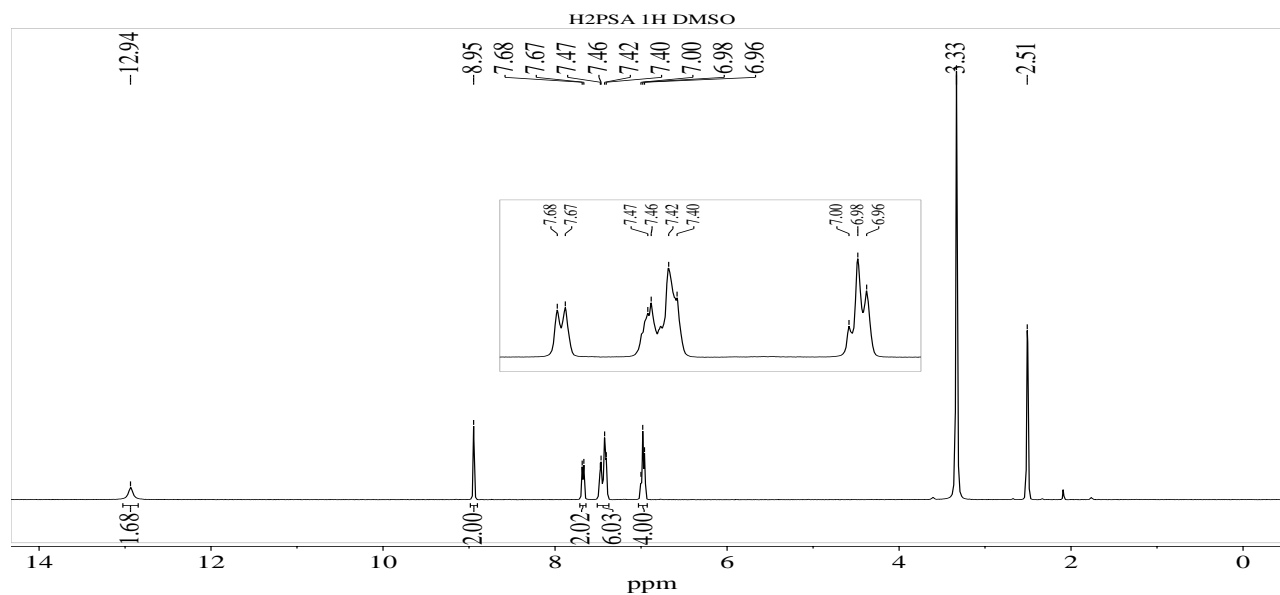
The <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra of the ligand, H<sub>2</sub>PSA, were obtained in DMSO-d<sub>6</sub> (Figures 1 and 2). In the <sup>1</sup>H-NMR spectrum of H<sub>2</sub>PSA, the weak broad absorption at 12.94 ppm (2H, s) was observed for the hydrogen bonded O-H [1]. The intense singlet peak at 8.95 ppm (2H, s) was ascribed to the frequency shifts of the azomethine protons (-N=C-H) [11]. The aromatic protons appeared as multiplet signals in the range 6.96–7.68 ppm. The <sup>13</sup>C-NMR spectrum showed the presence of aromatic carbons at 117-143 ppm. The absorption due to the azomethine carbon was seen at 161.81 ppm.

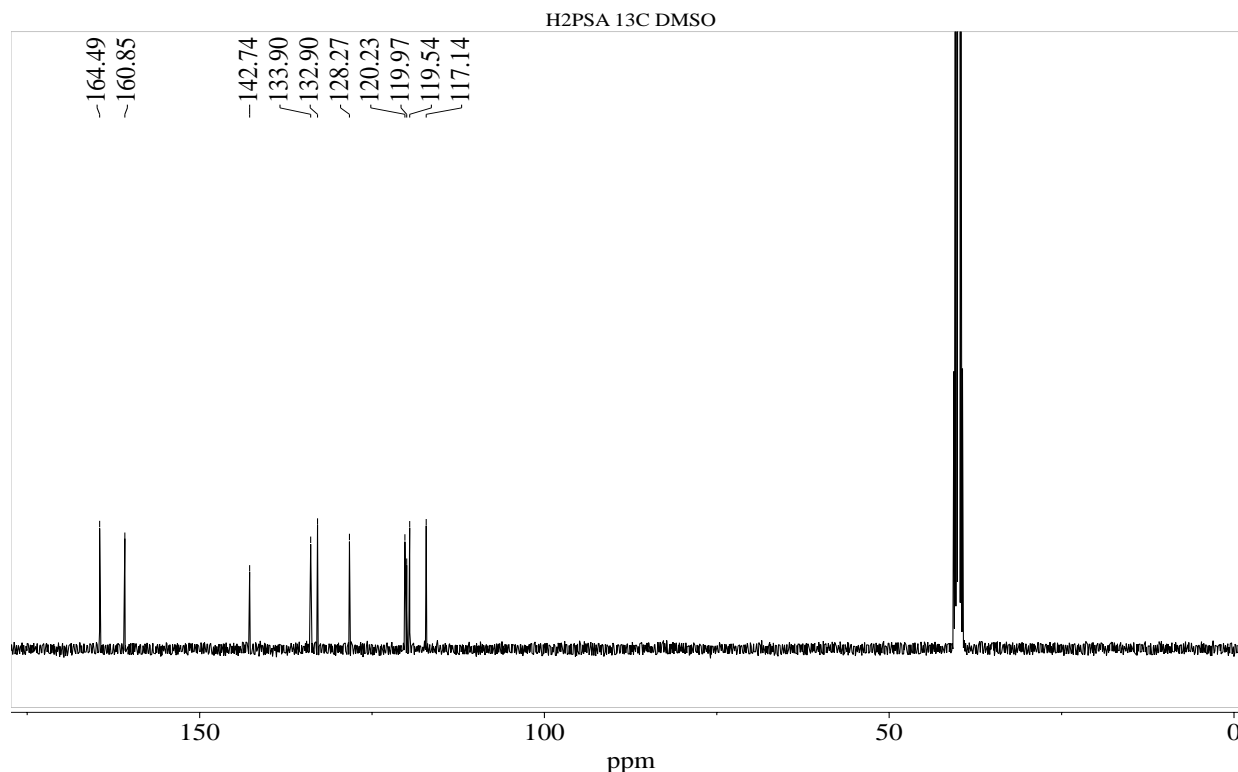
**Table 1:** Analytical data of the compounds

Compounds	Colour	M.pt. °C	C Found	H (Calculated)	N %	M	Yield (%)	$\mu_{\text{eff}}$ (BM)
H <sub>2</sub> PSA	Orange	164-166	---	---	---	---	97	---
C <sub>20</sub> H <sub>16</sub> N <sub>2</sub> O <sub>2</sub>								
[Ni(PSA)]	Red	328-330	64.18	3.57	7.45	16.25	65	D
C <sub>20</sub> H <sub>14</sub> N <sub>2</sub> NiO <sub>2</sub>			(64.40)	(3.78)	(7.51)	(15.73)		
[Cu(PSA)].2H <sub>2</sub> O	Brown	110-112	---	---	---	15.10	43	1.7
C <sub>20</sub> H <sub>18</sub> CuN <sub>2</sub> O <sub>4</sub>			---	---	---	(15.35)		
[Cu(PSA)(Phen)].H <sub>2</sub> O	Brown	340 <sup>Dec</sup>	66.53	3.88	10.17	11.20	68	2.2
C <sub>32</sub> H <sub>24</sub> CuN <sub>4</sub> O <sub>3</sub>			(66.71)	(4.20)	(9.73)	(11.03)		
[ZnSO <sub>4</sub> (PSA)].ZnSO <sub>4</sub>	Yellow	210-212	37.74	2.51	4.30	16.81	47	D
C <sub>20</sub> H <sub>16</sub> N <sub>2</sub> O <sub>10</sub> S <sub>2</sub> Zn <sub>2</sub>			(37.58)	(2.52)	(4.38)	(16.43)		

<sup>Dec</sup>Decomposition temperature<sup>D</sup>Diamagnetic**Table 2:** Infrared spectral data of the Schiff base, metal complexes and adduct.

$\nu$ (cm <sup>-1</sup> )	H <sub>2</sub> PSA	[Ni(PSA)]	[Cu(PSA)]	[Cu(PSA) (Phen)]	[ZnSO <sub>4</sub> (PSA)]. ZnSO <sub>4</sub>
O-H	3430	----	3399	3428	3416
C=N	1613	1608	1608	1607	1616
C-C	1585	1577	1577	1580	1585
	1450	1458	1457	1460	1463
C-O	1316	1340	1335	1337	1324
C-N	1277	1252	1255	1260	1245
M-N	----	544	559	561	550
M-O	----	458	440	437	451

**Figure 1:** <sup>1</sup>H-NMR spectrum of H<sub>2</sub>PSA at 400 MHz



**Figure 2.**  $^{13}\text{C}$ -NMR spectrum of  $\text{H}_2\text{PSA}$  at 400 MHz

### Electronic spectra

The electronic spectra of the Schiff bases and their complexes are summarized in Table 3. The spectrum of the Schiff base showed bands at 293, 330 and 392 nm. The first was attributed to  $\pi \rightarrow \pi^*$  while the second and third bands were attributed to  $n \rightarrow \pi^*$  transitions. These bands were not significantly affected by chelation as observed in the spectra of the metal complexes.

The spectrum of  $[\text{Ni}(\text{PSA})] \cdot \text{H}_2\text{O}$  showed two bands at 452 and 487 nm. These bands were assigned to  ${}^1A_{1g} \rightarrow {}^1A_{2g}$  and  ${}^1A_{1g} \rightarrow {}^1B_{1g}$  transitions respectively. The brownish red color of the complex, its diamagnetism and the position of the electronic

absorption bands of medium intensity are characteristic of a square planar geometry [1]. The spectrum of  $[\text{Cu}(\text{PSA})] \cdot 2\text{H}_2\text{O}$  showed two bands in the visible region at about 457 and 480 nm and were respectively assigned to  ${}^2B_{1g} \rightarrow {}^2A_{1g}$  and  ${}^2B_{1g} \rightarrow {}^2E_{1g}$  transitions in a square plane environment around the Cu(II) ion. The magnetic moment value of 2.2 B.M suggested a square planar geometry [1]. The Cu(II) adduct also displayed two broad bands at 451 and 464 nm, due to a distorted octahedral geometry. The electronic spectrum of Zn(II) complexes showed a charge transfer band at 469 nm, as no  $d \rightarrow d$  transition in the visible region.

**Table 3:** Electronic spectra data of the Schiff base, metal complexes and their adducts (nm).

Compounds	$\pi \rightarrow \pi^*$	$n \rightarrow \pi^*$	CT	$d \rightarrow d$
$\text{H}_2\text{PSA}$	293	330, 392	----	----
$[\text{Ni}(\text{PSA})]$	294	330	----	452, 487
$[\text{Cu}(\text{PSA})] \cdot 2\text{H}_2\text{O}$	292	339	----	457, 480
$[\text{Zn}(\text{PSA})\text{SO}_4] \cdot \text{ZnSO}_4$	296	338	469	----
$[\text{Cu}(\text{PSA})(\text{Phen})] \cdot \text{H}_2\text{O}$	295	327	----	451, 464

CT = Charge transfer

### Magnetic moments

The room temperature magnetic susceptibilities and effective magnetic moments of the metal complexes and adduct are recorded in Table 1. The effective magnetic moment (0.22 B.M) for the synthesized nickel(II) complex showed it is diamagnetic with no unpaired electron. This suggests a square planar geometry [11]. The spin-only magnetic moments of copper(II) compounds are expected to be 1.73 B.M corresponding to one unpaired electron. The value was found to be 1.70 B.M, which suggests a square planar geometry. The adduct had a magnetic moment of 2.20 B.M which is usually observed for mononuclear copper(II) complexes between the range of 1.82-2.20 B.M [1] and it is an indication of octahedral geometry [2]. The magnetic moments of most Zn(II) complexes are known to be diamagnetic, the observed values of 0 B.M for the synthesised Zn(II) complex corresponded to diamagnetism expected [2].

### Antimicrobial assay

The antimicrobial activity of the investigated compounds was tested at a concentration of 1000 µg/mL against six (6) microorganisms (*Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Aspergillus niger* and *Candida*

*albicans*), using the agar dilution method. The diameters of inhibition zones were measured (mm) and results summarized in Table 4. Most of the compounds showed activity at 1000 µg/mL. All the compounds were active against *Aspergillus niger* and *Candida albicans* in the range 12-18 mm. [Ni(PSA)] showed activity against all the bacterial and fungal strains used at 10-18 mm. The complexes showed higher inhibitory activity than the Schiff base.

### Minimum Inhibitory Concentration

The results of minimum inhibitory concentration (MIC) of some active complexes against *Bacillus subtilis*, *Escherichia coli*, *Aspergillus niger* and *Candida albicans* are shown in Table 6. Most of the complexes exhibited minimum inhibitory concentration at 1000 µg/mL in nearly all microorganisms tested. Amongst the complexes, [Ni(PSA)] and H<sub>2</sub>PSA exhibited higher antimicrobial activity at 250 µg/mL against *Aspergillus niger*. The variation in the effectiveness of different complexes against different organisms depends on the differences in the permeability of the cells of the microbes (Gabella, 2007). The minimum inhibitory concentrations (MIC) also showed that the complexes were most toxic against the fungi than the bacteria used for this assay.

**Table 4:** The diameters of inhibition zones (mm) and the minimum inhibitory concentration (MIC, µg/mL).

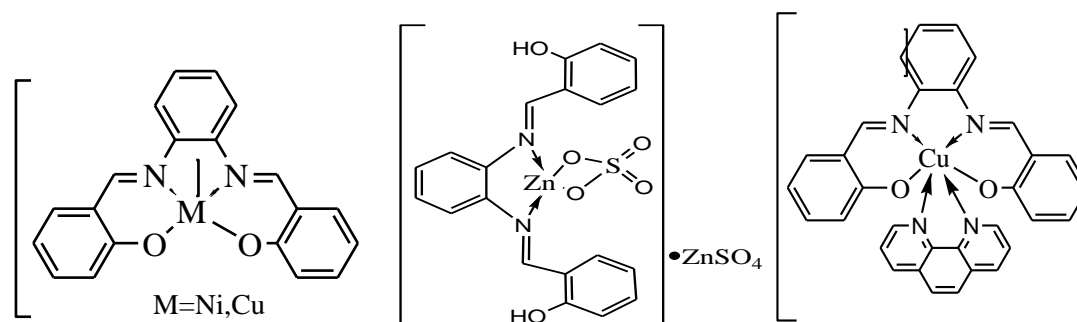
Compounds	<i>S. aureus</i>	<i>B. subtilis</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>A. niger</i>	<i>C. albicans</i>
H <sub>2</sub> PSA	8	14	8	8	18	12
H <sub>2</sub> PSA <sub>MIC</sub>	---	250	---	---	250	250
[Ni(PSA)]	10	16	10	10	18	16
[Ni(PSA)] <sub>MIC</sub>	---	750	---	---	250	1000
[Cu(PSA)].2H <sub>2</sub> O	8	8	8	12	14	12
[Cu(PSA)].2H <sub>2</sub> O <sub>MIC</sub>	---	---	---	1000	750	1000
[Zn(PSA)SO <sub>4</sub> ].ZnSO <sub>4</sub>	10	12	8	10	18	16
[Zn(PSA)SO <sub>4</sub> ].ZnSO <sub>4</sub> <sub>MIC</sub>	---	500	---	500	500	500
[Cu(PSA)(Phen)].H <sub>2</sub> O	8	8	8	8	18	16
[Cu(PSA)(Phen)].H <sub>2</sub> O <sub>MIC</sub>	---	---	---	---	500	---

### Conclusion

A tetradentate Schiff base ligand (H<sub>2</sub>PSA) formed by the 2:1 molar reaction of salicylaldehyde and *o*-phenylenediamine under reflux was synthesised. Ni(II), Cu(II) and Zn(II) metal complexes and Cu(II) adduct were synthesized and these compounds were characterized by their analytical and spectroscopic properties. The assignment of square planar geometry to Ni(II) and Cu(II) metal complexes, tetrahedral geometry to the

Zn(II) complex and octahedral geometry to the Cu(II) adduct was corroborated by analytical, spectroscopic and magnetic susceptibility data.

The antimicrobial assay showed that the compounds have moderate activity against the bacteria and fungi used. The metal complexes showed higher activity than the Schiff base. The proposed structures of the Schiff base, metal complexes and adduct are given below (Figure 3):



**Figure 3:** Proposed structures of the metal complexes and Cu(II) adduct

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