

# BIOLOGICAL INVESTIGATION OF SUBSTITUTED ACETOPHENONE DERIVED SCHIFF BASE NICKEL (II) COMPLEX

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**Abstract:** A tridentate ligand, 2-(((2-aminoethyl)imino)methyl)phenol obtained by the condensation of 2-hydroxyacetophenone and ethylenediamine and its Nickel complex has been synthesized and characterized by spectroscopic studies viz., FT-IR, <sup>1</sup>H NMR and UV-vis. The synthesized ligand and its complex has been tested against *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumonia*, *Salmonella typhi*, *Salmonella paratyphi 'A'*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* and the results suggested that Ni (II) complex has significant antimicrobial activity. A comparative study of minimum inhibitory concentration (MIC) values of Schiff base ligand and its Ni (II) complex.

**Keywords:** Antibacterial, 2-hydroxyacetophenone, Haemolytic assay, *Escherichia coli*, *Proteus mirabilis* and *Staphylococcus aureus*

## I. INTRODUCTION

Schiff bases have been studied as a class of ligands [1] and are known to coordinate with metal ions through the azomethine nitrogen atom. The metal based drugs, where metal plays an important role, occupy a prominent place in pharmaceutical chemistry; this has been highlighted in many reviews [2]. Other useful applications are sensor [3], electrodes [4], conducting polymer [5], energy storage [6], enzymatic application [7], solar cell [8], antiviral [9], antifungal [10], anti-inflammatory [11], antitumor [12], antiparasitic [13], antibacterial [14], anti-HIV [15], anticancer [16], etc. Nickel is usually divalent in its compounds, but it can also exist in the oxidation states 0, 1+, 3+, and 4+. In addition to the simple nickel compounds or salts, nickel forms a variety of coordination compounds. Currently, the bioinorganic chemistry of nickel is a topic of increasing interest because the study of the interactions of nickel (II) with Schiff bases offers an opportunity to understand various properties of nickel (II) complexes. Complex of nickel (II) ion with these Schiff bases have been prepared and characterized. The prepared Schiff base and nickel (II) complex has been tested for possible biological (antibacterial and haemolytic) activities.

Metal complexes of Schiff base ligands have recently been used as precursors in the preparation of nanostructures of the respective metal oxides. They are important compounds due to their wide range of biological activities and industrial application. The objectives of the present investigations (i) To synthesis of Schiff base ligand and its Ni (II) complex. (ii) To characterization by FTIR, <sup>1</sup>H NMR and UV-Visible spectroscopy. (iii) To study the antibacterial activity. (iv) To study haemolytic assay.

## II. EXPERIMENTAL PROCEDURE

### 2.1. Materials

All the chemicals used were chemically pure and AR grade. Solvents were purified and dried according to standard procedures. 2-hydroxyacetophenone and nickel chloride hexahydrate were obtained from Merck specialties Ltd., Mumbai, India

### 2.2. Synthesis of Schiff base ligand

The tridentate Schiff base was prepared by condensation of ethylenediamine with 2-hydroxyacetophenone (Scheme 1). Equimolar (0.5 mmol) quantity of 2-hydroxyacetophenone and ethylenediamine was dissolved in 20 mL of ethanol and the solution was refluxed for 4 h under constant stirring. This condensation reaction was carried out by using acid catalyst (few drops of glacial acetic acid). The formed water was removed from the reaction mixture using sodium sulfate (dehydrating agent). After completion of the reaction, the mixture was reduced to half of its original volume using a water bath and kept aside at room temperature. Yellow crystals of ligand were obtained from slow evaporation (Yield: 83%). The Schiff base is characterized by FTIR, UV-Vis and Proton NMR spectroscopy.

### 2.3. Synthesis of Schiff base Nickel (II) complex

Nickel(II) chloride (0.1 mmol) and the potential tridentate Schiff base ligand (0.2 mmol) were dissolved in acetone (20 mL) and the mixture was heated to reflux for 5 h and the reaction was monitored by TLC. After partial evaporation of the solvent,

solid metal(II) Schiff base complex (Scheme 2) were separated and dried in vacuum (Yield: 86%) and character by FTIR, UV-Vis and Proton NMR spectroscopy.

#### 2.4. Antibacterial Assay (MIC)

Seven bacterial isolates; *Escherichia coli*, *Proteus mirabilis*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Salmonella typhi*, *Salmonella paratyphi 'A'* and *Pseudomonas aeruginosa* procured from Microbiology Laboratory, KAPV Medical College, Tiruchrappalli, Tamilnadu were sub cultured periodically and had been stored in glycerol semi solid media. These pure isolates were inoculated in 1% Peptone water and incubated at 37°C for overnight.

The prepared acetophenone derivatives 2-((2-aminoethyl)imino)methyl)phenol and Bis(2-(1-((2-aminoethyl)imino)ethyl)phenoxy)zinc(II) were tested for Minimum Inhibitory Concentration for seven different bacterial isolates adopting the method of [17]. All the wells designed for the protocol were filled with 100µl of 1% peptone water and hundred micro liter of the product (mg/ml) was pipetted into each well of the first column of the plates designed for bacterial strains. Then a serial dilution was made to ensure that the first well of each organism loaded had 50µl of the product and the succeeding wells had half of volume of protein solution from the previous well in serially descending concentrations. Thereafter, 10µl of the bacterial suspension following the same volume of resazurin indicator were added and incubated for 24 hours. The MIC values were determined by visual observation of colour change. The colour change from purple to pink or colourless was recorded as the presence of bacterial growth whereas the retaining of original colour of the dye, purple means inhibition of growth. The experiments were done in triplicate and the data were presented in mean values.

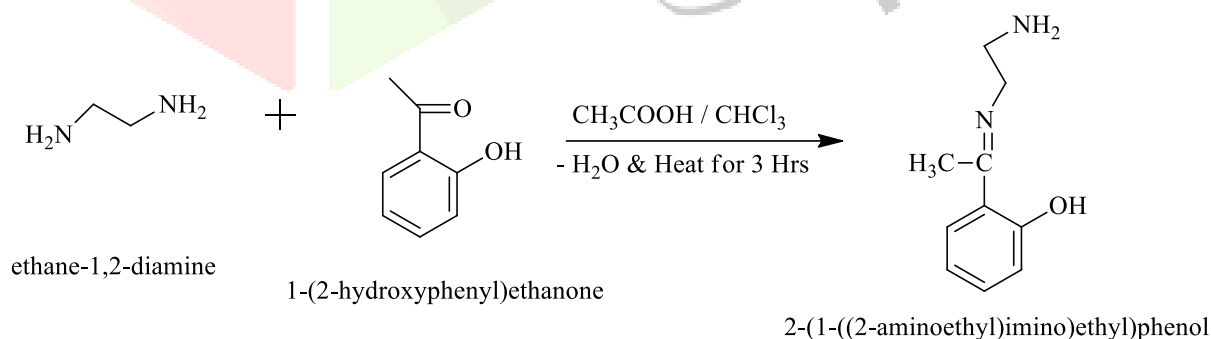
#### 2.5. Haemolytic Assay

The MHC (Minimum Haemolytic Concentration) of the products was carried out in micro well plates according to [18] with little modification. The human blood sample was gifted by a blood bank located at Tiruchirappalli, Tamilnadu, India. The red blood cells were sedimented and repeatedly washed with 0.9% NaCl. The cells were made into 4% suspension with normal saline. All the test wells were filled with 100 µl of normal saline. The first wells of the marked column for the product were filled with 100µl of the respective sample solutions and serially diluted to ensure that each first well had 50µl and serially descending concentration of the product. Then 100µl of 4% hRBCs was added in the wells of the respective rows. The 4% hRBCs alone and 4% hRBCs in 10% SDS were used as 0% (Negative Control) and 100% (Positive Control) haemolytic controls respectively. After 2 hours of incubation the button formation and absence of colour change was recorded as absence of haemolysis and the vice versa was recorded as presence of haemolysis. The MHC of a test sample is defined as the lowest concentration of protein at which 100% haemolysis occurs. The experiments were done in triplicate and the data were presented in mean values.

### III. RESULTS AND DISCUSSION

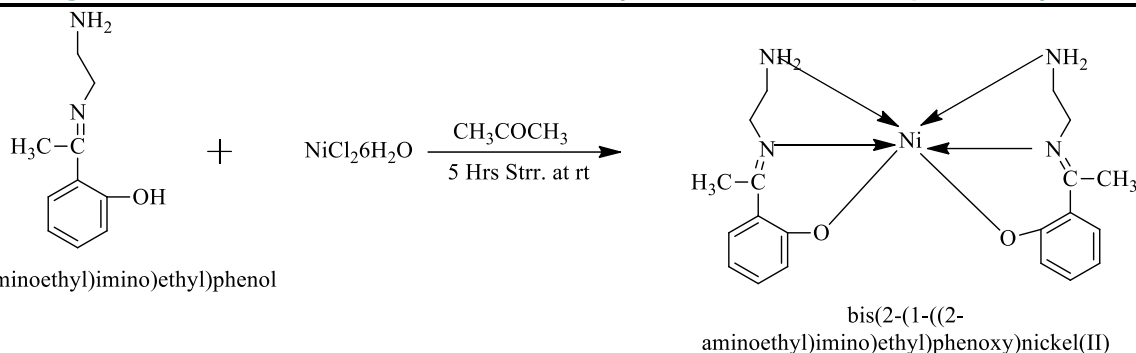
#### 3.1 Reaction Synthesis of Ligand and Complex

Schiff base ligand prepare by the condensation of derivative of acetophenone and ethylenediamine with molar equation, by Scheme 1



**Scheme 1** Preparation of 2-(1-((2-aminoethyl)imino)ethyl)phenol

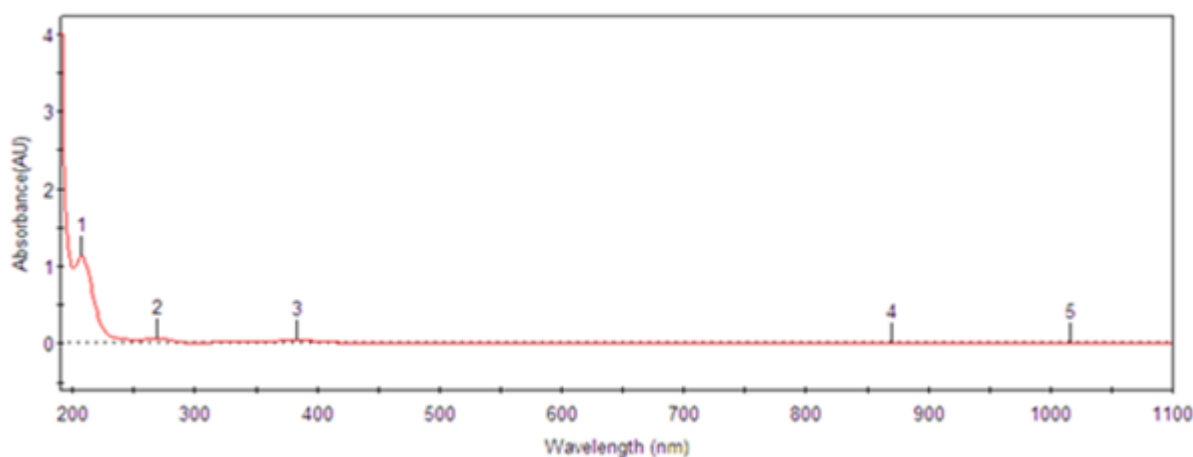
The Nickel (II) complex prepare by 2:1 ratio of Schiff base ligand and Zn(II) metal salt by **Scheme 2**.



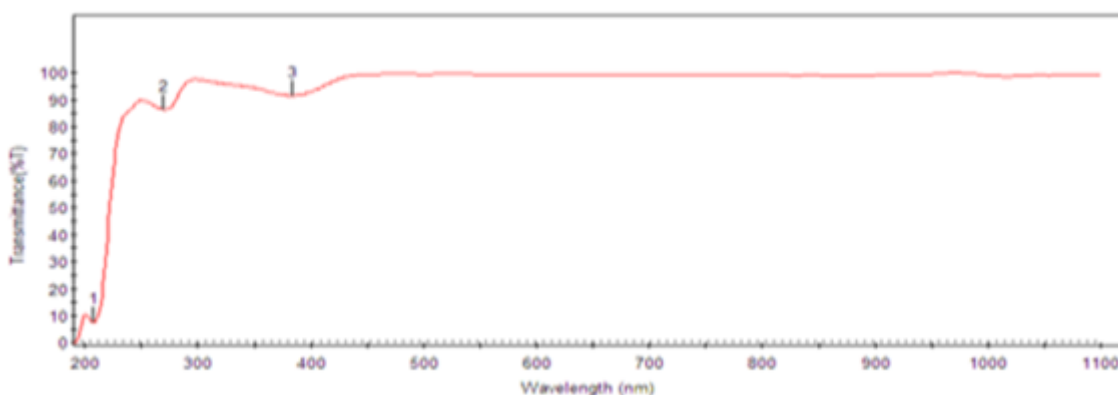
**Scheme 2** Bis(2-(1-((2-aminoethyl)imino)ethyl)phenoxy)nickel(II)

### 3.2 UV-Visible spectra

The UV-visible spectra of Preparation of 2-(1-((2-aminoethyl)imino)ethyl)phenol and Bis(2-(1-((2-aminoethyl)imino)ethyl)phenoxy)nickel(II) were recorded in aqueous solution ( $10^{-3}$  M) in the range of 200-1100 nm at room temperature. The electronic spectrum of the ligand shows a broad band at 378.90 nm and 98.9759 due to  $\pi$ - $\pi^*$  transition of the azomethine ( $-N=C<$ ) chromospheres, the intense absorption band at high energy, 0.1925 (AU) in the region 208.20 nm presumably for the  $\pi$ - $\pi^*$  transition of the benzylidene ring of ligand shows in **Fig. 1, 2**. The electronic spectrum of the Ni(II) complex shows a broad band at 360.00 nm and 95.635 due to d-d transitions of Ni (II) complex, the intense absorption band at high energy, 2.6704 (AU) in the region 207.00 nm shows in **Fig.3, 4**.



**Fig.1** - UV Spectrum of (1-((2-aminoethyl)imino)ethyl)phenol



**Fig.2** - Visible Spectrum of (1-((2-aminoethyl)imino)ethyl)phenol

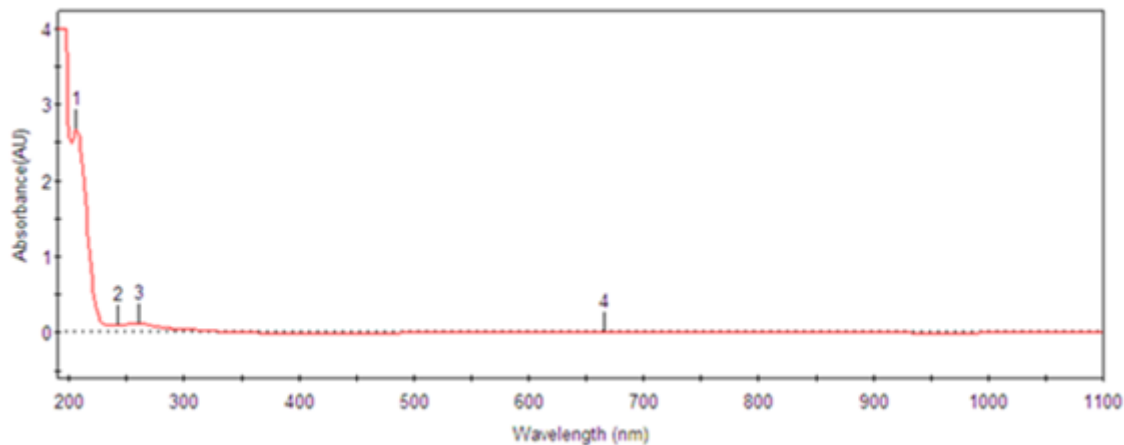


Fig3 - UV Spectrum of Bis(2-(1-((2-aminoethyl)imino)ethyl)phenoxy)nickel(II)

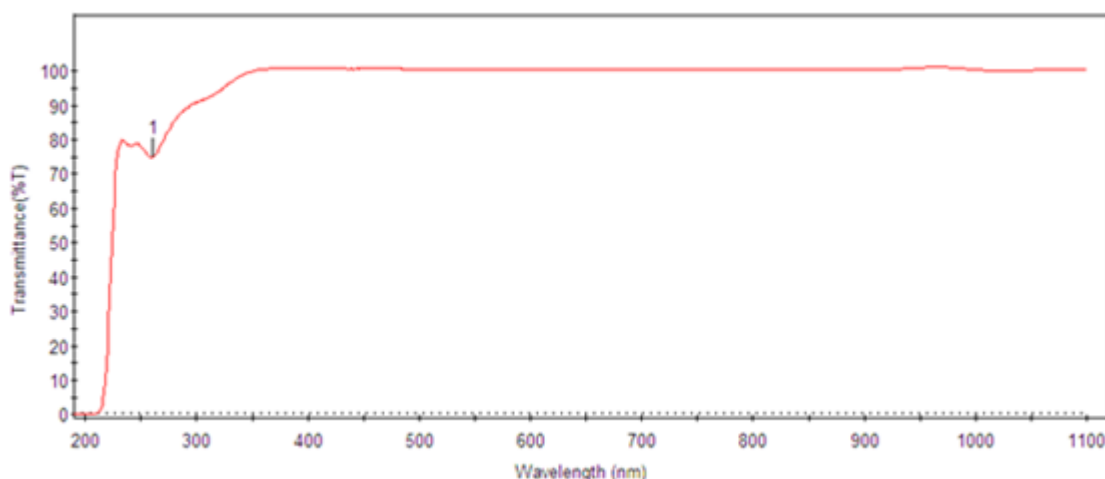


Fig 4 - Visible Spectrum of Bis(2-(1-((2-aminoethyl)imino)ethyl)phenoxy)nickel(II)

### 3.3 IR Spectra

The vibrational spectra is valuable information regarding the nature of functional group of the 2-(1-((2-aminoethyl)imino)ethyl)phenol and Bis(2-(1-((2-aminoethyl)imino)ethyl)phenoxy)nickel(II). The IR spectra of the Schiff base ligand data showed in Fig. 5 and Fig. 6, a broad band in the region 3480–3466  $\text{cm}^{-1}$  indicating the presence of  $-\text{NH}_2$  group. On complexation this band is shifted to lower frequency in the range (339–3440  $\text{cm}^{-1}$ ) showing the involvement of primary amine nitrogen in coordination to metal ion for all the Schiff base complexes [19]. A sharp peak in the region 1556  $\text{cm}^{-1}$  indicating azomethine group and its frequency is increasing 1591  $\text{cm}^{-1}$  after complexation. The free amine broad band at 3466  $\text{cm}^{-1}$  was decreasing to 3446  $\text{cm}^{-1}$  [20]. The frequency of 1403  $\text{cm}^{-1}$  indicating methyl group of 2-hydroxyacetophenone and which is decreasing after coordination to 1486  $\text{cm}^{-1}$ . The aromatic phenolic hydrogen stretching and bending frequency bands shows in the region 3100 and 1279  $\text{cm}^{-1}$  [21] were absent and form new sharp band form in the region 548.69  $\text{cm}^{-1}$  for confirming nickel metal oxygen covalent [22].

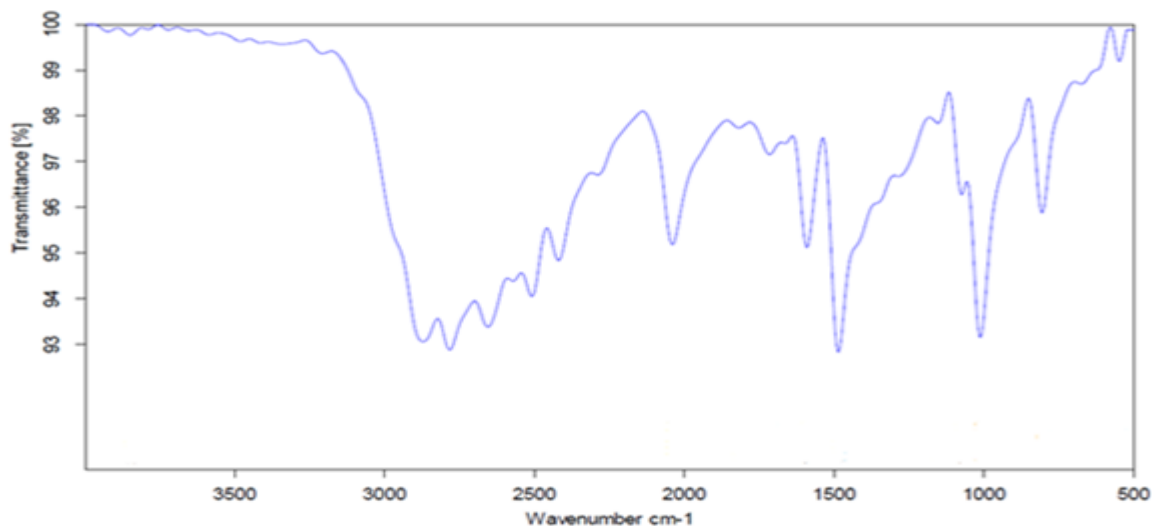
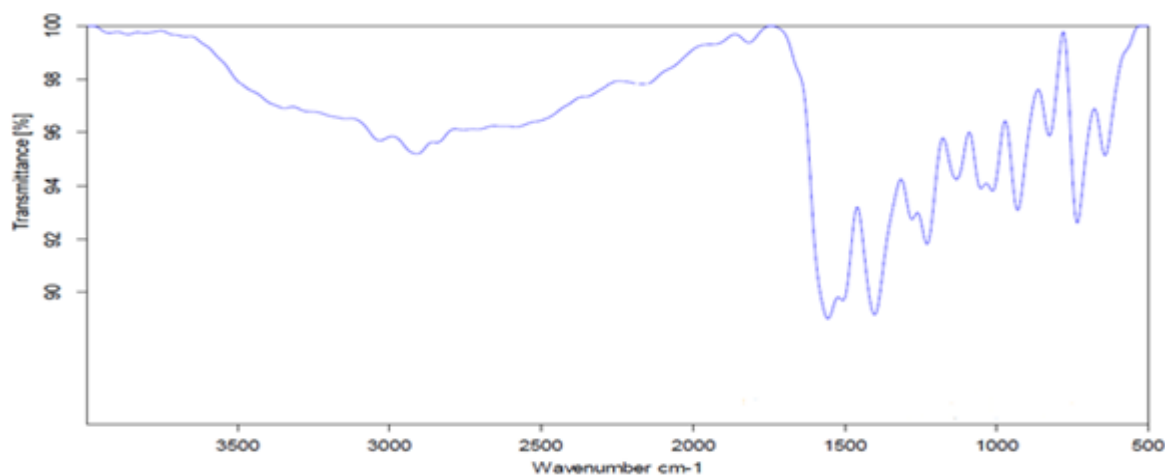


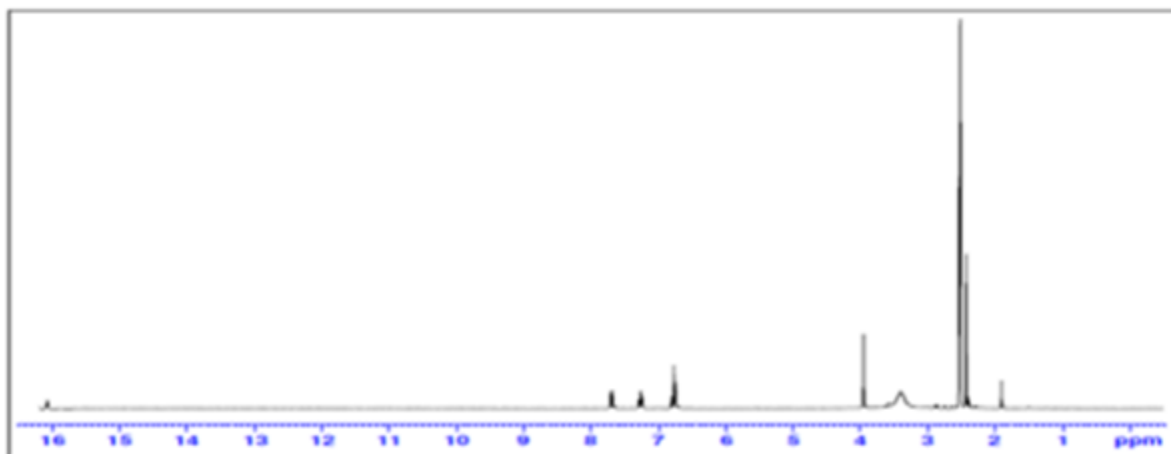
Fig.5 FTIR Spectrum of Bis(2-(1-((2-aminoethyl)imino)ethyl)phenoxy)nickel(II)



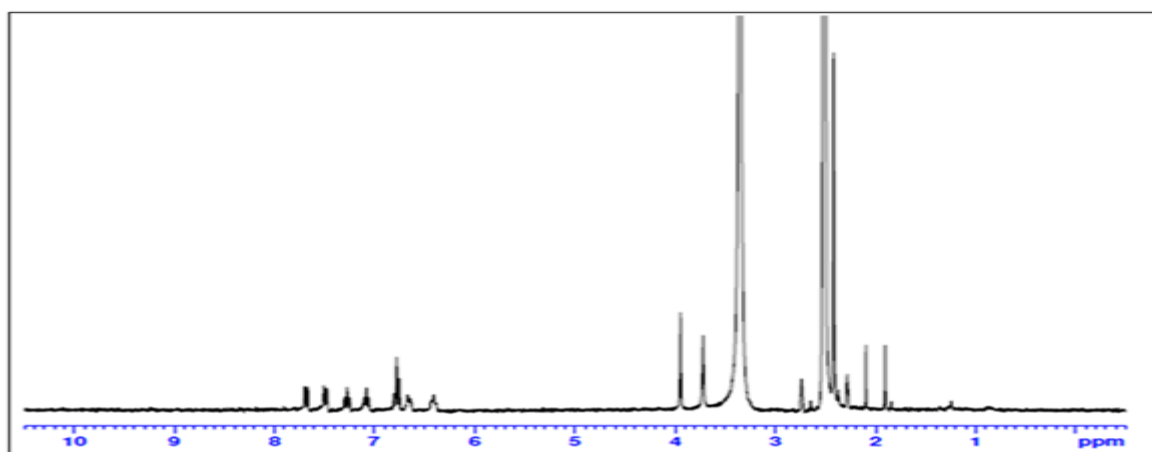
**Fig.6 FTIR of 2-(1-((2-aminoethyl)imino)ethyl)phenol**

### 3.4<sup>1</sup>HNMR Spectra

The <sup>1</sup>HNMR results show 2-(1-((2-aminoethyl)imino)ethyl)phenol and Bis(2-(1-((2-aminoethyl)imino)ethyl)phenoxy)zinc(II) in **Fig.7** and **Fig.8**, a sharp absorption singlet peak in the region 16.0 ppm which corresponds to phenolic alcohol of ligand, which peak is absent in Ni (II) complex. This indicates that the deprotonated phenolic Oxygen atom is involved in chelation. Four multiplets (2-doublets and 2-triplets) between the region 6.7 and 7.6 ppm, 7.6 (t, 1H) (J=8.1), 7.2 (t, 1H) (J=9.6), 7.2 (d, 1H) (J=3.3), 6.7 (d, 1H) (J=8.1) indicate protons shifts of benzylidene ring of ligand and decrease between 6.6 and 7.2 ppm, 7.2 (t, 1H) (J=6.9), 7.0 (t, 1H) (J=14.1), 6.7 (d, 1H) (J=8.7), 6.6 (d, 1H) (J=24.6) for Zn(II) complex. Two triplets between 3.0 and 4.0 ppm, 3.7 (t, 2H) (J=9.0), 2.8 (t, 2H) (J=9.9) indicate aliphatic alkane shifts of ligand and decrease its range after coordination between 2.8 and 3.8 ppm, 3.6 (t, 2H) (J=10.8), 2.5 (t, 2H) (J=10.5). A sharp singlet peak in the range 6.4 which corresponds to primary amine and decrease 5.11 ppm. This indicates that coordination bond formed between Nitrogen atom of amine and nickel metal atom. In multiplets, coupling constants are increasing after coordination respectively.



**Fig.7 <sup>1</sup>HNMR Spectrum of Preparation of 2-(1-((2-aminoethyl)imino)ethyl)phenol**



**Fig.8 <sup>1</sup>HNMR Spectrum of 2-(1-((2-aminoethyl)imino)ethyl)phenoxy)nickel(II)**

### 3.5 ANTIBACTERIAL STUDY

The acetophenone derivative of 2-(1-((2-aminoethyl)imino)ethyl)phenol and Bis(2-(1-((2-aminoethyl)imino)ethyl)phenoxy)zinc(II) complex were the aim of production and synthesis of antimicrobial compound is to inhibit the causal microbe without any side effects on the patients. In addition, it is worthy to stress here on the basic idea of applying any chemotherapeutic agent which depends essentially on the specific control of only one biological function and not multiple ones. The acetophenone derivatives of 2-(1-((2-aminoethyl)imino)ethyl)phenol and Bis(2-(1-((2-aminoethyl)imino)ethyl)phenoxy)zinc(II) complex involves in bacteriostatic show a remarkable biological activity against different types of Gram-positive (G+ve) and Gram-negative (G-ve) bacteria effect shows in **Table 1**. The importance of this unique property of the investigated Schiff base complexes lies in the fact that, it can be applied safely in the treatment of infections and some common diseases e.g. septicaemia, gastroenteritis, urinary tract infections and hospital acquired infections. These compounds showed significant antimicrobial activities against *Escherchia coli*, *Proteus mirabilis*, *Klebsiella pneumonia*, *Salmonella typhi*, *Salmonella paratyphi 'A'*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (G+ve) by well diffusion with serial dilution method up to the lowest concentration (15.62 µg) from highest concentration (250 µg). In the higher concentration (250 µg) of the prepared Schiff base and nickel complex against *Escherchia coli*, *Proteus mirabilis* and *Staphylococcus aureus* suppressed by ligand not nickel(II) complex. There is slight moderate growth of inhibition of *Klebsiella pneumonia* bacteria organism at the concentration of 125 µg of ligand complex and no effects for ligand. From the Table.1, both ligand and complex are very good activities against all bacterial strains of gram-positive and gram-negative bacteria in all high to lower concentrations. A strong result of bacterial growth of the Bis(2-(1-((2-aminoethyl)imino)ethyl)phenoxy)nickel(II) have *Salmonella typhi*, *Salmonella paratyphi 'A'* microorganisms have at the 125 µg only but inhibited growth of these organisms at the higher concentration (250 µg) and lower concentrations are 62.5, 31.25 and 15.62 µg. Hence, the acetophenone derivative Bis(2-(1-((2-aminoethyl)imino)ethyl)phenoxy)nickel(II) in this study could be a potential antibiotic for the enteric fever, typhoid. However, these compounds are 2-(1-((2-aminoethyl)imino)ethyl)phenol and Bis(2-(1-((2-aminoethyl)imino)ethyl)phenoxy)nickel(II) may be used for treating all infectious disease caused by the tested organisms.

Table 1: Minimum Inhibitory Concentrations of 2-(((2-aminoethyl)imino)methyl)phenol and Bis(2-(1-((2-aminoethyl)imino)ethyl)phenoxy)zinc(II) against human pathogenic bacteria.

Organism/ Concentration	250 µg		125 µg		62.5 µg		31.25 µg		15.62 µg	
	L	C	L	C	L	C	L	C	L	C
<i>Escherchia coli</i>	-	+	-	-	-	-	-	-	-	-
<i>Proteus mirabilis</i>	-	+	-	-	-	-	-	-	-	-
<i>Klebsiella pneumonia</i>	-	-	-	+/-	-	-	-	-	-	-
<i>Pseudomonas Aeruginosa</i>	-	-	-	-	-	-	-	-	-	-
<i>Salmonella typhi</i>	-	-	-	+	-	-	-	-	-	-
<i>Salmonella paratyphi 'A'</i>	-	-	-	+	-	-	-	-	-	-
<i>Staphylococcus aureus</i> (+ve)	-	+	-	-	-	-	-	-	-	-

- (+) Bacterial growth.  
 (-) Bacterial Inhibition.  
 (+/-) Bacterial growth Inhibition

Serial dilution Concentrations from (250 to 15.62)

µg – microgram

L - 2-(((2-aminoethyl)imino)methyl)phenol

C - Bis(2-(1-((2-aminoethyl)imino)ethyl)phenoxy) zinc(II)

### 3.6 HAEMOLYTIC ASSAY

The acetophenone derivatives are 2-(1-((2-aminoethyl)imino)ethyl)phenol and Bis(2-(1-((2-aminoethyl)imino)ethyl)phenoxy)zinc(II) haemolytic assay result in **Table 2**. There is no toxic effect of both ligand and its complex with human blood sample from highest concentration (250 µg) to lowest concentration (15.62 µg). Here, 10 % sodium dodecyl sulphate as positive control and saline as a negative control. From the table.2 2-(1-((2-aminoethyl)imino)ethyl)phenol and Bis(2-(1-((2-aminoethyl)imino)ethyl)phenoxy)zinc(II) have there is no toxicity in these tested concentrations and volumes also.

Table 2: Haemolytic Assay of 2-(((2-aminoethyl)imino)methyl)phenol and Bis(2-(1-((2-aminoethyl)imino)ethyl)phenoxy)zinc(II) against human pathogenic bacteria.

Compound / Conc.	250 µg	125 µg	62.5 µg	31.25 µg	15.62 µg	Positive Control	Negative Control
L	-	-	-	-	-	+	-
C	--	-	-	-	-	+	-

Negative control - Normal saline

Positive control - 10% Sodium Dodecyl Sulphate

Serial dilution Concentrations from (250 to 15.62)

µg – microgram

L - 2-(((2-aminoethyl)imino)methyl)phenol

#### IV. CONCLUSIONS

In the present research studies, our efforts were to synthesis and characterize of aceophenone derivative compounds by condensation method. These synthesized compounds were characterized by various physicochemical and spectral analyses. The compounds were tested against a large number of human pathogenic strains of Gram-negative bacteria including staphylococcus aureus (+ve). Compounds are exhibited good growth inhibition activity against all human pathogenic microorganisms and are promising to act as a potential antimicrobial agent. Based on the results obtained haemolytic assay work proves that cytotoxicity of the prepared 2-(1-((2-aminoethyl)imino)ethyl)phenol and Bis(2-(1-((2-aminoethyl)imino)ethyl)phenoxy)zinc(II) no toxic effect lowest concentration (15.62 µg).

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