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Synthesis, spectroscopic determination, and antimicrobial studies of Schiff base derived from 4-phenylthiosemicarbazide with 2-acetylpyridine and its Co(II) and Ni(II) complexes

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ABSTRACT

Condensation of 4-phenylthiosemicarbazide with 2-acetylpyridine in (1:1 molar ratio) ethanol produced Schiff base (2)-*N*-phenyl-2-[1-(pyridin-2-yl)ethylidene]hydrazine-1-carbothioamide (Acetylpyridine-4-phenylthiosemicarbazone). Interaction of the Schiff base with each of Co(II) and Ni(II) metal salts in (2:1 molar ratio) ethanol produced the Schiff base metal(II) complexes. The Schiff base and its Co(II) and Ni(II) complexes were characterized based on melting point/decomposition temperature, solubility, magnetic susceptibility, infrared spectra, molar conductance measurements, elemental, and gravimetric analyses. The Co(II) and Ni(II) metal complexes show moderate values of decomposition temperatures The Schiff base and the complexes were soluble in some common organic solvents. Infrared spectral data of the Schiff base and its complexes, indicated coordination of the Schiff base to the metal(II) ion via azomethine nitrogen. The effective magnetic moment of the Co(II) and Ni(II) complexes suggested an octahedral geometry. The molar conductance values of the complexes show that the complexes are electrolytes. The results of the elemental analysis of the ligand and its complexes are in good agreement with the calculated values, suggesting a 1:2 (metal-ligand) ratio. Antimicrobial screenings of the ligand and its complexes were conducted against Gram-positive (*Staphylococcus aureus*) and two Gram-negative (*Salmonella typhii, and Escherichia coli*) bacteria species. Also, three fungi mainly (*Candida albicans, Mucus indicus*, and *Aspergillus flavus*) were used. The results showed that both the ligand and the complexes are active against the species used.

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1. Introduction

Schiff base is a compound containing >C=NR group that is formed by the condensation of either an aldehyde or a ketone with a primary amine. Schiff base, therefore, is a weakly basic compound, product of the condensation of a carbonyl group (>C=O) to form an imine or an anil or azomethine (Pierra, 1987). Schiff base is named after Hugo Schiff, who carried out the first investigation leading to the formation of the product in 1864 (Nic et al., 2006). Schiff base chelates to active site of metal ion through N, O, and S donor atoms. Schiff bases of transition metal complexes were found to be important for their biological activity (Mishra et al., 2009). They have been used as analytical reagent, polymer-coating, ink, pigment, fluorescent materials, and catalytic reagent (Buhlmann et al., 1998).

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Thiosemicarbazones are class of sulfur (S) donor Schiff bases that are useful for transition metals due to their metal complexing ability (Abou-Melha et al., 2008). Thiosemicarbazone and their complexes have been studied for a considerable period and known to have high tendency to exhibit square planar or octahedral geometry (Konstantinovic et al., 2007; Prem et al., 2013). Thiosemicarbazone have great significant against tumor, tuberculosis, and leprosy and were found to exhibit activity (Ainscough et al., 2007). Clinical and experimental studies on the effect of thiosemicarbazones revealed their activities against cancer Marina et al. (2007). Drug synthesized from N-methyl derivatives of isatin1-β-thiosemicarbazone (methisazone) has been found to be active against small pox (Sau et al., 2003). The drug has been used also to treat patent with genital lesions caused by herpes simlex virus (Genova et al., 2004).

Pyridine is a basic hetero cyclic as well as aromatic compound. The pyridine ring occurs in many important compounds, including azines and vitamins niacin and pyridoxine found in B_{12}. Investigation and diagnosis, on Cu(II), Hg(II), Ni(II), Mn(II), and Co(II) complexes of Schiff base derived from 2-aminopyridine with 2-benzoylbenzoic acid, proved *in vitro* effective against *Escherichia coli* and *Staphylococcus aureus* (Rehab *et al.*, 2017). Series of 1-(5-substituted-2-oxoindolin-3-ylidene)-4-(substituted-pyridin-2-yl)thiosemicarbazide Schiff base and its Co(II) and Ni(II) complexes, were screened for *in vitro* antibacterial and antifungal and were found to exhibit good activity against *Baccilus subtilis, S. aureus, E. coli, Pseudomonas aeruginosa, Candida albicans*, and *Aspergilus niger* (Vijey et al., 2008).

2. Materials and Method

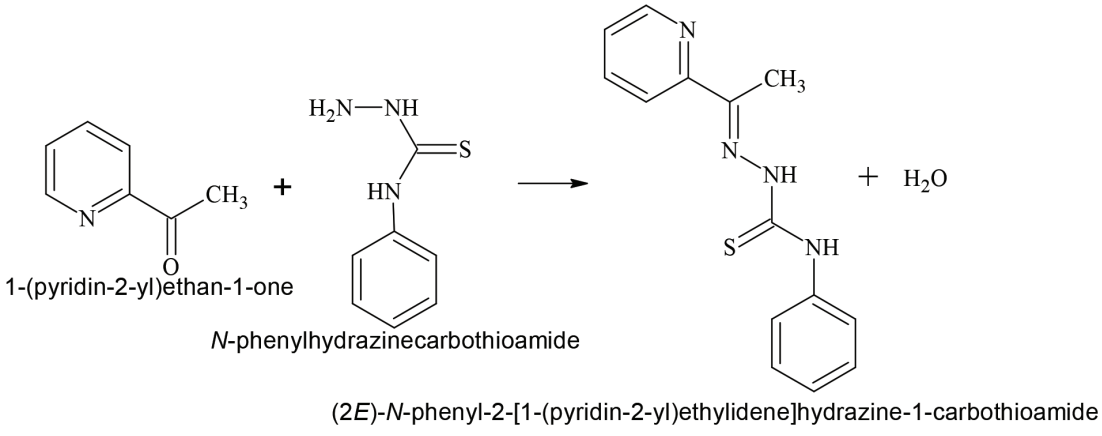
All the chemicals used in this work were of Analar Grade and were used without further purification. Glass wares used for the preparation were thoroughly washed with detergent, rinsed with distilled water, and dried in an oven. The main chemicals, 4-phenylthiosemicarbazide and 2-aceltylpyridine, were all purchased from Sigma Aldrich UK.

All weighing were carried out using Electrical Meter Balance AB 54. Magnetic susceptibility measurements of the complexes were determined using Sherwood Scientific MSB MK1 Magnetic Susceptibility Balance. Melting point/Decomposition temperatures were determined using a digital WRS-IB Microprocessor Melting Point Apparatus. Infrared spectral analyses were recorded using Shimadzu FTIR-8400S Fourier Transform Infrared Spectrophotometer in the range 4,500–250 cm^{-1}. Molar conductivity measurements were carried out using George Kent model 5003 conductivity metre. Metal and Elemental Analysis of C, H, and N were carried out at Robertson Microlit Laboratories, New Jersey (USA).

All the microbial isolates used in this were obtained from the Department of Medical Microbiology, Aminu Kano Teaching Hospital Kano, and identified at the Department of Microbiology. The antimicrobial screening was conducted in the Department of Microbiology, Bayero University Kano. Standard drugs; Ciprofloxacin for bacteria and Ketoconozole for fungi as reference standards were obtained from Department of Microbiology, Bayero University, Kano. Mueller-Hinton agar and Potato dextrose were used as a growth media for the microbes.

2.1. *Preparation of Schiff base [Acetylpyridine-4-phenylthiosemicarbazone (AP-PTSC)]*

2-acetylpyridine (0.01 mol, 1.12 cm^3) was added drop wise to a hot solution of 4-phenylthiosemicarbazone (1.67 g, 0.01 mol) in ethanol (70 cm^3), followed by the addition of three drops of acetic acid. The mixture



Scheme 1. Preparation of AP-PTSC Schiff base.

was refluxed with constant stirring for 2 hours. The solution was transferred into a refrigerator at 5°C for 24 hours. The precipitate obtained was filtered off, washed several times with ether and dried over P_2O_5 in a desiccator for 3 days (Scovill et al., 1982).

2.2. Preparation of the Schiff base metal (II) complexes

Co(II) and Ni(II) complexes for the Schiff base (AP-PTSC), derived from 2-acetylpyridine with 4-phenyl thiosemicarbazide, were prepared by the addition of 0.002 mol of the Schiff base in 20 cm^3ethanol to the solution containing 0.001 mol of each of the metal (II) salts in 10 cm^3ethanol. Each of the mixture was then refluxed for 3 hours with stirring. On cooling, the precipitate obtained was filtered, washed with diethyl ether, and dried over P_2O_5 in a desiccator for 3 days (Gehad et al., 2006).

2.3. Determination of melting point/decomposition temperature

The melting point of the Schiff base and the decomposition temperature of the metal(II) complexes were determined using microprocessor melting point apparatus (WRS-IB) and Gallenkamp melting point apparatus. The results obtained are shown in Table 1 (Aliyu and Zayyan, 2013).

2.4. Solubility test

The solubility of the Schiff base and each of the metal(II) complexes was tested using common organic solvents and distilled water. 20 mg of each compound was tested in 5 cm^3 of each of the corresponding solvent in a test tube, shaken thoroughly, and the results obtained are shown in Table 2 (Saha et al., 2009).

2.5. Magnetic susceptibility measurement

The magnetic susceptibility (χ_g) of complexes was determined using magnetic susceptibility balance MKI Sherwood Science Ltd via the expression below. The results obtained are shown in Table 3 (Lancanshire, 2010).

$$X_g = \frac{\mathrm{CL}\,(R_1 - R_o)}{m \times 10^9}$$

where m = sample mass (g) = $W_1 - W_0$, W_0 = weight of empty capillary tube, W_1 = weight of the capillary tube and the complex, R_o = initial reading from the balance for empty capillary tube, R_1= reading from the balance for the loaded capillary tube, L = length of the complex in the capillary tube (cm). C = balance calibration constant (C = 1).

2.6. Estimation of metals in the complexes

About 0.2000 g of each metal complex was placed in a 100 cm^3 beaker followed by 12.5 cm^3 of 20% nitric acid and heated to about dryness. The content of the beaker was allowed to cool at room temperature, and 12.5 cm^3 of distilled water was added while stirring. The content of the beaker was filtered and the filtrate was subjected to further analysis (Vogel, 1972).

2.7. Estimation of Cobalt(II) ion in $[Co(AP\text{-}PTSC)_2]Cl_2$

The percentage composition by weight of cobalt in the complex was calculated using gravimetric factor formula below and the results obtained are shown in Table 8.

$$\text{G. F of Co} = \frac{\text{Atomic weight of Co}}{\text{Molar mass of } [\mathrm{Co}\,(C_5H_5N)_4](SCN)_2}$$

2.8. Estimation of Ni(II) ion in $[Ni(AP\text{-}PTSC)_2]Cl_2$

The percentage composition by weight of nickel in the complex was calculated using gravimetric factor formula below and the results obtained are shown in Table 8.

$$\text{G. F of Ni} = \frac{\text{Atomic weight of Ni}}{\text{Molar mass of } [\mathrm{Ni}\,(C_4H_7O_2N_2)_2]}$$

2.9. Infrared measurements

Shimadzu FTIR-8400S Fourier Transform Infrared Spectrophotometer in the range 250–4,500 cm^{-1} was used. A small amount of powder sample (about 1%–2% of KBr amount) was taken and mixed with KBr powder and then analyzed using infrared analyzer. The results obtained are shown in Table 4.

2.10. Molar conductance measurement

Molar conductivity measurements were carried out on 1×10^{-3} $moldm^{-3}$ (0.001 M) solution of each of the metal complexes in Dimethyl Formamide (DMF) at 25°C. The molar conductance is calculated using the following equation (Mohd et al., 2013):

$$A_M = \frac{1{,}000L}{C}$$

where A_M = molar conductance, L = specific conductance, and C = concentration of the complex. The results are presented in Table 5.

2.11. Elemental analysis

The analysis was carried out using Perkin-Elmer CHN 2400 Elemental Analyzer based on the classical

Pregl–Dumas method. About 20 mg of each of the samples for analysis was encapsulated in an aluminium vial and inserted automatically into the Combustion Zone of the analyser using a single-sample auto injector. In the presence of excess oxygen and combustion reagents, the samples were combusted completely and reduced to the elemental gases CO_2, H_2O, and N_2. All the results were computed automatically and were given as percent weight of each element. The results are shown in Table 6.

3. Antimicrobial Test

The *in vitro* antimicrobial activity of the ligand (AP-PTSC) and its complexes were tested against Gram-positive (*S. aureus*) and two Gram-negative (*Salmonella typhii and E. coli*) pathogenic bacteria. Also, three fungi mainly (*C. albicans*, *Mucus indicus*, and *Aspergillus flavus*) were used. Muller-Hilton Agar media for bacteria while Potato Dextrose Agar for fungi were used and were prepared in distilled water. The ligands and the complexes were dissolved separately in Dimethyl Sulphoxide (DMSO) to obtain three different concentrations (15, 30, and 60 µg/ml), which were used to check the antimicrobial activities by well-diffusion method. The discs were saturated with the dissolved compounds in DMSO and then placed in petridishes containing the culture media. The petridishes were incubated at 37°C and the inhibition zone was measured after 24 hours for bacterial strain and 48 hours for fungal isolates and compared with the standard drugs (Ciprofloxacin for bacteria and Ketoconozole for fungi) (Sheikh et al., 2004).

4. Results

Table 1. Some physical properties of (AP-PTSC) Schiff base and its metal(II) complexes.

Ligand complex,	Color	Melting point (°C)	Decomposition temperature (°C)	Percentage (%) yield
AP-PTSC	Yellow	170	-	86.8
$[Co(AP\text{-}PTSC)_2]Cl_2$	Deep brown	-	288	78.4
$[Ni(AP\text{-}PTSC)_2]Cl_2$	Brown	-	219	76.2

AP-PTSC = Schiff base derived from 2-acetylpyridine and 4-phenylthiosemicarbazide.
$[M(AP\text{-}PTSC)_2]Cl_2$ = Metal (II) complex for the Schiff base derived from 2-acetylpyridine and 4-phenylthiosemicarbazide, where M = Co or Ni(II) ion.

Table 2. Solubility data of (AP-PTSC) Schiff base and its metal(II) complexes.

Ligand/Complex	Water	Methanol	Ethanol	N-hexane	Ether	Acetone	Dichloro Methane	Chloroform	DMSO	Benzene
AP-PTSC	IS	SS	SS	IS	IS	S	S	S	S	IS
$[Co(AP\text{-}PTSC)_2]Cl_2$	SS	S	S	IS	IS	S	S	S	S	IS
$[Ni(AP\text{-}PTSC)_2]Cl_2$	IS	SS	SS	IS	IS	S	S	S	S	IS

S=> Soluble, SS=> Slightly soluble while IS=>Insoluble

Table 3. Magnetic susceptibility values of AP-PTSC metal(II) complexes.

Complex	Gram susceptibility $(X_g)10^{-6}$	Molar magnetic susceptibility $(X_m)10^{-3}$	Magnetic moment μ_{eff} (B.M)	Magnetic property
$[Co(AP\text{-}PTSC)_2]Cl_2$	12.9	8.68	4.5	Paramagnetic
$[Ni(APPTSC)_2]Cl_2$	5.80	3.90	3.0	Paramagnetic

Table 4. Infrared spectra data (cm^{-1}) of AP-PTSC and its metal(II) complexes.

Compounds	υ (C=N)	υ (N-H)	υ (C=S)	υ(M-N)	υ(M-S)	υ (C-N)
AP-PTSC	1648	3,296	766	-	-	1,283
$[Co(AP\text{-}PTSC)_2]Cl_2$	1634	3,307	671	538	382	1,276
$[Ni(AP\text{-}PTSC)_2]Cl_2$	1544	3,340	754	461	409	1,346

AP-PTSC=> Schiff base derived from 2-acetylpyridine and 4- phenylthiosemecarbazide, $[M(AP\text{-}PTSC)_2]Cl_2$,=>Its Metal (II) complexes where M= Co, or Ni(II) ion.

Table 5. Molar conductance measurement for AP-PTSC metal(II) complexes.

Compound	FW $gmol^{-1}$	Conc. $Moldm^{-3} \times 10^{-3}$	Specific conductance $\times 10^{-4}$ $S\Omega^{-1}$	Molar conductance $\Omega^{-1}cm^2mol^{-1}$
$[Co(AP\text{-}PTSC)_2]Cl_2$	670.54	1.20	1.50	125.73
$[Ni(AP\text{-}PTSC)_2]Cl_2$	670.30	2.69	2.78	103.52

AP-PTSC=> Schiff base derived from 2-acetylpyridine and 4- phenylthiosemecarbazide, $[M(AP\text{-}PTSC)_2]Cl_2$,=>Its Metal (II) complexes where M= Co, or Ni(II) ion.

Table 6. Elemental analysis of AP-PTSC and its metal(II) complexes.

Ligand/Complex	%C (Calc) Found	%H (Calc) Found	%N (Calc) Found	%M (Calc) Found
AP-PTSC	(62.20) 62.40	(5.22) 5.70	(20.72) 20.80	
$[Co\ (AP\text{-}PTSC)_2]Cl_2$	(50.15) 50. 60	(4.21) 4.10	(16.71) 16.40	(8.79) 8.81
$[Ni\ (AP\text{-}PTSC)_2]Cl_2$	(50.17) 50.40	(4.21) 4.30	(16.72) 16.50	(8.76) 8.53

AP-PTSC=> Schiff base derived from 2-acetylpyridine and 4-phenylthiosemecarbazide, $[M(AP\text{-}PTSC)_2]Cl_2$,=>Its Metal (II) complexes where M= Co or Ni(II) ion.

Table 7. Zone of inhibition (mm) for Antibacterial of AP-PTSC and its metal (II) complexes.

Isolate	*S. aureus* (µg/disc)			*E. coli* (µg/disc)			*Salmonella typhirium* (µg/disc)		
Compd/conc.	**15**	**30**	**60**	**15**	**30**	**60**	**15**	**30**	**60**
AP-PTSC	6	8	11	7	9	11	6	8	10
$[Co(AP\text{-}PTSC)_2]Cl_2$	8	11	13	6	10	13	8	11	16
$[Ni(AP\text{-}PTSC)_2]Cl_2$	6	10	14	9	14	16	6	13	15
Ciproflaxacin		34			40			43	

AP-PTSC=> Schiff base derived from 2-acetylpyridine and 4-phenylthiosemecarbazide, $[M(AP\text{-}PTSC)_2]Cl_2$,=>Its Metal (II) complexes where M= Co or Ni(II) ion.

Table 8. Zone of inhibition (mm) for antifungal of AP-PTSC and its metal (II) complexes.

Isolate	*M. indicus* (µg/disc)			*A. flavus* (µg/disc)			*Candida Albican* (µg/disc)		
Compd/conc.	**15**	**30**	**60**	**15**	**30**	**60**	**15**	**30**	**60**
AP-PTSC	7	8	11	6	6	6	7	10	13
$[Co(AP\text{-}PTSC)_2]Cl_2$	8	11	14	10	12	17	12	13	14
$[Ni(AP\text{-}PTSC)_2]Cl_2$	10	15	17	9	11	14	10	13	16
Ketoconozole		27			32			38	

AP-PTSC=> Schiff base derived from 2-acetylpyridine and 4-phenylthiosemecarbazide, $[M(AP\text{-}PTSC)_2]Cl_2$,=>Its Metal (II) complexes where M= Co or Ni(II) ion.

5. Discussion

Condensation of 4-phenylthiosemicarbazide with 2-acetylpyridine in (1:1 molar ratio) in ethanol produced a Schiff base; (2)-*N*-phenyl-2-[1-(pyridin-2-yl) ethylidene]hydrazine-1-carbothioamide (2-acetylpyridine-4-phenylthiosemicarbazone) (AP-PTSC). Interaction of the Schiff base with Co(II) and Ni(II) metal salts in (2:1 molar ratio) in ethanol produced Schiff base complexes; $[Co(AP\text{-}PTSC)_2]Cl_2$ and $[Ni(AP\text{-}PTSC)_2]Cl_2$. The Schiff base AP-PTSC and its Co(II) and Ni(II) complexes were characterized based on melting point/decomposition temperature, solubility, magnetic susceptibility, infrared (IR) spectra, molar conductance measurements, elemental, and gravimetric analyses.

5.1. Physico-chemical properties of the Schiff base and its metal(II) complexes

The Schiff base is yellow powder with a yield of 86.8% and a melting point of 170°C. The complexes $[Co(AP\text{-}PTSC)_2]Cl_2$ and $[Ni(AP\text{-}PTSC)_2]Cl_2$ are deep brown and brown with decomposition temperature of 288°C and

219°C, a yield of 78.4% and 76.2% (Table 1). The variation in color of the Schiff base with its corresponding complexes is attributed to'd-d' orbital transition of electron between one energy level to another, by their magnitude of splitting, which in turn depends on the geometry of the complex, the nature of the ligand and charge transfer (Rodgers, 1994). The decomposition temperatures of the complexes are relatively high, indicating good thermal stability of the complexes.

Both the Schiff base and its complexes are soluble in acetone, dichloromethane, chloroform, and DMSO except $[Co(AP\text{-}PTSC)_2]Cl_2$ that is slightly soluble in water. The Schiff base and $[Ni(AP\text{-}PTSC)_2]Cl_2$ are slightly soluble methanol and ethanol but the $[Co(AP\text{-}PTSC)_2]Cl_2$ is completely soluble (Table 2). The solubility of the Schiff bases and their metal(II) complexes in the common solvents indicated their low polarity which can be used in order to determine the suitable solvents that could be utilized for subsequent spectroscopic measurements (Jones and Fleming, 2010).

The magnetic susceptibility as presented in Table 3 are 4.5 and 3 BM for $[Co(AP\text{-}PTSC)_2]Cl_2$ and$[Ni(AP\text{-}PTSC)_2]Cl_2$, indicating high spin octahedral geometry (Gehad et al., 2006).

The infrared spectra of the Schiff base shows a broad band at 1,648 cm^{-1}, that is attributable to υ(C=N) stretching vibration but shifted to a lower frequency at 1,634 cm^{-1} and 1,544 cm^{-1} in the spectra of the complexes. This indicates coordination of the Schiff base to the metal ions via azomethine nitrogen. Another frequency in the spectra of the Schiff base at 3,296 cm^{-1}, is assignable to υ(N-H) stretching vibration. This spectrum makes an upward shift at 3,307–3,340 cm^{-1} in the spectra of the complexes, supporting of coordination of the Schiff base to the metal(II) ion. The IR bands in the spectra of the complexes at 461–538 cm^{-1} and 382–409 cm^{-1} are assignable to υ(M-N) and υ(M-S), respectively is another clear indication for the coordination of the Schiff base to the metal(II) ion (Aurora et al., 2009; Awadelkareem et al., 2012) (Table 4).

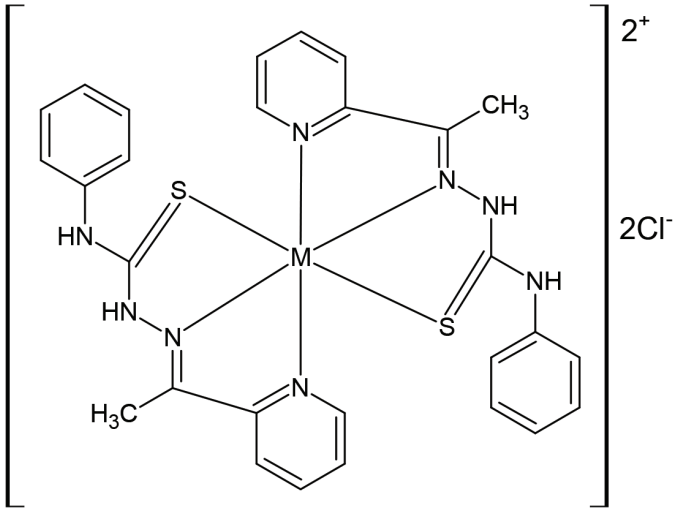


Figure 1. Proposed Structure of 2-acetylpyridine-4-phenylthiosemicarbazone (AP-PTSC) Metal(II) Complexes.

The $[Co(AP\text{-}PTSC)_2]Cl_2$ and$[Ni(AP\text{-}PTSC)_2]Cl_2$ were dissolved in DMF and the molar conductivities of their 10^{-3} M solutions were at the range of 103.52–125.73 $\Omega^{-1}cm^2mol^{-1}$ as shown in Table 5. These values indicate 2:1(ligand-metal ratio) electrolyte (Gehad et al., 2006; Mohd et al., 2013).

The results of the elemental analysis (C, H, and N) of the Schiff base and its respective metal(II) complexes are presented in Table 6. The values are in agreement with the proposed formulas of the ligand and its corresponding metal complexes since the values for the theoretically calculated percentage compositions of carbon, hydrogen, and nitrogen in the respective compounds are in agreement to the values of the elements experimentally found.

Antimicrobial test of the AP-PTSC Schiff base and its metal(II) complexes were carried out against three bacteria isolates; Gram-positive (*S. aureus,*), Gram-negative (*S. typhii, and E. coli*), and three fungi (*C. albicans, M. indicus and A. flavus*). Concentrations of 15, 30, and 60 (µg/disc) were used, standard drugs ciproflaxacin and ketoconozole for bacteria and fungi, were served as controls, (Tables 7 and 8). The results of the tests indicated moderate antimicrobial activity against the tested microorganisms when compared with the standards, and this activity increases by increasing concentration. Also, the metal complexes showed higher activity than free ligand, which can be explained on the basis of chelation theory, which states that the chelation tends to make the complex acts as a more powerful and potent bactericidal or bacteriostatic agent than the ligand (Sengupta et al., 1998).

From the results of analysis of the complexes and the available literature data, the general molecular structure below is proposed:

6. Conclusion

Schiffbase(2)-*N*-phenyl-2-[1-(pyridin-2-yl)ethylidene] hydrazine-1-carbothioamide, i.e., (2-acetylpyridine-4-phenylthiosemicarbazone)(AP-PTSC) together with its Co(II) and Ni(II) complexes were successfully synthesized and characterized. The decomposition

temperature indicated the good stability of the complexes. The IR values assignable to υ(N-H) and υ(C=N) stretching vibrations show coordination of the ligand to the metal ion via azomethine nitrogen. Molar conductance values range from 103.52–125.73 $\Omega^{-1}cm^2mol^{-1}$, indicating electrolytic nature of the complexes. The values of the magnetic susceptibility of the complexes indicated an octahedral geometry. The elemental analysis values indicates 2:1(ligand-metal ratio). The antimicrobial test shows that both the ligand and the complexes have good activities against the isolates used. Finally, the structures of the complexes have been proposed.

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