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Antibacterial and antifungal activities of Schiff base and its metal (II) complexes of Fe (II), Ni (II) and Co (II) derived from 2-hydroxy-1-naphthaldehyde and 2-amino-3-methylpyridine

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ABSTRACT

Aim and methods: The Schiff base was synthesized by condensation of 2-hydroxy-1-naphthaldehyde with 2-amino-3-methylpyridine and its complexes of Fe (II), Ni (II) and Co (II) chlorides were synthesized and characterized by melting point, solubility test, conductivity, measurement, magnetic susceptibility measurement and infrared (IR) spectroscopy. The Schiff base and its respective metal complexes were colored with sharp melting point and are soluble in ethanol, dimethylsulfoxide and methanol but insoluble in water and slightly soluble in other solvent. The conductivity value obtained indicates that the synthesized complexes are non-electrolytes while an octahedral geometry was suggested for all complexes based on the data obtained from magnetic susceptibility analysis. **Results:** The IR results revealed bands at 1596 cm^{-1} indicating the formation of azomethine (C=N) confirming the formation of Schiff base. 791 cm^{-1} , 683 cm^{-1} , 747 cm^{-1} for (M-N) and 447 cm^{-1} , 461 cm^{-1} , 451 cm^{-1} for (M-O) bands in the spectra of the complexes supporting coordination of Schiff base to respective metals. **Conclusion:** The in vitro antimicrobial screening of Schiff base and its metal complexes against *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumonia*, *Klebsiella pneumonia*, *Aspergillus niger* and *Candida albicans* showed that they are potential antibacterial and antifungal agents against the tested microorganisms.

Introduction

Metals that form compounds where electrons from *d* orbitals are ionized or otherwise involved in bonding are known as transition metals. Their characteristics include: the possibility of variable oxidation states; compounds with spectroscopic, magnetic or structural features resulting from partially occupied *d* orbitals; an extensive range of complexes and organometallic compounds including ones with very low oxidation state (zero or even negative); and useful catalytic properties shown by metals and by solid or

molecular compounds. Different transition metals display these features to different degrees, but together the properties form a sufficiently coherent pattern that the elements are best dealt with rather than individually or as groups. Although, formally part of the *d* block, the elements of group 12 do not show typical transition metal characteristics, as the *d* orbitals are too tightly bound to be involved in chemical bonding. These elements are better regarded as post transition [1].

Deficiency of some metal ions can lead to diseases such as pernicious anemia resulting from

iron deficiency, growth retardation arising from insufficient dietary zinc, and heart disease in infants owing to copper deficiency [2]. Transition metal complexes are still the most widely used chemotherapeutic agents and make a large contribution to medicinal therapeutics [3].

Schiff base is an imine or azomethine containing compound which was first reported by Hugo Schiff in 1864. It is considered as ligand due to its ability to donate a lone pair of electron to the central metal atom and it could be mono, Di, or polydentate ligand depending on the number of donor site it contain [4].

Schiff base complexes play crucial role in biological and medical processes, and it is evident that many organic compounds used in medicine do not have a purely organic mode of action, some are activated or bio transformed by metal ions metabolism. Many drugs possess modified toxicological and pharmacological properties in the form of metal complex and probably Schiff bases are versatile C=N (Imines) containing compounds possessing broad spectrum of biological activity and incorporation of metals in form of complexes showed some degree of antibacterial, antifungal, antitumor and anti-inflammatory activity [5].

The emergence of new pathogens such as corona virus and delta variants have further complicated the world's challenge in health sector [6]. Therefore to build a strong health sector in any nation; major advances towards the improvement of antimicrobial drugs have to be continuous process. These discoveries coupled with continuing developments for better antibiotics will be of very great achievement in modern science and technology [7]. In order to achieve this, several research works have been done and a lot more are still going on, all gearing towards the design of novel compounds that will serve as more potent antimicrobial drugs. Such efforts have been extended to cover plant extracts and synthetic materials [8]. The potentials of metal complexes in therapeutic applications have also been reported by various authors [9]. In bioinorganic chemistry the study of coordination chemistry of biologically important metal ions with ligands is creating a great impact in this area [10]. Schiff base is one of the compounds reported to possess remarkable antibacterial, antifungal, anticancer and antimalarial activities [11]. In view of the above this research work reported antibacterial and antifungal activities of Schiff base and its metal (ii) complexes derived

from 2-hydroxy-1-naphthaldehyde and 2-amino-3-methylpyridine.

Materials and Methods

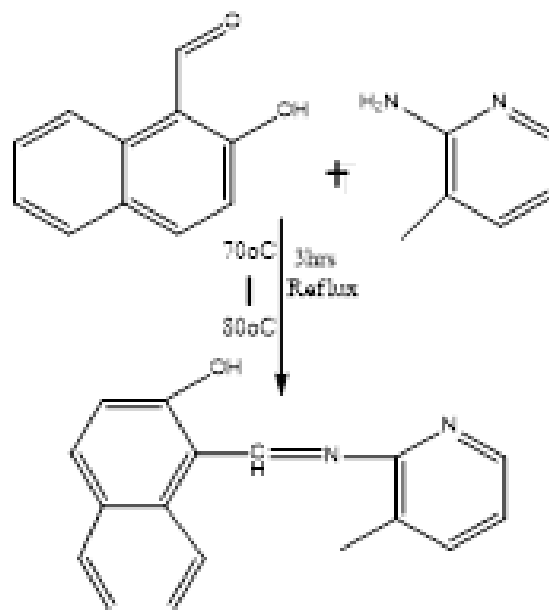
All reagents and solvents used for this research were of analytical grade and were purchased from sigma Aldrich and Merck and were used without further purification the melting point was recorded on hot stage gallenkamp melting point apparatus. the infrared spectra was recorded using agilent carry 630 FTIR spectrometer in the frequency range of 400-4000 cm^{-1} . The magnetic susceptibility was obtained at room temperature using magnetic susceptibility balance MK1 Sherwood. Conductivity measurement was carried out using Jan way conductivity meter 401

Sample collection

The four (4) bacterial species (*Escherichia coli*, *Staphylococcus aureus* *streptococcus pneumonia*, *klebsiella pneumoniae*), as well as the fungus species (*Aspergillus niger*, *Candida albicans*) were obtained from federal medical center nguru and identified at Microbiology Department of the Bayaro University Kano

Synthesis of Schiff base

Exactly 0.03mol (5.1654g) of 2-hydroxy-1-naphthaldehyde was mixed with 0.03mol (3.023ml) of 2-amino-3-methylpyridine in 50 cm^3 of ethanol. The mixture was heated under reflux at 70°C-80°C for 3hours and the solid product formed was separated by filtration, purified by recrystallization from ethanol, washed with ethanol, and then dried in desiccators over calcium chloride (CaCl_2) for 18hrs [4].



Synthesis of cobalt (II) complexes

An aqueous solution of a hydrated cobalt (II) chloride (0.01mol, 1.36g) in 10cm³ ethanol was added to an ethanolic solution of the prepared Schiff base ligand (0.02mol, 2.73g) the mixture was refluxed for 2hours. The precipitated complex formed was separated by filtration recrystallized and washed with ethanol and dried in a desiccator over phosphorus pentoxide (P₂O₅) for 48hrs. The same procedure was repeated for the synthesis of Cu (II), Fe (II) and Ni (II) chloride [12].

Media preparation and sensitivity test (antibacterial and antifungal)

The sensitivity test was carried out by preparing sterile Muller hinton agar and Potato dextrose agar media and carefully transferred into sterile Petri-dishes to an appreciable amount. The media was allowed to cool and solidify at room temperature. The Petri-dishes were marked to indicate fungi, bacteria and the positions of the three wells of different test concentrations (100ppm, 200ppm, 300ppm) for both the ligand and complexes. From the standardized inoculums of each isolate, uniform spreading (using a glass spreader) of 0.1 mL of bacteria and fungi(s) inoculums was done on the surface of dried Muller hinton agar and Potato dextrose agar. Each fraction of metals (II) complexes and Schiff base (ligand) petri-dishes were placed at the marked positions. For the fungi. The petri-dishes were kept in a cool dry place for 72 hours which the plate were observed for the presence for zones of inhibition as evidence of antifungal activities, while for bacteria the Petri-dishes were kept in an incubator for 24 hours at 37°C. The degree of sensitivity was determined by measuring the diameter of visible zones of inhibition to the nearest millimeters with respect to each isolate and test concentration and the result was recorded [13].

Results and Discussion**Physical data of Schiff base and metals complexes**

In the present study, we were able to demonstrate the interaction between 2-hydroxy-1-naphthaldehyde and 2-amino-3-methylpyridine with resultant orange yellow Schiff base and its metal complexes of Fe (II), Ni (II), and Co (II) exhibiting various colour ranging from dark blue, greenish yellow and light yellow respectively. This is typical for transition metal complexes due to d-d electrons transition [14]. The purity and stability of the Schiff base and metal complexes is established by the observance of sharp melting/decomposition

temperature and for Schiff base and metal complexes were 188 °C, 258 °C, 260 °C, and 264 °C, respectively. The complexes showed a steady trend of higher melting points than the ligand which may be due to the inter-molecular bonding as a result of metallic lattice and increase in molecular weight [15]. The consistent range difference of +2 °C melting points observed indicates that the synthesized compounds are presumed pure [16]. An appreciable percentage yield of all new compounds was obtained which range from 69% to 76%. The ligands and their metal (II) complexes were observed to be non-hygroscopic solids, air and photo stable under normal [17].

Solubility of schiff base and its metal (II) complexes

Solubility of a compound in various solvents depends on the nature of the compound, type of bonding and solvent [18]. The solubility test was carried out in methanol, ethanol dimethylsulphoxide, water, chloroform and acetone both the Schiff base and metals complexes were found to be soluble in some solvent such as dimethylsulfoxide, methanol and ethanol. This is due to the fact that polar solvents dissolve polar compounds due to similar attractive force between them [19]. However, they are slightly soluble in acetone and chloroform because many non-polar solvents are able to dissolve compounds containing oxygen atom which is used in forming a bond with polar hydrogen of the carbon-hydrogen in the solvent as [18]. Both Schiff base and complexes are insoluble in water [20].

Magnetic susceptibility result

Magnetic susceptibility measurement carried out at room temperature showed that the effective magnetic moment of the complexes as determined from the magnetic susceptibility balance were found to be 5.12BM, 4.33BM and 3.0BM for Fe (II), Ni (II), and Co (II) respectively. Which suggest high spin complexes and are paramagnetic in nature [21]. All the magnetic moment values of the metal (II) complexes prepared were found within the range of octahedral complexes [22]. .

Infra-red (IR) spectroscopy analysis result

The IR analysis carried out for Schiff base showed an absorption band at 3365cm⁻¹ which could be attributed to V(O-H) vibration frequency. This is a consequence of strong intramolecular hydrogen bonding between the hydroxyl proton and the imine nitrogen (OH...NH). The presence of the hydroxyl

group was further substantiated with the appearance of the phenolic C–O stretch band at 1196 cm^{-1} [23]. The band shifted to different frequency of 3309 cm^{-1} , 3298 cm^{-1} and 3301 cm^{-1} for Fe (II), Ni (II), and Co (II) complexes respectively. However, the weak OH band of the Schiff base ligands was still evident in the spectra of most of the complexes, undergoing a blue or red shift upon chelation with the metal (II) ions. This indicates that the phenolic OH was not deprotonated in the metal complexes but rather coordinates as neutral species [23]. The band at 1596 cm^{-1} could be due to $\nu(\text{C}=\text{N})$ [24]. On complexation with metal (II) ions the $\nu(\text{C}=\text{N})$ band shifted to higher frequency [16] at the following regions; 1614 cm^{-1} , 1611 cm^{-1} and 1626 cm^{-1} respectively, indicating coordination of the Schiff base ligands *via* the imine nitrogen [18]. The coordination of the Schiff base ligands to the metal (II) ions was further substantiated by the appearance of new bands in the far-infrared spectra of the complexes at (791 cm^{-1} 683 cm^{-1} 747 cm^{-1}) which were absent in the spectra of Schiff base ligand and could be assigned to $\nu(\text{M}-\text{N})$ similarly the bands at (447 cm^{-1} 461 cm^{-1} and 451 cm^{-1}) could be due to $\nu(\text{M}-\text{O})$ stretching frequencies [25].

Conductivity data

Electrolytic conductivity is the measure of the mobility of ions present in the solution. The ionic mobility in turn depends on the charge and size of metal ions and interaction with solvent molecules. When a metal ion forms a complex with solvent molecule or some ligands, the conductivity reduced [26]. In this study the electrical conductivity of divalent metal ions (M^{+2}) in 10^{-3} M DMSO solution was studied. It is observed that molar conductance value of Fe (II), Ni (II) and Co (II) complexes determined in 10^{-3} M DMSO solution at room temperature are (29.7, 20.5, 14.79, $\text{Ohm}^{-1}\text{cm}^2\text{ mol}^{-1}$) their molar conductance value are low which suggest a non-electrolytic nature [23], It could also be due to the fact that the metal (II) ions form a stable complexes with DMSO solution, and therefore mobility and conductance of these metal (II) complexes is lowered [26]. Thus it has been established that ionization is an important factor in the activity of synthetic drugs [27].

Antibacterial and antifungal results

The Schiff base ligand and the metals (II) complexes were screened for their *in vitro* antibacterial and antifungal activities at different concentration of 100ppm, 200ppm and 300ppm against bacterial isolates (*Staphylococcus aureus*, *Escherichia coli*,

Streptococcus pneumonia, and *Klebsiella pneumoniae*) using Ciprofloxacin (500mg) as control. The Schiff base shows significance activity against tested *organism* at all concentrations with inhibitory zone ranging 3 mm to 8mm but the activity is higher in *Staphylococcus aureus* with inhibitory zone of 5mm-8mm as compared to that of *Escherichia coli*, *Streptococcus pneumonia*, and *Klebsiella pneumoniae* with inhibitory zone ranging from 3mm-6mm. The metal co mplexes on the other hand showed higher activities compared to Schiff base ligand because of chelation and π -electron delocalization, which increase the lipophilic character, favoring its permeation into the bacterial membrane, causing the death of the organisms [28]. The activities of both Schiff base and its metal (II) complexes increase as the concentration increases [4] as shown in figure 1- 4. It was also revealed that cobalt complex showed higher activity as compared to iron and nickel complexes [14] which reported that the effect of metal ions on the biological activity of a given bio-active compound is metal specific. Both the complexes are less active than control [4]. The study also revealed that the minimum inhibitory concentration for (*Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumonia*, and *Klebsiella pneumoniae*) of Schiff base and metal complexes was found to be 200ppm, 100ppm, 150ppm and 200ppm respectively as shown in Table 6-9. The minimum inhibitory concentration of metal (II) complexes is higher than that of Schiff base ligand. The increasing activity of the metal complexes against the Schiff base ligand could be explained on the basis of oxidation state, overtone concept and chelation theory [29] which reduces polarity of the metal ion by partial sharing of the positive charge with donor atoms of the ligand (imine & oxygen). This atomic shake-up increases the lipophilic character, favouring the permeation through lipid layers of the bacterial membrane and consequently restricting further growth of the organism [9].

The antifungal activity of the Schiff base and its metal (II) complexes as carried out against fungal isolates (*Aspergillus niger* and *Candida albicans*) using Ketoconazole (200mg) as control, The results of the findings revealed that metals (II) Complexes have higher activity compared to Schiff base with inhibitory zone of 6mm-14mm and 5mm-8mm metal complex to Schiff base respectively. The better activities of the metal complexes are attributed to the metal ions, since the metal-free

ligand has low antifungal activity [30]. The activity of metal complexes differs from metal to metal with Iron, and nickel having the higher activity of 8mm-13mm, and 8mm-12mm as compared with cobalt with inhibition zone of 5mm-8mm for *Aspergillus niger* while for *Candida albicans* cobalt showed higher activity of 10mm-15mm, followed by iron with inhibitory zone of 10mm-11mm but nickel showed less activity of 5mm-8mm as shown in

figures (5,6). This could be due to the fact that chelation of free ligands with metals ions selectively enhanced the biological activity of the compound [23]. The activity increases with increase in concentration of both Schiff base ligand and metal (II) complexes [4]. The activity of both Schiff base and metal (II) complexes at all concentration is less than that of control with inhibitory zone of 16mm.

Table 1. Physical properties and analytical data of Schiff base and its metal (II) complexes.

Compound	Color	Melting point	Decomposition Temperature (°C)	Percentage Yield
Ligand	Orange yellow	188		76
[FeL ₂ Cl ₂]	Dark blue		258	72
[CoL ₂ Cl ₂]	Greenish yellow		260	69
[NiL ₂ Cl ₂].	Light yellow		264	68

L= Ligand, C₁₇H₁₄N₂O

Table 2. Solubility of Schiff base and its metal (II) complexes

Compound	Water H ₂ O	Ethanol CH ₃ CH ₂ OH	Methanol CH ₃ OH	DMSO (CH ₃) ₂ SO	Chloroform CHCl ₃	Acetone CH ₃ COCH ₃
Ligand	IS	S	S	S	SS	SS
[FeL ₂ Cl ₂]	IS	S	S	S	SS	SS
[CoL ₂ Cl ₂]	IS	S	S	S	SS	SS
[NiL ₂ Cl ₂]	IS	S	S	S	SS	SS

Key S = soluble SS = sparingly soluble IS = Insoluble

Table 3. Magnetic susceptibility of metal (II) complexes.

Compound	Magnetic susceptibility (cm ³ g ⁻¹)	Molar magnetic susceptibility (cm ³ mol ⁻¹)	B.M(μ _{eff})	Magnetism
[FeL ₂ Cl ₂]	153.32 x 10 ⁻⁷	1.101 x 10 ⁻⁶	5.12	Paramagnetic
[CoL ₂ Cl ₂]	109.36 x 10 ⁻⁷	78.881 x 10 ⁻⁶	4.33	Paramagnetic
[NiL ₂ Cl ₂]	52.62 x 10 ⁻⁷	37.943 x 10 ⁻⁶	3.0	Paramagnetic

L= Ligand, C₁₇H₁₄N₂O

Table 4. The infrared spectral data of Schiff base and its metal (II) complexes.

Compound	ν(OH) cm ⁻¹	ν(C-O) cm ⁻¹	ν(C=N)cm ⁻¹	ν(M-N)cm ⁻¹	ν(M-O)cm ⁻¹
Ligand	3365	1149	1596		
[FeL ₂ Cl ₂]	3309	1190	1614	791	747
[CoL ₂ Cl ₂]	3298	1186	1611	683	661
[NiL ₂ Cl ₂]	3301	1167	1626	747	661

L= Ligand, C₁₇H₁₄N₂O

Table 5. Conductivity measurement of complexes in DMSO solution (1X10⁻³ moldm⁻³).

Compound	Concentration (mol ⁻¹ dm ⁻³)	Specific conductance Ohm ⁻¹ cm ⁻¹	Molar conductance Ohm ⁻¹ cm ² mol ⁻¹
[FeL ₂ Cl ₂]	1 x 10 ⁻³	29.7x10 ⁻⁶	29.7
[CoL ₂ Cl ₂]	1 x 10 ⁻³	20.5x10 ⁻⁶	20.5
[NiL ₂ Cl ₂]	1 x 10 ⁻³	14.79x10 ⁻⁶	14.79

L= Ligand, C₁₇H₁₄N₂O

Table 6. Minimum inhibitory concentration (MIC) of Schiff base ligand and its metal complexes.

compound	Clinical isolate	concentration			
	<i>S. aureus</i>	50ppm	100ppm	150ppm	200ppm
Ligand		-	-	-	+
[FeL ₂ Cl ₂]		-	-	+	+
[CoL ₂ Cl ₂]		-	+	+	+
[NiL ₂ Cl ₂]		-	-	+	+

L= Ligand, C₁₇H₁₄N₂O

Table 7. Minimum inhibitory concentration (MIC) of Schiff base ligand and its metal complexes.

Compound	Clinical isolate	concentration			
	<i>E. coli</i>	50ppm	100ppm	150ppm	200ppm
Ligand		-	-	-	+
[FeL ₂ Cl ₂]		-	+	+	+
[CoL ₂ Cl ₂]		-	+	+	+
[NiL ₂ Cl ₂]		-	-	+	+

L= Ligand, C₁₇H₁₄N₂O

Table 8. Minimum inhibitory concentration (MIC) of Schiff base ligand and its metal complexes

compound	Clinical isolate	concentration			
	<i>S. pneumoniae</i>	50ppm	100ppm	150ppm	200ppm
Ligand		-	-	-	+
[FeL ₂ Cl ₂]		-	-	+	+
[CoL ₂ Cl ₂]		-	+	+	+
[NiL ₂ Cl ₂]		-	+	+	+

L= Ligand, C₁₇H₁₄N₂O

Table 9. Minimum inhibitory concentration (MIC) of Schiff base ligand and its metal complexes.

compound	Clinical isolate	concentration			
	<i>K. pneumoniae</i>	50ppm	100ppm	150ppm	200ppm
Ligand		-	-	-	+
[FeL ₂ Cl ₂]		-	+	+	+
[CoL ₂ Cl ₂]		-	+	+	+

L= Ligand, C₁₇H₁₄N₂O

Figure 1. Sensitivity test for antibacterial activity of Schiff base and its metal (II) complexes against clinical isolate (*Staphylococcus.aureus*) using well diffusion method.

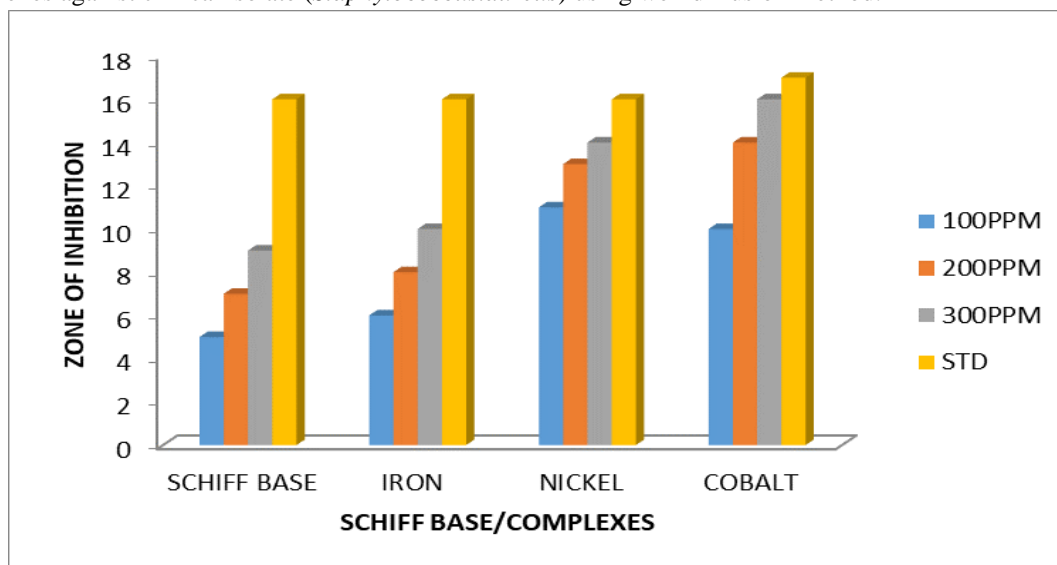


Figure 2. Sensitivity test for antibacterial activity of Schiff base and its metal (II) complexes against clinical isolate (*Escherichia coli*) using well diffusion method.

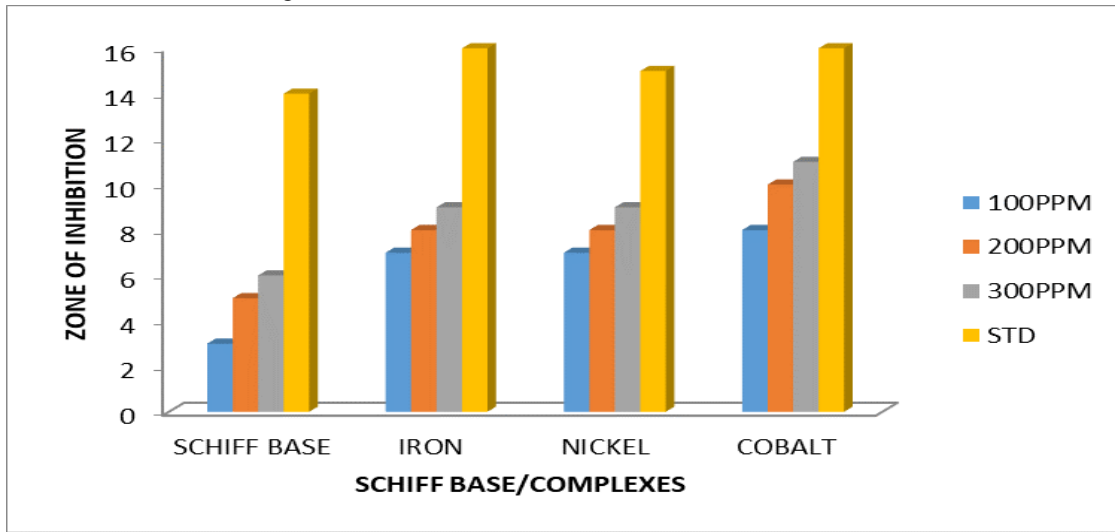


Figure 3. Sensitivity test for antibacterial activity of Schiff base and its metal (II) complexes against clinical isolate (*Streptococcus pneumoniae*) using well diffusion method.

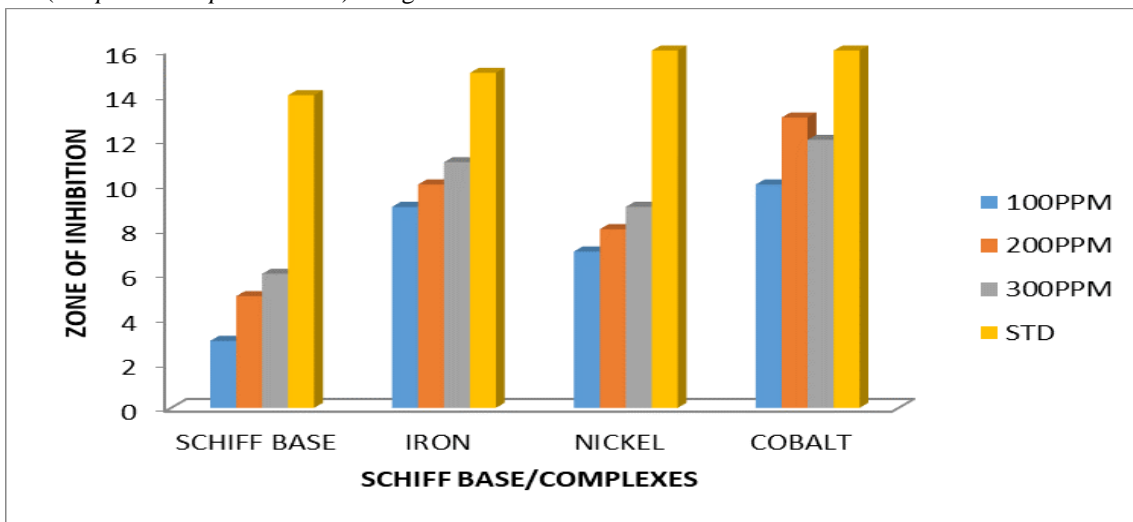


Figure 4. Sensitivity test for antibacterial activity of schiff base and its metal (II) complexes against clinical isolate (*Klebsiella pneumoniae*) using well diffusion method.

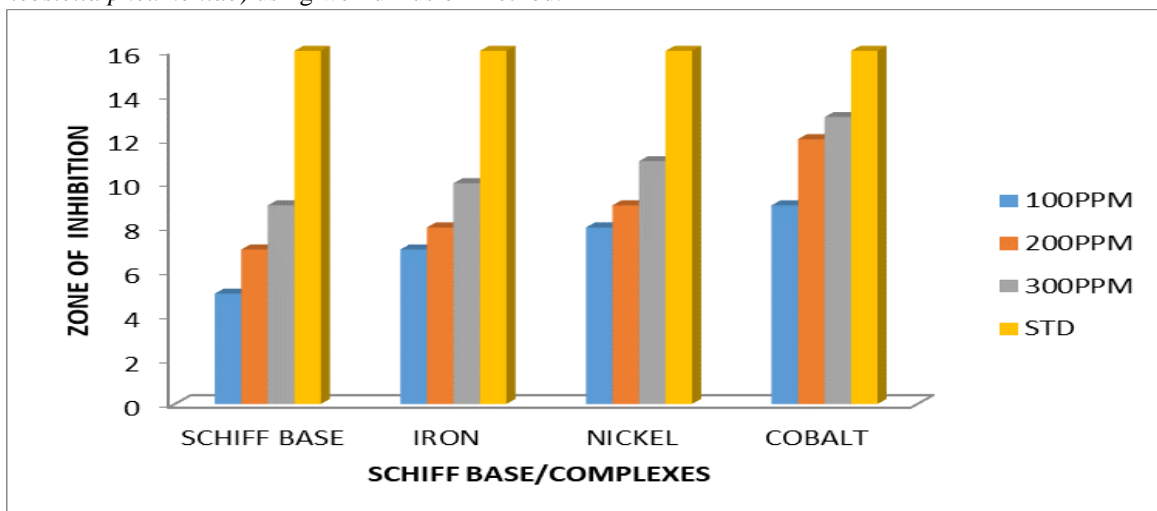


Figure 5. Sensitivity test for antifungal activity of Schiff base and its metal (II) complexes against clinical isolate (*Aspergillus niger*) using well diffusion method.

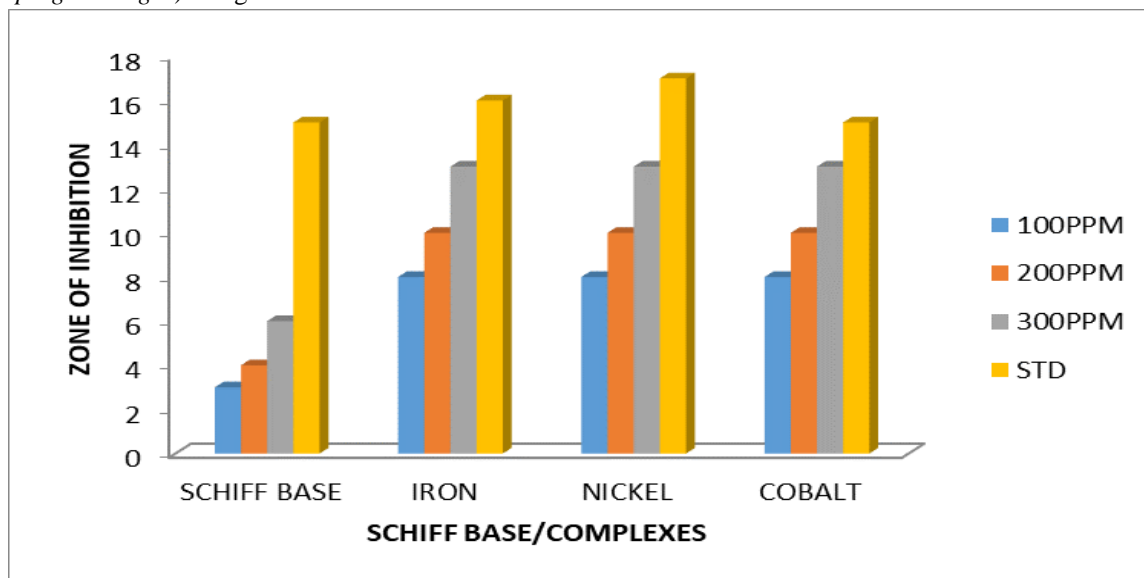
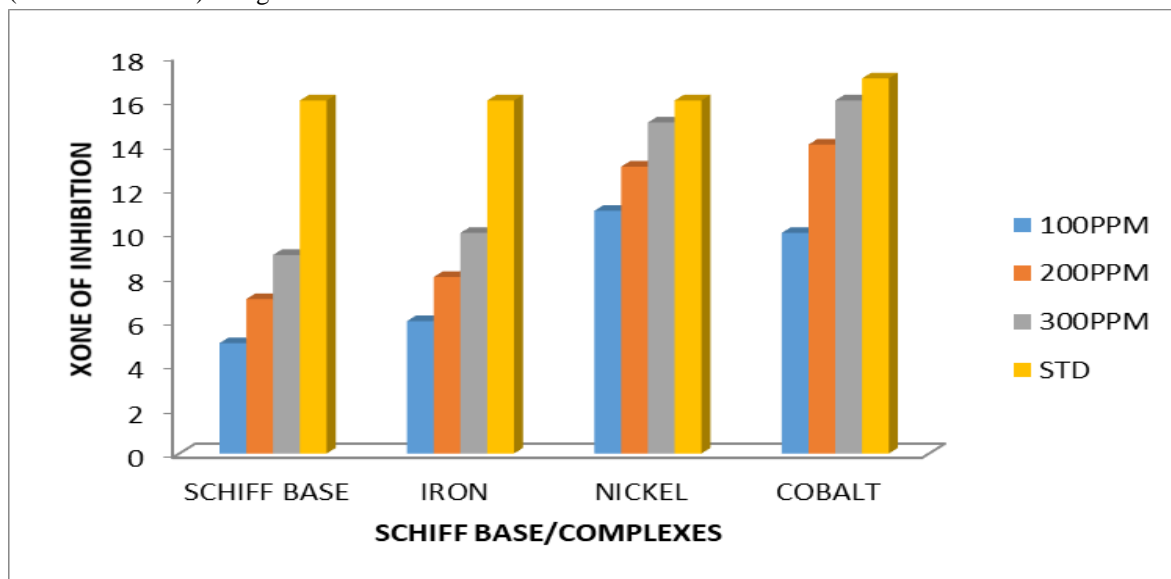


Figure 6. Sensitivity test for antifungal activity of Schiff base and its metal (II) complexes against clinical isolate (*Candida albican*) using well diffusion method.



Conclusion

The Schiff base of 2-hydroxy-1-naphthaldehyde with 2-amino-3-methylpyridine and its complexes of Fe (II), Ni (II) and Co (II) chlorides was synthesized and characterized. Based on the data obtained it was revealed that both Schiff base and complexes are colored with sharp melting point and are soluble in ethanol, dimethylsulfoxide and methanol but insoluble in water and slightly soluble in other solvent. The conductivity value obtained indicates that the synthesized complexes are non-electrolytes while an octahedral geometry was suggested for all complexes based on the data

obtained from magnetic susceptibility analysis. The IR results revealed bands at 1596cm^{-1} indicating the formation of azomethine ($\text{C}=\text{N}$) confirming the formation of Schiff base. 791cm^{-1} , 683cm^{-1} , 747cm^{-1} for (M-N) and 447cm^{-1} , 461cm^{-1} , 451cm^{-1} for (M-O) bands in the spectra of the complexes supporting coordination of Schiff base to respective metals. The in vitro antimicrobial screening of Schiff base and its metal complexes showed that they are potential antibacterial and antifungal agents against the tested microorganisms.

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Competeing interests: None.

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