

BIOLOGICAL STUDY OF Co(II), Cu(II) AND Ni(II) SCHIFF BASE COMPLEXES DERIVED FROM 2-HYDROXY-1-NAPHTHALDEHYDE AND HYDRAZINE MONOHYDRATE

¹S. A Dailami, ²U. Sani, ³I. Bala, ⁴M .A Funtua & ⁵A .I Sadiq

^{1&.4}Department of Chemistry,
Federal University Lokoja, Kogi, Nigeria

^{2&.5}Department of Chemistry,
Bayero University, Kano, Nigeria

³Department of Microbiology,
Bayero University, Kano, Nigeria

Abstract

Transition metal complexes of Co(II), Cu(II) and Ni(II) with a new Schiff base ligand (H₂L) derived from the ethanolic condensation of 2-hydroxy-1-naphthaldehyde and hydrazine monohydrate were successfully synthesized and separated. The Schiff base ligand and all the metal(II) complexes prepared were characterized on the basis of melting point/decomposition temperature, solubility, conductivity, FT-IR spectra and elemental analyses. Sharp melting point/decomposition temperature indicated the compounds are probably pure. The IR spectra of the free ligand showed band at 1655cm⁻¹ which is assigned to the (-C=N-) stretching vibration of the azomethine. This band was observed at lower frequencies in the spectra of the metal(II) complexes, which indicates complexation. The conductivity measurement showed that all the complexes are non electrolyte while elemental analysis result revealed 1:1 Metal-Schiff base ratio and tetrahedral nature of the metal(II) complexes. Solubility results indicated that all the compounds are soluble in protonic solvents such as methanol and ethanol, and insoluble in aqueous media. The in vitro Antimicrobial assay showed that the test compounds are active against most of the tested isolates at high concentration (500 g per disc). Antioxidant activity of the phenolic Schiff base against DPPH free radicals was ascertained by employing probit analytical method using SPSS 16.0 software. The IC₅₀ value for the phenolic Schiff base revealed its promising use as antioxidant.

Keyword: *Biological study, Complexes, Hydrazine, Monohydrate.*

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Background to the Study

The chemistry of Schiff bases marked its beginning in 1864 with the pioneering work of a Nobel Prize Winner and German Scientist Hugo Schiff (1). Since then the transition metal complexes of Schiff bases continue to be of interest even after a hundred years of study.

Schiff bases are simply the condensation products of primary amines with carbonyl compounds (2). They are compounds containing azomethine group ($R_1N=CR_2R_3$) and have the general structure as follows:

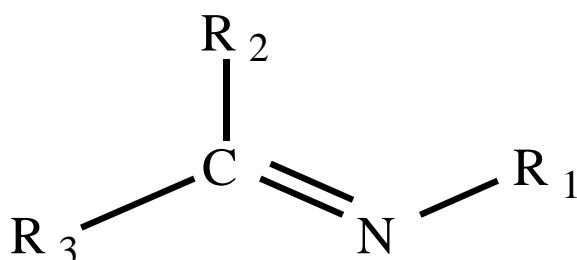


Figure 1: General Structure of Schiff Bases

Where R₁, R₂ and R₃ are alkyl, aryl, cyclo-alkyl, heterocyclic group etc., which may be variously substituted.

Phenolic Schiff bases have been widely reviewed due to their various applications in different spheres, ranging from inorganic chemistry, physical analytical chemistry to biochemistry and biology (3, 4). They are reported as effective corrosion inhibitors on mild steel such as Copper and Aluminium (5). They are known for their antibacterial (6-9), antifungal (10, 11) and anticancer (3) activities. Due to the presence of OH group, phenolic Schiff bases are reported as powerful antioxidants and good radical scavengers (12, 13). In general, the free radical scavenging activity of phenols is mainly attributed to the hydrogen atom transfer of the -OH, -NH and -SH groups (usually attached to aromatic rings) to the free radicals.

Experimental

Analar grade metal (II) chlorides were used to prepare all the metal (II) complexes. 2-hydroxy-1-naphthaldehyde and hydrazine monohydrate were obtained from Sigma Aldrich. All solvents were used as purchased without further purification.

Bacterial and fungal (Clinical) isolates were obtained and identified at the Department of Microbiology, Faculty of Sciences, Bayero University, Kano.

Preparation of the Schiff Base

A solution of 0.5mole hydrazine monohydrate in 25cm³ ethanol was added slowly to a solution of 0.1mole 2-hydroxy-1-naphthaldehyde in 25cm³ ethanol to form the reaction mixture. The mixture was then refluxed with magnetic stirrer for an hour. The solid crystals obtained on cooling the reaction mixture was filtered, washed successively with ethanol and diethyl ether, recrystallised from methanol and then dried over CaCl₂ for a week (14).

Preparation of the Schiff Base Metal Complexes

All the Schiff base metal (II) complexes were prepared by addition of the mixture of the Schiff base (0.1mole) and sodium hydroxide (0.2mole) in hot ethanol (50cm³) into an aqueous solution of the metal (II) chloride (0.1mole). The mixture was refluxed for 2hours while stirring magnetically. The solid crystals obtained were washed with ethanol and diethyl ether, recrystallised from methanol and then dried over CaCl₂ for a week. (14).

Biological Study

Antibacterial Activity Test

The in vitro antibacterial property of the Schiff base ligand and its Co(II), Cu(II) and Ni(II) complexes were assayed using four gram negative (*E. coli*, *K. pneumonia*, *P. aeruginosa* and *P. mirabilis*) and one gram positive (*S. aureus*) bacterial (clinical) isolates by Disc Diffusion Technique (15).

The suspension of each microorganism was rubbed using sterile swab on a solidified nutrient agar in Petri dishes. Three different concentration (500 µg, 250 µg and 125 µg) per disc of the test compounds in DMSO were prepared and placed on the culture media before incubation at 37°C for 24hrs. Activities were determined by measuring (in mm) the diameter of the zone showing complete inhibition. The results obtained were compared with the activity of Augmentin (30 µg) as a standard antibacterial drug.

Antifungal Activity Test

The in vitro antifungal property of the Schiff base ligand and its Co(II), Cu(II) and Ni(II) complexes were assayed using three fungal isolates (*C. albicans*, *F. solani* and *A. fumigates*) by Disc Diffusion Technique (15). Potato Dextrose Agar were used to prepare the culture media and incubated at room temperature. The results obtained were compared with the activity of Ketoconazole (600 µg) as a standard antifungal drug.

Antioxidant Activity Test

The free radical scavenging activity of the phenolic Schiff base and its metal (II) complexes against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was studied according to the procedure described by Aktumsek et al, 2013 (16).

Each sample of stock solution (1.0 mg/ml) of the test compounds was diluted through the final concentration; 1000, 500, 250, 125, 62.5, 31,30, 15,63 and 7.81 µg/ml. A total of 50µM DPPH methanolic solution (3.8ml) was added to the sample solution (0.1ml each) and allowed to react at room temperature for 30mins in dark. The reduction capability of the DPPH radicals was determined from the decrease in its absorbance at 517nm which can be induced by antioxidants.

Inhibition of DPPH radical (I %) were calculated using the relation

$$I\% = \frac{(A_{\text{blank}} - A_{\text{sample}})}{A_{\text{blank}}}$$

Where A_{blank} = Absorbance of the reagents without the test compound

A_{sample} = Absorbance of the reagents with the test compound

The concentration corresponding to the 50% inhibition (IC_{50}) was determined using Probit Analysis by means of SPSS 16.0 software. The IC_{50} values obtained are compared with that of Ascorbic Acid as a standard antioxidant. Lower IC_{50} value indicates higher activity.

Results and Discussions

Three new Schiff base complexes derived from ethanolic condensation of 2-hydroxy-1-naphthaldehyde and hydrazine monohydrate were successfully prepared in good yield (Table 1). Sharp increase in melting /decomposition temperatures indicated the compounds are probably pure and the magnetic measurement showed that all the complexes are paramagnetic (Table 1). Solubility test revealed that all the compounds are soluble in protonic solvents such as methanol and ethanol, insoluble in Distilled water, Ether, Nitrobenzene and Carbon tetrachloride while slightly soluble in Acetone, Acetonitrile and Dimethylformamide (Table 2).

Table 1: Physical and Analytical Data of the Schiff base and the Metal (II) Complexes

Compounds	Colour	Yield (%)	Melting Temp. ($^{\circ}C$)	Elemental Analysis Calc. (Found)			Magnetic (μ_{eff}) Temp. (K)
				%C	%H	%N	
Schiff base (L)	Yellow	78	260	77.64 (77.21)	4.70 (4.53)	8.23 (8.11)	—
CoL	Light Brown	61	310	66.52 (66.01)	3.53 (3.40)	7.05 (7.00)	3.14
CuL	Blue	80	290	65.75 (64.01)	3.49 (3.15)	7.06 (6.88)	2.12
NiL	Dark Brown	75	278	66.55 (65.99)	3.53 (3.11)	6.97 (6.88)	3.05

L = N,N'-Bis(2-hydroxy-1-naphthyl)hydrazine

Table 2: Solubility of the Schiff base and the Metal (II) Complexes

Solvents	Schiff base (L)	CoL	CuL	NiL
Distilled Water	IS	IS	IS	IS
Methanol	S	S	S	S
Ethanol	S	S	S	S
Ether	IS	IS	IS	IS
Acetone	SS	SS	SS	SS
Nitrobenzene	IS	IS	IS	IS
Carbon tetrachloride	IS	IS	IS	IS
Acetonitrile	SS	SS	SS	SS
Dimethylsulfoxide	S	S	S	S
Dimethylformamide	SS	SS	SS	SS

L = N,N'-Bis(2-hydroxy-1-naphthyl)hydrazine

Conductivity measurement of the complexes revealed the non electrolytic nature of the compounds (Table 3) as molar conductance value 120-160 $\text{Ohm}^{-1}\text{cm}^2\text{mol}^{-1}$ is expected for 1:1 electrolytic (17).

Table 3: Molar conductance of the Schiff base Metal (II) Complexes

Schiff base Complex	Electrical Conductance ($\text{Ohm}^{-1}\text{cm}^2$)	Molar Conductance ($\text{Ohm}^{-1}\text{cm}^2\text{mol}^{-1}$)
CoL	7×10^{-6}	7.00
CuL	9×10^{-6}	9.00
NiL	5×10^{-6}	5.00

L = N,N'-Bis(2-hydroxy-1-naphthyl)hydrazine

Elemental analysis for carbon, hydrogen and nitrogen content in the Schiff base and the metal complexes revealed 1:1 Metal-Schiff base ratio (Table 1). The IR spectra of the Schiff base and the metal complexes were recorded on Shimadzu 8400S FT-IR spectrophotometer in the range $4000\text{-}400\text{cm}^{-1}$. The spectra of the ligand showed a sharp band at 1655cm^{-1} and 3434cm^{-1} attributed to $\nu(\text{-HC=N-})$ and $\nu(\text{-OH})$ stretching vibrations respectively (Table 4). The 3434cm^{-1} $\nu(\text{-OH})$ disappeared in the spectra of the metal (II) complexes indicating deprotonation of the phenolic OH of the Schiff base (18). The $\nu(\text{-HC=N-})$ vibration in the metal complexes shifted to lower frequencies (1611-1599) which indicates the formation of coordinate bond between the metal ions and the azomethine nitrogen.

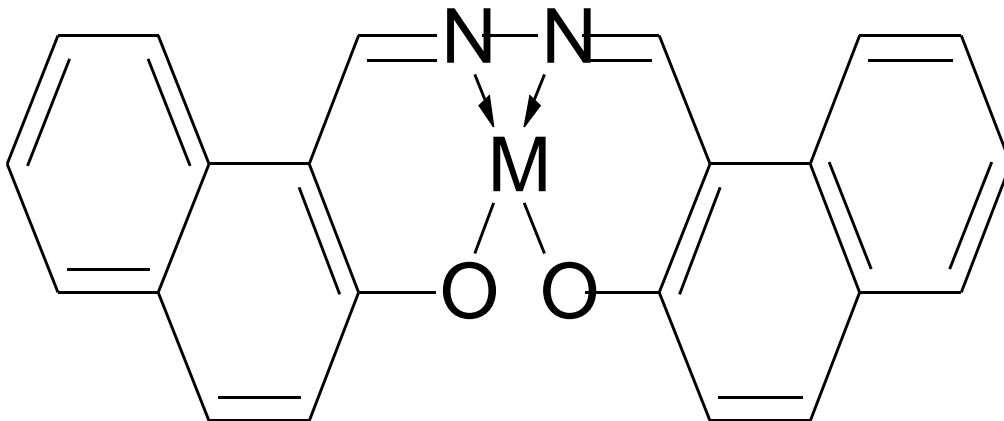
Two new bands ($532\text{-}512\text{cm}^{-1}$ and $771\text{-}721\text{cm}^{-1}$) appear in the far infra red region in the spectra of the complexes, which suggests the formation of M-O and M-N bonds, respectively (19).

Table 4: Selected Vibration Frequencies of the Schiff base and its Metal (II) Complexes

Compound	(OH)	(C=N)	(M-O)	(M-N)
Schiff base (L)	3434	1655	—	—
CoL	—	1611	532	721
CuL	—	1602	505	743
NiL	—	1599	512	771

L = N,N'-Bis(2-hydroxy-1-naphthyl)hydrazine

Proposed Structure of the Schiff base Metal (II) Complexes



M = Co^{2+} , Cu^{2+} and Ni^{2+}

The *in vitro* antimicrobial activity of the Schiff base and its Co(II), Cu(II) and Ni(II) complexes were assayed using Disc Diffusion Technique (15). Diameter of the zones of inhibition by the Schiff base and its metal (II) complexes at concentrations of 500, 250 and 125 $\mu\text{g}/\text{disc}$, against the five bacterial and three fungal isolates used are presented in Tables 5 and 6 respectively. The antibacterial activity results (Table 5) revealed that all the test compounds are active against, *S. aureus* at all concentrations, *P. mirabilis*, *E. coli* and *P. aeruginosa* at high concentrations while *K. pneumoniae* resist all the test compounds except Cu(II) complex at high concentration (500 μg). Although a limited number of isolates were tested, some predictions can be made as to possible outcome of the *in vivo* treatment efficiency since, in hospitals treatment is based on tests on one clinical isolate. The results obtained were compared with activity of a standard antibacterial drug (Augumentin), and the test compounds show potential bactericidal activity. The antifungal activity results (Table 6) revealed that all the test compounds are active fungicidal agents. These results are compared with that of Ketoconazole as a standard antifungal drug.

The enhanced antimicrobial activity of the metal chelates over their corresponding chelating agent may be explained on the basis of Overtone's concept (Anjaneyula and Rao, 1986), and the Tweedy's chelation theory (20, 21). According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid-soluble materials due to which, lipophilicity is an important factor which controls the antimicrobial activity. On chelation, the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further, it increases the delocalisation of π -electrons over the whole chelate ring and enhances the lipophilicity of the complexes. This increased lipophilicity enhances the penetration of the complexes into lipid membranes and thus blocking the various metabolic activities of microorganisms. The higher activity of the metal complexes can be attributed to the involvement of a metal ion in the normal cell processes (22).

- Generally, this can be achieved through the following properties of the metal complexes:
- The complex should possess sufficient lipid solubility to permit transport of metal ions across the membranes.
 - The metal complexes should be highly thermodynamically stable to reach the reaction site without being dissociated or even completely deactivated.

Table 5: Sensitivity of Bacterial Isolates to the Schiff base and its Metal (II) Complexes

Isolates	Schiff base (L)			CoL			CuL			NiL		
	($\mu\text{g}/\text{disc}$)	($\mu\text{g}/\text{disc}$)	($\mu\text{g}/\text{disc}$)	($\mu\text{g}/\text{disc}$)	($\mu\text{g}/\text{disc}$)	($\mu\text{g}/\text{disc}$)	($\mu\text{g}/\text{disc}$)	($\mu\text{g}/\text{disc}$)	($\mu\text{g}/\text{disc}$)	($\mu\text{g}/\text{disc}$)	($\mu\text{g}/\text{disc}$)	($\mu\text{g}/\text{disc}$)
	500	250	125	500	250	125	500	250	125	500	250	125
E. coli	07	NZI	NZI	11	09	NZI	13	10	08	14	07	NZI
K. pneumoniae	NZI	NZI	NZI	NZI	NZI	NZI	07	NZI	NZI	NZI	NZI	NZI
P. aeruginosa	07	NZI	NZI	10	08	NZI	10	07	NZI	14	11	08
P. mirabilis	08	07	NZI	11	09	07	12	07	NZI	09	07	06
S. aureus	10	08	07	14	10	07	15	12	08	12	11	07

L = N,N'-Bis(2-hydroxy-1-naphthyl)hydrazine

Table 6: Sensitivity of Fungal Isolates to the Schiff base and its Metal (II) Complexes

Isolates	Schiff base (L)			CoL			CuL			NiL		
	($\mu\text{g}/\text{disc}$)	($\mu\text{g}/\text{disc}$)	($\mu\text{g}/\text{disc}$)	($\mu\text{g}/\text{disc}$)	($\mu\text{g}/\text{disc}$)	($\mu\text{g}/\text{disc}$)	($\mu\text{g}/\text{disc}$)	($\mu\text{g}/\text{disc}$)	($\mu\text{g}/\text{disc}$)	($\mu\text{g}/\text{disc}$)	($\mu\text{g}/\text{disc}$)	($\mu\text{g}/\text{disc}$)
	500	250	125	500	250	125	500	250	125	500	250	125
C. albicans	10	07	NZI	14	10	08	18	15	10	13	10	09
F. Solani	07	06	NZI	16	10	07	16	13	11	10	08	06
fumigates	12	10	08	14	12	08	19	15	09	15	13	08

L = N,N'-Bis(2-hydroxy-1-naphthyl)hydrazine

Table 7: Probit Concentrations of the Schiff base and the Metal (II) complexes for 50% Inhibition (IC₅₀)

Compound	IC ₅₀ (µg/ml)
Phenolic Schiff base (L)	2.98
CoL	-3.57
CuL	-7.82
NiL	-5.93
Standard (Ascorbic Acid)	4.11

L = N,N'-Bis(2-hydroxy-1-naphthyl)hydrazine

The results for the antioxidant activity of the phenolic Schiff base ligand and the metal (II) complexes are presented in Table 7. The IC₅₀ values showed that only the ligand possesses radical scavenging activity against DPPH radicals. Ascorbic acid was employed as the standard antioxidant for comparison. The IC₅₀ value of the ligand was found to be 2.98 µg/ml whereas that of Ascorbic Acid was 4.11 µg/ml. The lower concentration value (IC₅₀) of the Schiff base indicates that the phenolic ligand has good antioxidant activity as compared to the Ascorbic acid standard used (23). All the metal complexes show negative IC₅₀ values which indicated the absence of phenolic OH available for proton donation, and hence void of radical scavenging activity, since negative concentration values are not possible.

Conclusion

Spectral data and elemental analyses results showed that all the metal (II) complexes synthesised are tetrahedral in shape. Antimicrobial activity results indicated that the Schiff base ligand and its metal (II) complexes are promising antibacterial and antifungal agents. The enhanced activity in most of the metal (II) complexes than the parent ligand has been explained on the basis of chelation theory. The antioxidant activity results obtained indicated that all of the metal(II) complexes are void of radical scavenging property as they have negative IC₅₀ values. The Schiff base ligand, however, showed radical scavenging activity with IC₅₀ value of 2.98 µg/ml.

Recommendation

Due to limited analytical equipments for further characterization and biological study, this work therefore recommends the following;

- i. NMR Spectroscopy (¹H-NMR and ¹³C-NMR) should be carried out to further confirm the structure of the synthesized compounds

- ii. X-ray Crystallography to know the exact position of the atoms, bond length and bond angles between the atoms
- iii. In vivo antimicrobial study should be carried out to study the effects of the synthesized compounds on living models (especially mammals)
- iv. Application of the synthesized Schiff base in Dye Technology may also be studied as well. This is because the Schiff base possess very intense yellow colour.

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