

## Fe(III) and Cr(III) Complexes with Phenolic Schiff Base: Synthesis, Physico-Chemical Characterisation, Antimicrobial and Antioxidant Studies

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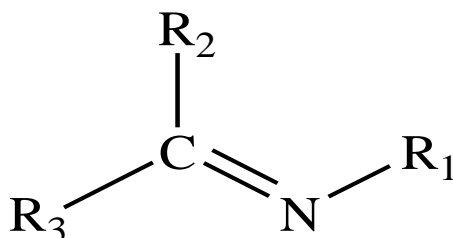
**Abstract:** Iron (III) and Chromium (III) complexes with a tetradentate phenolic Schiff base were prepared by refluxing method. The phenolic Schiff base was formed by the condensation of 2-hydroxy-1-naphthaldehyde and hydrazine monohydrate in methanol. The resulting precipitates were isolated by filtration and purified by recrystallisation. The Schiff base and the metal (III) complexes were characterized by elemental analysis, m.p, FT-IR, molar conductivity and magnetic measurements. Sharp range in melting/ decomposition temperature shows the compounds are probably pure. The IR spectra of the Schiff base and the metal (III) complexes are compared and discussed. Elemental analysis results for CHN revealed 1:1 Metal-Schiff base ratio and the magnetic data suggests an octahedral geometry in the complexes. Both the complexes were paramagnetic and electrolytes. The *in vitro* antimicrobial studies revealed an improved activity in the metal (III) complexes compared to the Schiff base which may be attributed to an improved stability of the metal (III) complexes. The radical scavenging activity of the phenolic Schiff base was determined using DPPH method and the results were analyzed by probit analysis using SPSS 16.0 software. The lower IC<sub>50</sub> value of 2.98 µg/ml<sup>1</sup> obtained indicates the promising use of the Schiff base as antioxidant.

**Keywords:** Schiff base, 2-hydroxy-1-naphthaldehyde, Complex, Antimicrobial and Antioxidant

### I. Introduction

Schiff bases have been known since 1984 when a Nobel Prize winner Hugo Schiff reported the condensation of primary amines with carbonyl compounds. Since then the research area keeps expanding enormously.

Schiff bases are simply the condensation product of primary amines with carbonyl (aldehyde or ketone) compounds (Ashraf *et al.*, 2011). They are compounds containing azomethine (imine) group and have the general structure as follows;



Scheme 1: General Structure of Schiff bases

Where R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub> are Alkyl, Aryl, Cycloalkyl, Heterocyclic group etc which may be variously substituted.

Many studies revealed that Schiff bases prepared from aromatic aldehydes were easily formed and are substantially more stable. Aromatic aldehydes having ortho-substituted hydroxyl group have aroused researchers' interest because of their ability to form phenolic Schiff bases that act as bidentate ligands (Kalaivani *et al.*, 2012). The ortho-positioned -OH group serves as an additional factor of stability during complexation.

Phenolic Schiff bases have been reported in their biological properties such as, antibacterial (Khan *et al.*, 2009; Chohan *et al.*, 2006; Chohan *et al.*, 2004 and Kabeer *et al.*, 2011), antifungal (Guo *et al.*, 2007 and Chohan *et al.*, 2006) and antioxidant (Mohammed Khan *et al.*, 2012a,b) activities. In general, the antibacterial and antifungal activities of Schiff bases are attributed to the presence of lone pairs of electrons on the nitrogen atom and electron-donating character of the double bond of the azomethine group. The radical scavenging activity

(antioxidant property) of phenols however, is attributed to the hydrogen atom transfer of the –OH, –NH and –SH groups in the Schiff base to the free radicals (Mohammed Khan *et al.*, 2012)

## II. Experimental

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

Bacterial and fungal suspensions were obtained and identified at the Department of Microbiology, Faculty of Sciences, Bayero University Kano.

### Preparation of the Schiff Base

A solution of hydrazine monohydrate (0.1 mol) in methanol was added to a solution of 2-hydroxy-1-naphthaldehyde (0.2 mol) in 20ml methanol. The mixture was refluxed for about an hour on magnetic stirrer. The product formed was cooled in an ice bath and the resulting precipitate was collected by filtration, washed successively with methanol and diethyl ether and dried (Aliyu and Sani, 2012)

### Preparation of the Metal (Iii) Complexes

Aqueous solution of the metal (III) chloride (0.1 mol) was added to a mixture of the methanolic solution of the schiff base (0.1 mol) and sodium hydroxide (0.2 mol). The mixture was refluxed for about two (2) hours on magnetic stirrer. The precipitates formed was cooled in an ice bath, filtered, washed successively with methanol and diethyl ether, recrystallised from methanol and dried (Aliyu and Sani, 2012).

### Antimicrobial Studies

The *in vitro* antimicrobial activity of the Schiff base and the metal (III) complexes was tested against two bacterial (*S. aureus* and *E. coli*) and two fungal (*C. albicans* and *F. solani*) isolates using Disc Diffusion Technique (Sharma *et al.*, 2009). Stock solutions of the test compounds were prepared by dissolving a required quantity in DMSO followed by serial dilution to prepare the various concentrations used (1000µg, 500µg, 250µg). Nutrient Agar and Potato Dextrose Agar were used as the bacterial and fungal media respectively. The medium was poured into petri plates and allowed to solidify. Suspension of the tested microorganism (0.5ml) was spread over the medium by the help of a sterile swab. The three different concentrations (1000µg, 500µg, 250µg) of the stock solutions of the test compounds were applied on a 6mm diameter sterile disc. After evaporating the solvent, the discs were placed on the inoculated plates before incubation at suitable optimum temperature for 24-48hrs. Activities were determined by measuring (in mm) the diameter of the zone showing complete inhibition. The results obtained were compared with the activities of Augumentin (30µg) and Ketoconazole (600µg) as the standard antibacterial and antifungal drugs respectively.

### Antioxidant Activity Test

The radical scavenging activity of the phenolic Schiff base and its metal (III) complexes against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals was studied according to the procedure described by Lu *et al.*, (2013). 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 %) was calculated using the relation;

$$I\% = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100 \quad \dots \dots \dots (1)$$

$A_{\text{blank}}$

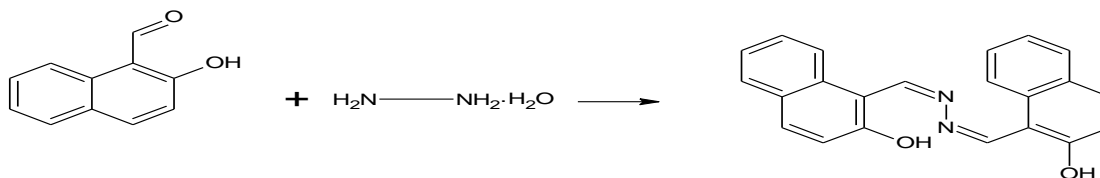
Where  $A_{\text{blank}}$  = Absorbance of the reagents without the test compound

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

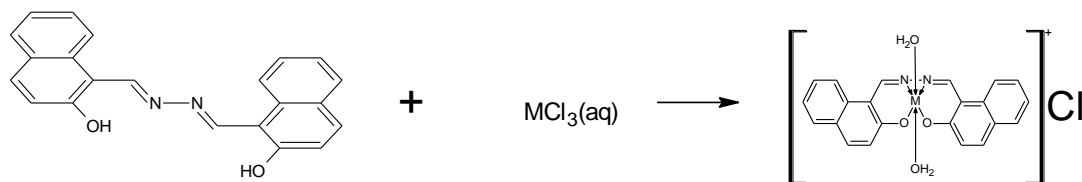
The concentration corresponding to the 50% inhibition ( $IC_{50}$ ) was determined by Probit Analysis using SPSS 16.0 software. The  $IC_{50}$  values obtained are compared with that of Ascorbic Acid as a standard antioxidant. Lower  $IC_{50}$  value indicates higher activity.

## III. Results and Discussion

The synthetic routes for the formation of the Schiff base and the metal (III) complexes are presented in the following schemes;



Scheme 2: Formation of the Schiff base



M= Fe and Cr

Scheme 3: Formation of the Metal (III) Complexes

### Elemental Analysis and Magnetic Susceptibility

Both the Schiff base and the metal (III) complexes were prepared in good yield (Table 1). Sharp melting/ decomposition temperatures indicated the probable purity of the compounds. The magnetic measurement results (Table 1) for the Fe (III) and Cr (III) complexes give magnetic moment values of 3.67 BM and 3.11 BM respectively. These values indicate paramagnetism and presence of three or more unpaired electrons in the metal ions (Huheey *et al.*, 1993). The magnetic moment values also suggest Jahn-Teller distortion in the structure of the complexes.

The elemental analysis data (Table 1) obtained is consistent with the calculated results from the empirical formula of the compounds. The analytical data confirms 1:1 Metal-Schiff base stoichiometry in all the metal complexes.

Table 1: Physico-Chemical and Analytical Data of the Schiff base and the M(III) Complexes

Compound	M.wt. (g/mol)	Colour	% Yield	Melting Temp. ( $^{\circ}$ C)	Elemental Analysis Calc. (Found)			Magnetic (BM)
					%C	%N	%H	
L	340	Yellow	62	298	77.65 (76.90)	8.24 (8.00)	4.71 (4.52)	-
[FeL]	430	Brown	58	>360	61.40 (60.87)	6.51 (5.99)	4.19 (4.01)	3.67
[CrL]	426	Green	77	326	61.97 (61.12)	6.57 (5.82)	4.23 (3.97)	3.11

L= N,N'-bis(2-hydroxy-1-naphthyl)hydrazinediiminato

### Solubility

The Schiff base and the metal (III) complexes were insoluble in water, diethyl ether and acetonitrile, soluble in DMSO and DMF, and slightly soluble in  $\text{CH}_3\text{Cl}$ ,  $\text{CCl}_4$  and Acetone. The Schiff base was found to be Soluble in methanol and ethanol while the complexes were only slightly soluble (Table 2).

Table 2: Solubility of the Schiff base and the M(III) complexes

Solvent	Schiff base (L)	[FeL]	[CrL]
Water	IS	IS	IS
Methanol	S	SS	SS
Ethanol	S	SS	SS
Diethyl ether	IS	IS	IS
Acetone	SS	SS	SS
Acetonitrile	IS	IS	IS
DMSO	S	S	S
DMF	S	S	S
Chloroform	SS	SS	SS
Carbontetrachloride	SS	SS	SS

L= N,N'-bis(2-hydroxy-1-naphthyl)hydrazinediiminato

KEY: S= Soluble, SS= Slightly Soluble, IS= Insoluble

### Molar Conductivity

The molar conductivity (in DMF) of the metal (III) complexes fall in the range  $110 \text{ Ohm}^{-1}\text{cm}^2\text{mol}^{-1}$ - $128 \text{ Ohm}^{-1}\text{cm}^2\text{mol}^{-1}$  for the Fe(III) and the Cr(III) complexes respectively (Table 3). This result agrees with the molar conductance value expected for 1:1 electrolytes in DMF (Geary, 1971).

**Table 3:** Molar Conductance of the M(III) complexes

Compound	Electrical Conductance (Ohm <sup>-1</sup> cm <sup>2</sup> )	Molar Conductance (Ohm <sup>-1</sup> cm <sup>2</sup> mol <sup>-1</sup> )
[FeL]	110x10 <sup>-6</sup>	110
[CrL]	128x10 <sup>-6</sup>	128

L= N,N'-bis(2-hydroxyl-1-naphthyl)hydrazinediiminato

### IR Spectra

The IR spectra of the Schiff base and the metal(III) complexes were recorded on Shimadzu 8400S FT-IR spectrophotometer in the range 4000-400cm<sup>-1</sup>. The spectra of the Schiff base showed a sharp band at 1655cm<sup>-1</sup> and 3434 cm<sup>-1</sup> attributed to the ν(-HC=N-) and ν(-OH) stretching vibrations respectively (Table 4). The ν(-OH) band disappeared in the spectra of the metal (III) complexes which indicated deprotonation in the phenolic site of the Schiff base(Raman *et al.*, 2004).

The ν(-HC=N-) stretching vibration occur at the lower region (1606-1599cm<sup>-1</sup>) in the spectra of the metal (III) complexes. This indicates coordination of the nitrogen of the azomethine to the metals.

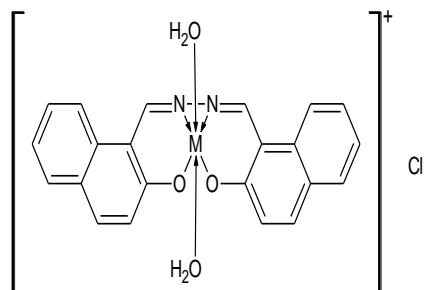
Three new bands appear in the spectra of the metal (III) complexes (916-911cm<sup>-1</sup>) corresponding to coordination of the metals to water molecule, 463cm<sup>-1</sup> corresponding to ν(M-N) coordination and (555-537cm<sup>-1</sup>) corresponding to ν(M-O) covalent bond formation(Hamrit *et al.*, 2000). The appearance of bands at lower frequencies in the spectra of the metal (III) complexes indicates improved stability.

**Table 4:** IR Spectral Data of the Schiff base and the M(III) Complexes

Compound	ν(OH) (cm <sup>-1</sup> )	ν (C=N) (cm <sup>-1</sup> )	ν (M-N) (cm <sup>-1</sup> )	ν (M-O) (cm <sup>-1</sup> )	ν (H <sub>2</sub> O) (cm <sup>-1</sup> )
L	3434	1615	-	-	-
[FeL]	-	1606	463	537	916
[CrL]	-	1599	463	555	911

L= N,N'-bis(2-hydroxyl-1-naphthyl)hydrazinediiminato

Based on the above physical and analytical data, the following structure of the metal (III) complexes is proposed.



**Scheme4:** Proposed Structure of the M(III) Complexes

### Antimicrobial Screening

The *in vitro* antimicrobial properties of the Schiff base and the metal (III) complexes were tested against two bacterial and two fungal isolates using Disc Diffusion Method(Sharma *et al.*, 2004). The diameter of the zones showing complete inhibition at three different concentrations (1000µg, 500µg and 250µg) per disc was presented (Tables 5 and 6). Both the Schiff base and the metal (III) complexes were active against all the tested isolates at all concentrations, except *F. solani* which resisted the Schiff base at 250µg/disc. 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 the activity of a standard antibacterial drug (Augumentin, 30µg) and a standard antifungal drug (Ketoconazole, 600µg) respectively.

The enhanced antimicrobial activity of the metal chelates over their corresponding chelating agent (Schiff base) may be explained on the basis of Overtone's concept(Maruvadaet *al.*, 1994)and the Tweedy's chelation theory(Thangadurai, and Natarajan 2001). 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 (Robertson, 1995)

Generally, this can be achieved through the following properties of the metal complexes:

- (a) The complex should possess sufficient lipid solubility to permit transport of metal ions across the membranes.
- (b) The metal complexes should be highly thermodynamically stable to reach the reaction site without being dissociated or even completely deactivated.

The values for the minimum inhibition concentrations (MIC) shows that the Schiff base is more active against the unicellular fungus (*Candida albicans*) followed by the gram positive bacterium (*Staphylococcus aureus*). Since lower MIC value indicates higher drug activity, the compounds are therefore promising antimicrobials subject to *in vivo* study outcomes.

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

Isolate/ Conc.	Schiff base (L) (µg/disc)			[FeL] (µg/disc)			[CrL] (µg/disc)			STD (µg/disc)
	1000	500	250	1000	500	250	1000	500	250	
<i>E. coli</i>	14	11	09	18	13	11	16	10	07	19
<i>S. aureus</i>	12	08	07	15	10	08	14	11	08	22

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

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

Isolates/ Conc.	Schiff base (L) (µg/disc)			[FeL] (µg/disc)			[CrL] (µg/disc)			STD (µg/disc)
	1000	500	250	1000	500	250	1000	500	250	
<i>C. albicans</i>	20	14	09	23	15	11	21	18	10	18
<i>F. Solani</i>	10	07	NZI	19	14	09	16	11	08	15

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

**KEY:** NZI = No Zone of Inhibition

**Table 7:** Minimum Inhibition Concentrations (MIC) of the Schiff base

Microorganism		500 (µg/ml)	250 (µg/ml)	125 (µg/ml)	62.50 (µg/ml)	31.25 (µg/ml)	15.63 (µg/ml)	MIC Value (µg/ml)
<i>Bac teri</i>	<i>E. coli</i>	-	-	-	+	+	+	125
	<i>S. aureus</i>	-	-	-	-	+	+	62.50
<i>Fungi</i>	<i>C. albicans</i>	-	-	-	-	-	+	31.25
	<i>F. solani</i>	-	-	+	+	+	+	250

### Antioxidant Activity

Only the phenolic Schiff base showed antioxidant activity against DPPH free radicals with an IC<sub>50</sub> value of 2.98µg/ml. The metal (III) complexes were void of radical scavenging activity since they give negative IC<sub>50</sub> values which is possibly due to deprotonation of the phenolic -OH group of the Schiff base and the absence of any other group with hydrogen acidic enough to scavenge the DPPH free radicals in the complexes (El Hassane, 2014).

The result obtained was compared with the activity of a standard antioxidant (Ascorbic acid), and the Schiff base was found to be more active than the standard. The high radical scavenging activity of Schiff base may be attributed to the electron donating character of the double bond of the azomethinewhich makes the aromatic ring more electron rich, and subsequently making the phenolic hydrogen more acidic.

**Table 7:** Antioxidant Activity of the Schiffbase and the M(III) Complexes

Compound	IC <sub>50</sub> (µg/ml)
Phenolic Schiff base (L)	2.98
[FeL]	-1.57
[CrL]	-0.82
Standard (Ascorbic Acid)	4.11

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

## IV. Conclusion

Magnetic moments and elemental analyses results showed that all the metal (III) complexes synthesised are octahedral in shape. Antimicrobial activity results indicated that the Schiff base ligand and its metal (III) complexes are promising antibacterial and antifungal agents. The enhanced activity in the metal (III)

complexes than the free ligand has been explained on the basis of chelation theory. The antioxidant activity results indicated that the metal(III) complexes are void of radical scavenging property. The Schiff base, however, showed good radical scavenging activity with (low) IC<sub>50</sub> value of 2.98 µg/ml which suggests its promising use as antioxidant.

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