

Synthesis, Characterization and Chemosensing Application of Schiff Base derived from 2-Pyridinecarboxaldehyde and 2-Nitroaniline and its Fe (II) and Cu (II) Complexes

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ABSTRACT

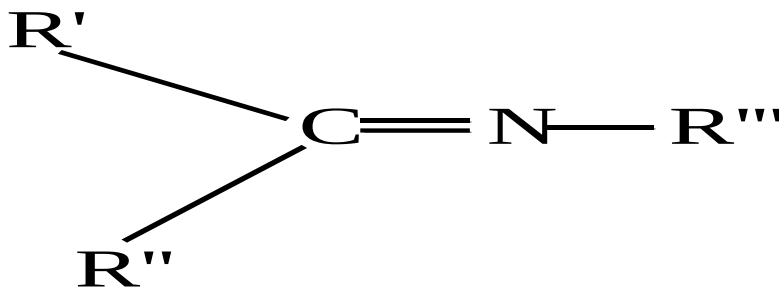
A Schiff base ligand was synthesized by refluxing 2-nitroaniline with 2-pyridinecarboxaldehyde in ethanol for three hours. The resulting ligand was further refluxed with metal (II) chlorides to yield Fe (II) and Cu (II) complexes in good yields, each exhibiting distinct colors. The ligand and its metal complexes were characterized using melting point, decomposition temperature, solubility tests, FTIR spectroscopy, magnetic susceptibility, conductivity measurements, and elemental analysis. Elemental data confirmed a 1:2 metal-to-ligand stoichiometry. The FTIR spectrum of the Schiff base displayed a strong absorption band at 1620 cm⁻¹, attributed to the azomethine (C=N) stretch, which shifted to lower frequencies in the complexes indicating coordination through the azomethine nitrogen. Conductivity studies revealed non-electrolytic behavior, while magnetic susceptibility measurements confirmed their paramagnetic nature. The Schiff base also exhibited potential as a fluorescence chemosensor, showing notable absorption changes at 295 and 395 nm and a visible color transition from light yellow to yellowish-brown, demonstrating high selectivity and sensitivity toward Cr³⁺ and Cd²⁺ ions compared to other analyte. The sensing performance was further evaluated through determination of limit of detection (LOD), which reflects the sensor's sensitivity, and binding constant (K_a), which indicates the strength of the analyte-sensor interaction. Antimicrobial screening showed that the metal(II) complexes possessed enhanced antibacterial and antifungal activities compare to the Schiff base.

Keywords: Complexes, chemosensor, 2-pyridinecarboxaldehyde, Schiff base, azomethine, 2-nitroaniline

INTRODUCTION

Schiff base (also known as imine or azomethine) is a chemical compound containing carbon and nitrogen double bond (-HC=N-) in which the nitrogen atom is connected to aryl or

alkyl group. These compounds were named after Hugo Schiff, a German scientist, who first reported them in 1864 (Suleiman *et al.*, 2023). Schiff's bases have the following general structure (Figure 1.1)



Where R', R'' and R''' are aryl or alkyl group.

Figure 1: General structure of Schiff base

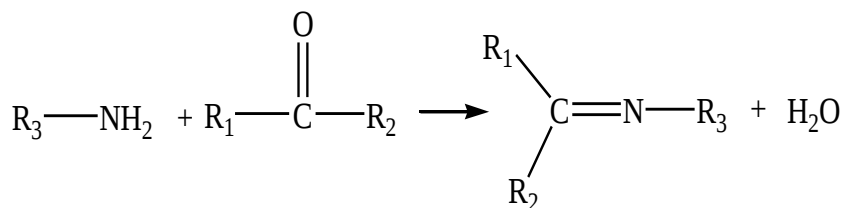
Schiff bases are usually colored and transparent solids. They are used in the determination of metal amounts and in the identification of carbonyl compounds due to their precise melting points (Subasi, 2022). The carbon-nitrogen double bond in Schiff bases rotates more easily than the carbon-carbon double bond, which allows stereo isomers to transform into each other. The reason for this: polarization occurs in the azomethine bond due to the fact that nitrogen is more electronegative than carbon. The stereo isomers of Schiff bases cannot be isolated with a few exceptions due to the very small energy difference between them (Subasi, 2022). If only an electronegative group is attached to the nitrogen atom, stereo isomers become isolated, since this group reduces the ease of rotation around the azomethine bond. Since the electronegative group attached to the nitrogen atom in the

azomethine group will push the negative charges of the nitrogen atom toward the carbon, this will cause a decrease in polarization and an increase in the character of the covalent double bond. All compounds containing an azomethine group show basic properties due to the unshared electron pairs on the nitrogen atom and the electron donating feature of the double bond (Subasi, 2022). Schiff bases show weaker basic properties compared to their corresponding amines. The reason for this is that while the nitrogen atom in amines undergoes sp³ hybridization, this hybridization turns into sp² hybridization when the imine structure is formed. Since the s character will increase in hybridization, the basicity will decrease greatly (Subasi, 2022).

The most common method for preparation of Schiff bases involved acid-catalyzed condensation reaction of an amine

and aldehyde or ketone under refluxing conditions (Kilic *et al.*, 2009). The first step in this reaction involved nucleophilic addition which leads to the formation of unstable carbinol

amine intermediate which loses a molecule of water in the second step forming the corresponding imine as shown in Scheme 1 (Ha *et al.*, 2010).



Where R₁, R₂ and R₃ may be an alkyl or an aryl group

Scheme 1: The General Schematic Formation of Schiff Base

Transition metals are known to form complexes and Schiff bases have often been used as chelating ligands in the field of coordination chemistry. Their metal complexes have been of great interest for many years (Mahadevi, 2023). It is well known that N, O and S atoms play a key role in the coordination of metals at the active sites of numerous Schiff bases (Ibrahim *et al.*, 2022). These compounds are majorly used in industries and also have significant biological activities, including antioxidant, antibacterial, antifungal, antiviral, antitumor, and anti-inflammatory. The majority of these compounds show excellent catalytic activities. Schiff bases are considered to be the most versatile ligands as they form complexes with the metal atoms (Ibrahim *et al.*, 2022). They serve as models for biologically important species and find applications in biomimetic catalytic reactions. It is known that the existence of metal ions bonded to biologically active compounds may enhance their activities (Ibrahim *et al.*, 2022).

The sensing mechanism of the Schiff base chemosensor, including its selectivity and sensitivity. The electron configuration of both the ligand and the metal ion, as well as the structural stiffness and the capacity of the Schiff base chemosensor to attach to the metal ion, is a parameter that controls the selectivity and high sensitivity of Schiff base chemosensors. (F.M, Feffer *et al.*, 2005, J, T Rademacher *et al.*, 1997). Therefore, various mechanisms, such as chelation, intra/intermolecular charge transfer (ICT), hydrolysis, chelation enhancement quenching effect (CHEQ), and ring-opening mechanisms, this have been proposed for the interaction between metal ions and various SB chemosensor. Also the mechanisms underlying the chemosensory applications of Schiff base derivatives are: (i) Charge transfer (CT): intramolecular charge transfer (ICT), metal ligand charge transfer (MLCT), and twisted intra/intermolecular charge transfer (TICT) are examples of Charge transfer (CT) processes. A ratiometric signal is produced by intramolecular charge transfer (ICT) based chemosensor when intramolecular charge transfer (ICT) process is enhanced or suppressed. On the other hand, transition metal complexes like copper (ii) complex with halide, chromate ion, ruthenium, rhenium, and iridium, etc., frequently exhibit metal ligand charge transfer (MLCT), in which Charge transfer (CT) occurs from a ligand to a transition metal cation. Through the influence of metal ligand charge transfer (MLCT) energy level by analytes, it can also be employed for chemosensor design. Additionally, twisted intramolecular charge transfer (TICT) is a potent intramolecular Charge transfer (CT) that takes place in the excited state and requires solvent relaxation surrounding the molecule to produce an ongoing rotation of the electron donor and acceptor until it is twisted roughly 90 degrees (K, Rotkiewicz *et al.*, 1973, W, Rettig *et al.*, 1988). Since intramolecular rotation and charge separation in the twisted intramolecular charge transfer (TICT) state depend on

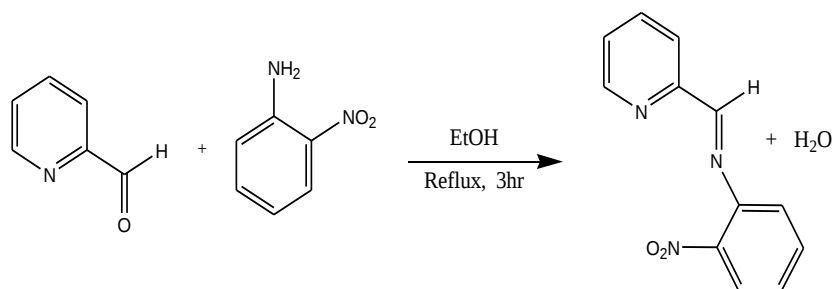
polar solvent relaxation, the fluorescence behavior is extremely sensitive to micro polarity and/or steric barrier for molecular rotation (De silva, 1997, A, Ito 2010, W, Rettig, 1994). (ii) Energy transfer (ET): Depending on the interaction distance between the energy donor and energy acceptor, Energy transfer can be divided into two categories: electronic energy transfer and fluorescence resonance energy transfer. While fluorescence resonance energy transfer requires a specific amount of spectral overlap between the emission spectrum of the donor and the acceptor, electronic energy transfer also known as Dexter electron transfer. As a result, the chemosensors based on the energy transfer mechanism are highly dependent on distance. (iii) Excimer/excplex: An excimer is a complex that is created when a fluorophore interacts with another fluorophore of the same structure in the ground state while it is stimulated. The resulting complex is known as an exciplex, if the fluorophore in the excited state differs from the fluorophore in the ground state. A dual emission from the monomer and excimer/excplex is frequently recorded at the same time because the emission spectra of an excimer/excplex are red-shifted in comparison to that of the monomer. The excimer/excplex band can therefore be monitored to detect excimer/excplex production or deformation in response to interaction with a guest species (A, Ito, 2010, C, Lodeiro, 2009, JS, Wu, 2005).

MATERIALS AND METHODS

All chemicals used were of analytical grade and used without further purification. Glassware was thoroughly cleaned, rinsed with distilled water, and oven-dried at 110 °C. Magnetic susceptibility of the complexes was measured using a Sherwood MK1 balance, while melting points and decomposition temperatures were determined with a Gallenkamp melting point apparatus (Gemini BV4947). FTIR spectra were recorded on an Agilent Cary 630 spectrophotometer, molar conductance was measured with a Jenway 4010 meter, and elemental analyses were conducted at OEA Labs, UK, using a CE Instruments EA1110 analyzer. The antimicrobial activity of the Schiff base and its complexes was evaluated *in vitro* against bacterial and fungal strains, using Ampiclox and Ketoconazole as reference drugs at Bayero University, Kano.

Preparation of the Schiff base

A 2-nitroaniline (0.01 mol, 1.37 g, 1.29 cm³) solution in 20 cm³ ethanol was added to the 20 cm³ ethanolic solution of 2-pyridinecarboxaldehyde (0.01 mol, 1.07g, 0.95cm³) with stirring and the reaction was refluxed for 3 hours. The solid product was separated by filtration, purified by crystallization from ethanol, washed with diethyl ether, and dried in a desiccator over anhydrous calcium chloride (Salihuet *et al.*, 2022, Ogbonda *et al.*, 2023, Suleiman *et al.*, 2023)



Scheme 1: Synthesis of the Schiff base

TLC Monitoring

TLC was performed on silica gel 60 F254 Aluminium plates using n-hexane ethyl acetate (7:3, v/v) as eluent. The Spots were visualized under UV light at 254 nm.

Rf Value: SB=0.52 2-nitroaniline=0.31, 2-pyridinecarboxaldehyde=0.68.

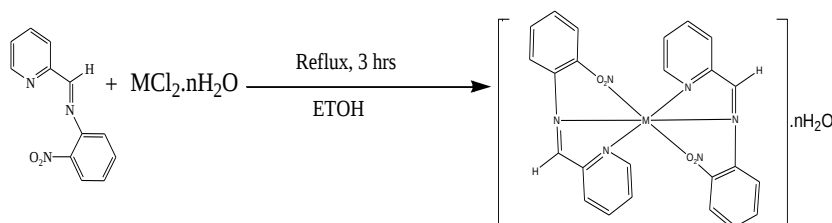
Disappearance of the 2-nitroaniline spot and appearance of new spot with Rf 0.52 confirmed schiff base formation.

Yield Optimization

Optimal condition 1:1 molar ratio, 3 drops AcOH, ethanol as solvent, 3h reflux at 78 °C. Under these conditions yield 89.28%.

Synthesis of the Complexes

Schiff bases (0.01 mol, 1.37 g) solution in hot ethanol 20 cm³ and ethanolic solution of the metal (II) chloride (0.01 mol), was mixed, stirred and reflux for 3 hours. The product obtained was then reduced to half its volume and left overnight. The product obtained was then filtered, recrystallized in ethanol, wash with cold diethyl ether and dried (Salihuet *al.*, 2022, Suleiman *et al.*, 2023, Dasilvaet *al.*, 2011, Mundeet *al.*, 2009).



Where $1 \leq n \leq 2$, and M = Fe (II) and Cu (II),
Scheme2: Synthesis of the complexes

Preparing Cd²⁺ and Cr³⁺ Solution for Fluorescence Detection Using Schiff Base Chemosensors

A 0.89g CdCl₂ and CrCl₃ was weighed and dissolved in 100 cm³ distilled water to obtain the stock solution, the stock solution was further dissolved in 100 cm³ of buffer solution to prepare the working solution. Also, 1.78g of the Schiff base chemosensor was dissolved in DMSO, where equal volume of CdCl₂ and SB chemosensor were mixed together, and the mixture was incubated for 30 minutes to allow for complexes formation (Abu Tahaet *al.*, 2025, Bharatet *al.*, 2023, Lianlianet *al.*, 2013).

Antibacterial and Antifungal Test

The method used by (Salihu *et al.*, 2022, Yusha'u & Salisu 2011) was used in atest, in which the schiff base and its

metal(II)complexes were assayed by the ager disc diffusion method using cultures of *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*. The samples were separately dissolved in dimethylsulfoxide (DMSO) to have four different concentrations (500, 1000, 2000 and 4000) µg/disc. Each of these was separately placed on the surface of the culture and incubated at 37°C for 24. The diameter of the zone of inhibition produced by the Schiff base ligand and its metal (II) complexes were taken and recorded. A similar procedure was applied in antifungal by using *Candida Albicans*, *Tinea Capitis*, and *Tinea Pedis* fungal isolates and incubated for 72 hours

RESULTS AND DISCUSSION**Results****Table 1: Physical Properties of the Schiff Base and its Metal (II) Complexes**

Compound	Colour	Decomposition Temperature(°C)	Melting Point(°C)	Percentage Yield (%)
Ligand (L)		-	93	90.81
[FeL ₂].2H ₂ O	Brown	170	-	88.42
[CuL ₂].2H ₂ O	Brown	175	-	86.22

L = Ligand (C₁₂H₉N₃O₂)

Table 2: Solubility Test of the SB and its Metal (II) Complexes

Compound	Water	Ethanol	Methanol	Acetonitrile	Ether	DMSO	DMF	nHexane
Ligand (L)	IS	SS	SS	IS	SS	S	S	IS
[FeL ₂].2H ₂ O	IS	S	SS	IS	IS	S	S	IS
[CuL ₂].2H ₂ O	IS	SS	SS	IS	IS	S	S	SS

Key:

L = Ligand (C₁₂H₉N₃O₂)

IS = Insoluble

S = Soluble

SS = Slightly Soluble

DMSO = Dimethyl sulfoxide

DMF = Dimethyl formamide

Table 3: Conductivity Measurement of the Metal (II) complexes in (10⁻³) IN DMSO

Complex	Concentration (mol dm ⁻³)	Specific Conductance (Ohm ⁻¹ cm ⁻¹)	Molar Conductance (Ohm ⁻¹ cm ² mol ⁻¹)
[FeL ₂].2H ₂ O	1 × 10 ⁻³	2.66 × 10 ⁻⁵	26.66
[CuL ₂].2H ₂ O	1 × 10 ⁻³	2.00 × 10 ⁻⁵	20.00

L = Ligand (C₁₂H₉N₃O₂)**Table 4: Magnetic Parameters of Metal (II) Complexes**

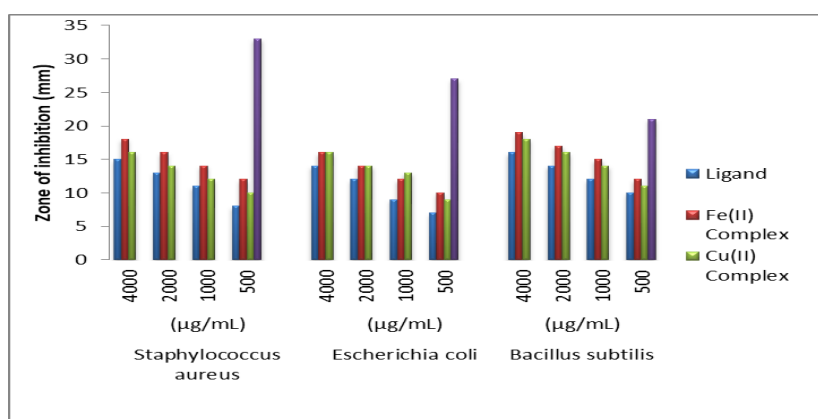
Complex	Magnetic Susceptibility	Molarmagnetic susceptibility	μ _{eff} (BM)
[FeL ₂].2H ₂ O	7.4862 × 10 ⁻⁶	3.1906 × 10 ⁻³	2.76
[CuL ₂].2H ₂ O	5.9550 × 10 ⁻⁷	2.3682 × 10 ⁻⁴	1.00

L = Ligand (C₁₂H₉N₃O₂)**Table 5: Infrared Spectral Bata of the Schiff Base and its Hetal (II) complexes**

Complex	ν(OH) cm ⁻¹	ν(C=N)cm ⁻¹	ν(M-N) cm ⁻¹	ν(M-O) cm ⁻¹
Ligand (L)	3339	1620	-	-
[FeL ₂].2H ₂ O	3349	1568	745	428
[CuL ₂].2H ₂ O	3341	1594	741	443

L = Ligand (C₁₂H₉N₃O₂)**Table 6: Microanalysis Data of the Schiff Base and its Metal (II) Complexes**

Compound	M. wt. (g/mol)	% Calculated (found)		
		C	H	N
L	227.216	32.12(31.68)	2.48(1.98)	8.43(9.24)
[FeL ₂].2H ₂ O	511.25	28.2(28.16)	2.51(1.76)	8.37(8.22)
[CuL ₂].2H ₂ O	518.93	33.36(32.71)	2.48(2.23)	4.43(3.98)

L = Ligand (C₁₂H₉N₃O₂)**Figure 2: Antibacterial activities SB and its complex**

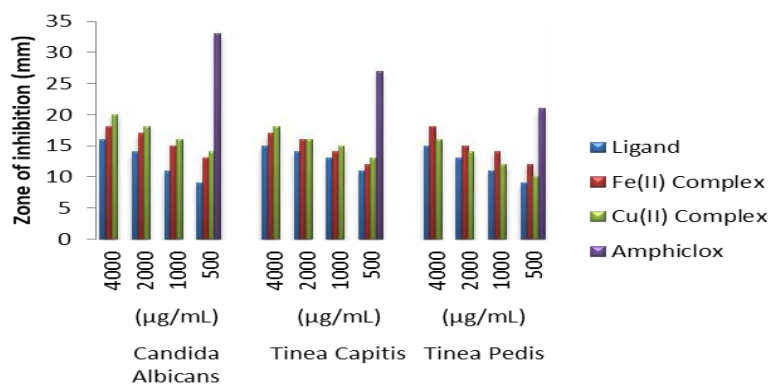
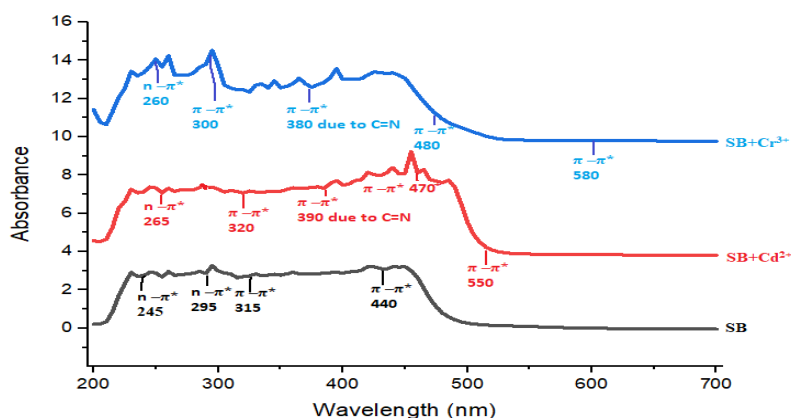


Figure 3: Antifungal activities SB and its complexes

Figure 4: UV-Spectra of SB, Cd²⁺ and Cr³⁺

Discussion

The Schiff base and its metal (II) complexes were prepared in good yield, and their physical properties were analyzed. The percentage yield of the Schiff base was 90.81 % while that of the complexes were 88.42 and 86.22 %, which indicated that the methodology was very efficient for the synthesis. The Schiff base was yellow while the Fe(II) and Cu(II) complexes were brown and dark brown respectively, this showed that there is transformation and formation of the desired compounds. Also, it was found that the melting point of the Schiff base was 93°C and the decomposition temperature of the metal (II) complexes were 170°C and 175°C, this is an indication that the complexes are thermally stable compared to the Schiff base which is due to the coordination of metal ion, as similar research was conducted and reported by (Salihu *et al.*, 2021). This is presented in Table 1. The solubility test carried out for the Schiff base and their corresponding complexes in the following solvents, distilled water, ethanol, methanol, dimethylsulphoxide, diethyl ether, hexane and dimethylformamide. The Schiff base and the complexes are soluble in most of the highly polar solvent such as dimethylsulphoxide and dimethylformamide but poorly soluble in non-polar solvent; such as methanol and hexane, this is an indication that the compounds are polar in nature or there is a presence of polar group attached to the compounds, this is an indication that the compounds are polar in nature or there is a presence of polar group attached to the compounds as similar reported by (Salihu *et al.*, 2021, Gehadet *et al.*, 2005). This result is contained in Table 2

The molar conductance of the metal (II) complexes were relatively low and are in the range 26.66 and 20.00 Ω⁻¹cm²mol⁻¹ for Fe (II) and Cu (II) complexes. These low values suggest their non-electrolytic nature as similar reported by

(Muzammilet *et al.*, 2015). This contained in Table 3. The effective magnetic moments of the complexes were 2.76 and 1.00 B.M showing their paramagnetic nature. These magnetic susceptibility value of the complexes are consistent with octahedral geometry around the central metal ion as similar research was conducted and reported by (Salihu *et al.*, 2019, Gehadet *et al.*, 2005). This contained in Table 4. The infrared spectra of the Schiff base showed a prominent band at 1620cm⁻¹ due to ν(C=N) linkage appeared indicating that condensation between 2-nitroaniline and 2-pyridinecarboxaldehyde has taken place resulting into formation of the Schiff base. There is a shifting from 1620 cm⁻¹ to range 1568-1594 cm⁻¹ which indicate the formation of the complexes. Further evidence of the co-ordination of the Schiff base with metal ions was shown by the emergence of new peaks due to ν (M–N) in the range of 745-741 cm⁻¹, ν (M–O) in the range of 428- 443 cm⁻¹. The spectra showed a strong band at range of 3345-3347 cm⁻¹, suggesting the presence of free hydrogen in the metal complexes, a similar research was conducted and reported by Salihu *et al.*, 2023. (Table 5). The elemental analysis for C, H and N for all the complexes determined showed a good agreement between the calculated and observed values and the result suggested 1:2 metal to Schiff base as similar reported by (Salihu *et al.*, 2021, Abdullahi *et al.*, 2020). This result is contained in Table 6. The antibacterial activity of the Schiff base and their metal (II) complexes were determined using agar well diffusion method and the results presented in (Figure 2). The diameters of zone of inhibition were measured for each treatment. The Schiff base showed little activity at low concentration for most of the isolates (*Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*), but relatively improved with an increase in the concentration of the compounds. Upon complexation,

there has been increase in the activity, with most of the complexes showing significant inhibition which also increase with increase in concentration. Cu(II) complexes were found to be the most active, which indicate the tendency of this complex to be used as a potential drug (Salihu et al., 2021, Siraj et al., 2021). The antifungal activity of the Schiff base and their metal(II) complexes were also determined using agar well diffusion method and the results presented in figure 3. The diameters of zone of inhibition were measured for each treatment. The Schiff base showed little activity against all the fungal isolate (*Candida Albicans*, *Tinea Capitis*, and *Tinea Pedis*) at higher concentration but no activity at lower concentration. However, complexes recorded a much higher activity against all the tested fungal strains which increases with increases in concentration. However, they were found to be lower in activity than the Ketoconazole control used. (Siraj et al., 2021). The UV-vis absorption studies of the Schiff base chemosensor derived from 2-nitroaniline and 2-pyridinecarboxaldehyde (SB1) were investigated and it showed absorptions peaks centered at 245 nm, with absorbance (2.919), 295 nm with an absorbance (3.275), 295 nm 315nm with absorbance (2.641), and 440nm (3.215). The absorption band at 245 nm, 295 nm, 315 nm, and 440 nm, corresponds to the $n-\pi^*$ and $\pi-\pi^*$ transition. These peaks of the SB chemosensor disappeared upon the addition of Cr^{3+} , and Cd^{2+} to SB solution, and new absorption bands 260, and 265. The band at 295 nm on the SB shifted bathochromically to 300 nm and 320 nm. Also, there is a red shift from 315 nm on the SB to 390 and 380 nm for Cd^{2+} , and Cr^{3+} . The bathochromic shift at 390 and 380 nm was due to the sensing ability of the chemosensors and the $\pi-\pi^*$ transition on C=N. The 480 and 470 nm appeared due to the $\pi-\pi^*$ for Cr^{3+} and Cd^{2+} , however, for the SB chemosensor there is hypsochromic shift at 440 nm. These spectral changes occur due to the intramolecular charge transfer (ICT) process during the metal-ligand complex formation. However, there is Red shift and hypochromic shift for the absorbance on the all heavy metals. Hence, the increase in the wavelength and the decrease in the absorbance was due to the change in the chemical environment. The sensing performance was further evaluated through determination of limit of detection (LOD), which reflects the sensor's sensitivity, and binding constant (K_a), which indicates the strength of the analyte-sensor interaction.

CONCLUSION

The Schiff base complexes were prepared from 2-pyridinecarboxaldehyde and 2-nitroaniline. FTIR, conductivity measurement, solubility, magnetic susceptibility, UV-visible analysis, and CHN micro-analysis were used to evaluate the complexes' compositions and structures. Antimicrobial research on Schiff bases and complexes suggests that metal complexes have higher activity than Schiff bases, which can be used as a potential drug. The Schiff base can be used as an environmental remediation as it captures the selected hazardous metal ions (Cd^{2+} and Cr^{3+}).

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