

VANILLIN AND 4-NITROANILINE DERIVED SCHIFF-BASE AND ITS NICKEL(II) COMPLEX: SPECTRAL ANALYSIS AND ANTIBACTERIAL INVESTIGATION

Kasanesh Misganaw², Ponnusamy Thillairasu¹, Gemechu Shumi¹, Habtamu Debebe^{3*} and Sintayehu Berhanu²

¹Department of Chemistry, Wollega University, P.O. Box 395, Nekemte, Ethiopia

²Department of Chemistry, Bonga University, P.O. Box 334, Bonga, Ethiopia

³Department of Chemistry, Borana University, P.O. Box 19, Yabelo, Ethiopia

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ABSTRACT. Ni(II) metal complex of Schiff base (SB) ligand 2-methoxy-4-[(4-nitrophenyl) iminomethyl]phenol (MNIMP); derived from vanillin and 4-nitroaniline was synthesized in 1:2 M:L by refluxing solution containing metal salt (NiCl₂·6H₂O, 97.0%) and SB in ethanol and characterized based on powder (XRD), NMR, FT-IR, UV-Vis spectrophotometer, and physicochemical measurements. The FT-IR spectra revealed that the complex is coordinated through azomethine nitrogen and phenolic oxygen of the bi-dentate ligands. The chemical shift $\delta = 9.8$ ppm in ¹H-NMR of SB; corresponds to the azomethine proton of the ligand suggesting the presence of -C=N- linkage and ¹³C-NMR confirms the presence of ¹⁴C in the ligand and the complex. The shift to a lower wavelength of UV-Vis spectra of the complex compared to the ligand signifying complex formation. The powder (XRD) pattern put forward the crystalline phase of the complex through nickel(II) occupying the center of octahedron geometry. The higher molar conductance value of the complex confirms its electrolytic nature. *In-vitro* antibacterial activities for synthesized compounds have been evaluated towards human pathogenic microbes *Staphylococcus aureus* and *Escherichia coli* and compared with standard, gentamicin. The result demonstrated that Ni(II) complex; [Ni(MNIMP)₂(H₂O)₂]Cl₂ has greater zone of inhibition against the tested organism than free MNIMP ligand as antibacterial agent.

KEY WORDS: Derivatization, Schiff base ligand, Vanillin, Nickel (II) complex, Bi-dentate, Antibacterial activity

INTRODUCTION

Transition metals can exhibit a wide variety of coordination properties and reactivity, which can be used to form complex with biologically active organic molecule as ligands. The structure, properties and biological importance of these complex is depends upon the central metal atom and surrounding ligand molecules they are connected to the metal atom by coordinate bond. In particular, metal complexes of N and O donor atom chelating ligands have attracted considerable attention because of their interesting biological activities. Those atoms play a key role in the coordination of metals at the active sites of numerous metallo-organic compounds and as donor atoms in the formation of dative bond with the corresponding ligand [1, 2].

Coordination compounds combining Schiff base ligands with nitrogen and oxygen donor atoms are designed and synthesized extensively since the metal–ligand interaction controls and enhances the biological, chemical, and electrochemical activities of the metal centers. Metal complexes having bi-dentate Schiff base ligands with both nitrogen and oxygen donor atoms and containing halogen groups are interesting due to their antibacterial properties. Complex formation between transition metal and the Schiff Base ligand results in foundation of coordinate complexes and in recent studies, researchers have shown significant interest in various amino acid Schiff base ligands [3, 4].

Vanillin is a phenolic organic compound with aldehyde and ether functional groups. Condensation product of vanillin (aldehyde as a center of reaction) and 4-nitroaniline (primary amines with -NH₂ as a center of reaction) yields carbon-nitrogen double bonded organic

*Corresponding authors. E-mail: habtamudebebe8@gmail.com

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compound called Schiff bases (SBs). The functional feature of SBs are represented by the azomethine group (-C=N-), where the substituents are alkyl, aryl, or heterocyclic groups [5, 6].

A number of SB compounds are used as ligands by considering their metal chelating ability, physiological and pharmacological activities. Specifically, the highly stable complexes formed by SBs with transition metals often lead to compounds with strongly enhanced biological activities. It is known that the existence of metal ions bonded to biologically active compounds may enhance their activities and chelation; causes change in the biological properties of the ligands and also the metal moiety. It has been reported that chelation is the cause and cure of many diseases caused by microbial like bacteria and fungi [7, 8].

The growing importance of the Schiff bases and their metal complexes in modern coordination chemistry is attributable to recent observations about their antimicrobial and oxygen carrier properties [9, 10]. Specifically, SBs derived from aromatic amines and aldehydes have a wide variety of applications in many fields. A search through literature reveals that vanillin Schiff confers good biological activities, have better complexation and possess polyvalent metal ions [11, 12]. However, there was no information on the combined ligand of Schiff base of vanillin and 4-nitroaniline with nickel(II) metal complex. In view of this, we herein report the derivatization, characterization and antibacterial investigation of Schiff base (2-methoxy-4-[(4-nitrophenyl)iminomethyl]phenol (MNIMP)) and its nickel(II) complex; $[\text{Ni}(\text{C}_{14}\text{H}_{12}\text{N}_2\text{O}_4)_2(\text{H}_2\text{O})_2]\text{Cl}_2$.

EXPERIMENTAL

Chemicals and reagents

Vanillin (99.5%), 4-nitroaniline (99.0%), nickel chloride ($\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, 97.0%) were used as a precursor to synthesis of the complexes. Silver nitrate AgNO_3 (0.1 M) and HNO_3 (99%) were used for tested to chloride complex. DMSO (99.0%), distilled water, petroleum ether, ethanol (99.8%), acetone (99.0%), methanol (99.8%), hexane (99.0%), acetonitrile (99.9%), conc. H_2SO_4 and THF (99.8%) were used to prepare the ligand and complex solutions. All the chemicals were purchased from India and solvents used were analytical grade and dried by the standard procedures.

Characterization of the complex and physical measurements

The progress of the reaction and the purity was monitored by thin layer chromatography (TLC). Molar conductivity of the complex was measured at room temperature with synthesized solution in DMSO using a microprocessor based conductometer which gives valuable information about the coordination number, the nature of counter ions present in the complex and structure of the complexes. The specific conductance value recorded with EC-214 a microprocessor using this, the molar conductance of the metal complex was calculated using the following expression [13].

$$\Lambda_M \left(\text{in } \frac{\text{Scm}^2}{\text{mol}} \right) = 1000 \frac{k}{C}, \quad (1)$$

where, k = specific conductance ($\mu\text{S}/\text{cm}$), C = concentration (mol/cm^3) and Λ_M = molar conductance.

In test of chloride counter ion, the formation of white precipitate suggests the presence of chloride in the sample. Disappearance of the white precipitate on adding ammonia solution and regeneration of the white precipitate on dropping few drops of concentrated nitric acid confirms the presence of chlorides [14]. Magnetic susceptibility measurement of the complex was recorded using a MSB-AUTO Magnetic balance (Sherwood Scientific) at 19 °C. It used to determine the extent of electron pairing, the stereochemistry and metal-ligand interactions in the complexes

[15]. The instruments generate gram susceptibility (χ_g) data for a given paramagnetic substance from which the magnetic moment was calculated using the formula given below [21].

$$\mu_{\text{eff}} \text{ (in BM)} = 2.824 \times \sqrt{T \times \chi_{\text{MCorr}}}, \quad (2)$$

where, μ_{eff} = effective magnetic moment, χ_m = molar of magnetic susceptibility and T = Kelvin temperature.

Additionally, from the effective magnetic moment of the complex, the number of unpaired electrons present in the metal ions is calculated using the relation given below [16, 17].

$$\mu_{\text{eff}} = \sqrt{n(n+2)}, \text{ where, } n = \text{is number of unpaired.} \quad (3)$$

Elemental analyses (carbon, hydrogen, oxygen and nitrogen) were performed on a thermo finnigan Flash EA-1112 elemental analyzer. Elemental analysis of a compound enables one to determine the empirical formula of the compound [18].

Structural characterizations

The powder X-ray diffraction patterns were collected in the scan range 2θ between 10° and 90° using an advanced diffractometer equipped with source $\text{Cu}\alpha = 1.54 \text{ \AA}$. This was used to elucidate the structure and crystallite size of the complex. FTIR spectra of the synthesized compounds were recorded on a Shimadzu 4000 instrument using KBr pellet within the range of 400 ± 10 to $4000 \pm 10 \text{ cm}^{-1}$ and applicable to examine the functional groups. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra of compounds were recorded on Bruker Avance II-400MHz. NMR spectrometer in deuterated DMSO solvents using TMS as internal standard. Chemical shift values are expressed in δ (ppm). It used to elucidate the structure and locating precisely the donor sites of the ligand. The UV-Vis spectrophotometer (electronic spectrum) was used to identify the geometry and charge transfer of the complex. The absorbance was recorded by the Sanyo UV-Vis spectrophotometer model (SP65, UK) [19].

Pharmacological reagents

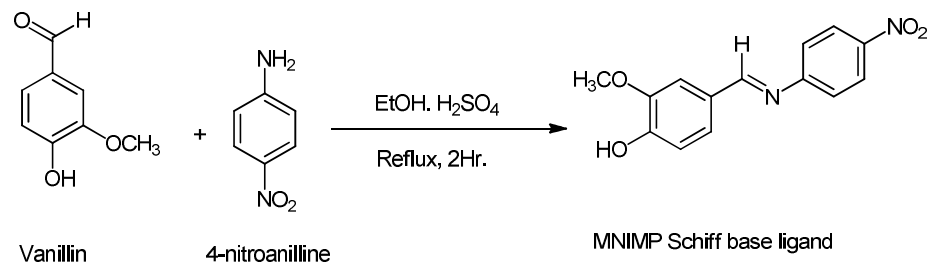
Reagents for biochemical assays such as nutrient agar (NA, Micro master laboratories, India) and standard gentamicin (Research products international (RPI), India), were used for the antimicrobial studies.

Synthesis of MNIMP ligand

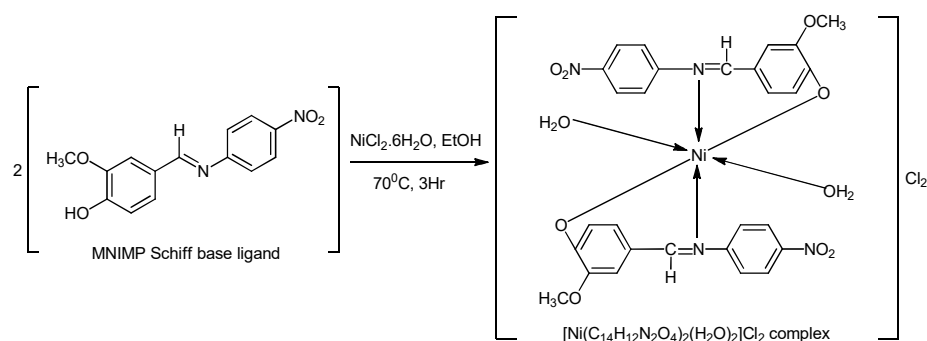
MNIMP Schiff base ligand was synthesized from the reaction (0.1521 g, 0.001 mol) of vanillin and (0.1381 g, 0.001 mol) of 4-nitroaniline with pure alcoholic medium of 15 mL ethanol with the addition of five drops of H_2SO_4 to the solution. The reaction mixture was left to reflux for 2 h to precipitate the product in a yellow color and the resulting precipitate filtered through suction filtration. Finally, the precipitate washed with distilled water, ethanol and with a 50:50 mixture of ethanol and petroleum ether then dried and stored in desiccators containing calcium chloride (Scheme 1) [20].

Synthesis of Ni(II) metal complex

A Schiff base of the metal complex was synthesized by adding $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ metal salt (0.2379 g, 0.001 mol) to an ethanolic solution (20 mL) of MNIMP Schiff base ligand (0.5544 g, 0.002 mol) with stirring. The mixture was heated under reflux for 3 h at 70°C . Then a greenish yellow colored precipitate solution is obtained the solution were filtered while it is hot and washed with ethanol and recrystallized in methanol and diethyl ether. After that, the product was collected and dried over anhydrous CaCl_2 (Scheme 2) [21].



Scheme 1. Synthetic scheme of MNIMP SB ligand.

Scheme 2. Synthetic scheme of $[\text{Ni}(\text{MNIMP})_2(\text{H}_2\text{O})_2]\text{Cl}_2$ complex and proposed structure.

Antibacterial test: The synthesized Nickel (II) complex at concentrations of $10\mu\text{g/mL}$ is tested for antibacterial activities against gram positive bacteria *S. aureus* and gram negative bacteria *E. coli* at 37°C for 24 hours. The method used to evaluate the antimicrobial activity is disc diffusion method. The lowest concentration inhibiting growth of the organism is recorded as MIC to check the accuracy of the drug concentrations and have been compared standard; Gentamicin [22].

RESULTS AND DISCUSSION

The solubility of the Schiff base ligand and its Ni(II) complex are checked with different solvent polarities (Table 1). According to the result, the solubility behavior of Schiff base and the complex have polar nature characteristics (like dissolve like principle) [23]. When a AgNO_3 is added to the Ni(II) complex solution, a cruddy white precipitate (AgCl) is formed in the solution, which revealed the presence of chloride groups as counter ions (outside the complexes sphere) which also in good agreement with the results of molar conductance [24]. The molar conductance of metal complex was measured and given in Table 1.

The Ni(II) complex shows a molar conductance of $114\text{S}\cdot\text{cm}^2\cdot\text{mol}^{-1}$ within the range of $110\text{--}195\text{S}\cdot\text{cm}^2\cdot\text{mol}^{-1}$ as 1:2 electrolyte in DMSO. The specific molar conductance values obtained for Ni (II) complex is $114\mu\text{S}/\text{cm}$ (Table 1). The molar conductance value of the complexes is high indicating that the complex behaved electrolytes due to presence of chorine ions outside the coordination sphere [25]. This verifies that outer sphere counter anions are contained in complexes and suggests that the chloride in the complexes is in the ionization sphere or $[\text{Ni}(\text{L})_2]\text{Cl}_2$.

Table 1. Preliminary test and physical measurements of MNIMP ligand and $[\text{Ni}(\text{MNIMP})_2(\text{H}_2\text{O})_2]\text{Cl}_2$ complex.

Preliminary Tests and Physical Measurement		Compounds		
		MNIMP ligand	$[\text{Ni}(\text{MNIMP})_2]\text{Cl}_2$	
a	Molecular formula	$\text{C}_{14}\text{H}_{12}\text{N}_2\text{O}_4$ ligand	$[\text{Ni}(\text{C}_{14}\text{H}_{12}\text{N}_2\text{O}_4)_2(\text{H}_2\text{O})_2]\text{Cl}_2$ complex	
b	Molecular weight (g/mol)	272	710	
c	Color	Yellow	Yellowish green	
d	Melting point (°C)	181.0-184.7	299.4-302.8	
e	Elemental analysis (%, Exp./Calc.)	C (%), Exp./Calc.)	60.40 (59.98)	55.32 (54.85)
		H (%), Exp./Calc.)	4.41 (4.28)	3.98 (3.803)
		N (%), Exp./Calc.)	10.29 (9.86)	9.28 (8.973)
f	Conductivity	Specific conductivity	-	$1.14 \times 10^{-4} \text{ S.cm}^{-1}$
		Molar conductance	-	$114 \text{ S.cm}^2\text{mol}^{-1}$
g	Solubility in polar and non-polar solvent	Solubility	Solubility	
	Methanol	Soluble	Soluble	
	Ethanol	Soluble	Soluble	
	Acetone	Soluble	Soluble	
	Acetonitrile	Soluble	Soluble	
	DMSO	Soluble	Soluble	
	THF	Soluble	Soluble	
	Water	Slightly soluble	Slightly Soluble	
Hexane	Insoluble	Insoluble		

Remark^c: Maximum acceptable difference between results: C (0.25%), H (0.25%) and N (>0.5% nitrogen content = 10%, <0.5% nitrogen content = 0.05%). Remark^d: The ranges of molar conductance of metal complexes with 1:1, 1:2 and 1:3 ratios of cationic and anionic species in DMSO at 25 °C is non-electrolyte (0-50), 1:1 electrolyte (50-90), 1:2 electrolytes (110-195), 1:3 electrolytes (200-240 $\text{S.cm}^2\text{mol}^{-1}$).

The elemental analysis data was applied to determine the percentage composition of C, H and N. The acceptable differences between the test results and certified values are presented in Table 1. The results revealed that the % C, H, and N are in good agreement with the proposed structures. From the data obtained it was found that the compounds analyzed as $[\text{M}(\text{L-L})_2]$ indicating a 1:2 mole ratio of metal:ligand (M:L), which is in a good agreement with the proposed molecular formula $[\text{M}(\text{C}_{14}\text{H}_{12}\text{N}_2\text{O}_4)_2(\text{H}_2\text{O})_2]$ [26].

The high melting point value of the complex than the ligand suggests that good chelating effect of the ligands; it is stable and not decomposed easily. Both decomposition temperatures and stability have direct relationship with melting point (determined using a melting point apparatus with sealed capillary tubes containing a few milligrams of sample) [27]. The results of proposed molecular formula, color and melting point are summarized in Table 1.

Many transition metal salts and complexes are paramagnetic due the partially filled d-orbitals. The measured gram magnetic susceptibility of Ni(II) is 7.506×10^{-6} . The result indicates that the complex is paramagnetic subsequently the value is positive. The spin free nickel(II) complex is exhibiting magnetic moment in the range 2.82-3.4 BM with specific value of 3.25 BM which corresponding to two unpaired electrons. This follows to conclude that the Ni(II) ion exist in d^8 ($t_{2g}^6 e_g^2$) octahedral environment in its complex. This is in a good agreement with literature data for Ni^{2+} in octahedral environment [28, 29].

Spectral characterization

FT-IR spectral. The FT-IR spectrum of ligand and their Ni(II) complex are given in Figure 1a. The FT-IR spectrum of ligand is found within the range of $400\text{-}4000 \text{ cm}^{-1}$. A strong band is appeared in the range of frequency $1600\text{-}1675 \text{ cm}^{-1}$ which belongs to the C=N of azomethine

group and conform the formation of the Schiff base. The absence of any frequency in 1700 cm^{-1} (frequency of C=O) and around 1300 cm^{-1} (frequency of N-H) in spectra of ligand indicated the completion of the reaction between aldehyde and amine [30, 31]. The band at 1458.5 and 1111.0 cm^{-1} indicate the presence of $-\text{NO}_2$ and $-\text{OCH}_3$ group. The peak appeared at 1587.4 cm^{-1} is also due to the presence of $-\text{C}=\text{C}-$ stretching frequency of the aromatic group of the free ligand. The FT-IR spectrum of the metal complexes exhibit broad bands in the range of $3354\text{--}3423\text{ cm}^{-1}$ indicating the presence of coordinated water molecules. Frequency of (M-N) and (M-O) are observed in the far-infrared region. These bands are absent in the spectra of the ligand. Based on the data we could assign the bands at $408\text{--}443$ and $524\text{--}599\text{ cm}^{-1}$ to M-N and M-O vibrations, respectively (Figure 1a and Table 2).

Due to the larger dipole moment change in the vibration of the M-O band in comparison to that of the -M-N- band, the band (M-O) usually occurs in the higher frequency region and the band is usually sharper and stronger than (M-N). There is no peak around $300\text{--}350\text{ cm}^{-1}$ indicating the absence M-Cl bond in the complexes. The spectra of complex show stretching vibration band at region between $1500\text{--}1700\text{ cm}^{-1}$ is shifted to lower frequencies compared with ligand and it is accepted for most complex (Figure 1a and Table 2).

The frequency corresponding to the azomethine group (C=N group (1632.0 cm^{-1})) in the ligand is shifted to higher frequency at 1647.5 cm^{-1} in the spectra of the complex (Table 2). This is due to the interaction of (C=N) nitrogen to the metal ion as a result of rear donation of π -electrons by the metals. This is attributed to the bonding affinity of nitrogen atom of the azomethine group of the Schiff base to the respective metal ions directly linked to the donation of electrons from nitrogen to the empty d-orbital's of the metal atom and decreasing the planar property after complexation [31]. The spectra of ligand show band at 1458.5 cm^{-1} and 1111.0 cm^{-1} due to presence of $-\text{NO}_2$ and $-\text{OCH}_3$ group respectively, no appreciable change of signal is observed in the spectra of complex. It indicates that $-\text{NO}_2$ and $-\text{OCH}_3$ group do not participate in coordination with metals (Figure 1a and Table 2).

The broad band spectra for the ligand at 3485.0 cm^{-1} is related to the stretching vibration of $-\text{OH}$ in phenol, the disappearance of this band at the spectrum for complex indicate the deprotonation of phenol group to coordination with metal ion. For the metal complex new band occurred at 566.2 cm^{-1} on the site of reaction (M-O). This supports the coordination mode of ligand through oxygen atom of the phenolic group. Also bands observed at frequency 408.0 cm^{-1} indicates the formation of M-N. All the FT-IR data suggested that the metal is bonded to the Schiff bases through the phenolic oxygen and the imino-nitrogen (Figure 1a and Table 2). The selected vibrational frequencies for the ligand and its metal complexes are summarized in Table 2. This occurrence indicates that there is coordination between the metal and the lone pairs of electron on the nitrogen atom of the ligand [32].

Table 2. FT-IR spectral data and electronic spectral data of MNIMP ligand and $[\text{Ni}(\text{MNIMP})_2(\text{H}_2\text{O})_2]\text{Cl}_2$.

Compound	FT-IR spectral data (ν) (where ν is wavenumber in cm^{-1})							Electronic spectral data (in nm)		
	(C=N-)	(C=C-)	(-O-H)	(-NO ₂)	(-OCH ₃)	(M-O)	(M-N)	Benzene ring ($\pi\text{--}\pi^*$)	-C=N- ($\pi\text{--}\pi^*$)	-C=N- ($n\text{--}\pi^*$) (nm)
Schiff base	1632.0	1587.4	3485.0	1458.7	1111.0	–	–	239.1	260.7	388.0
Ni complex	1647.5	1586.8	3364.2	1458.4	1111.8	566.2	408.0	275.8	311.1	372.8

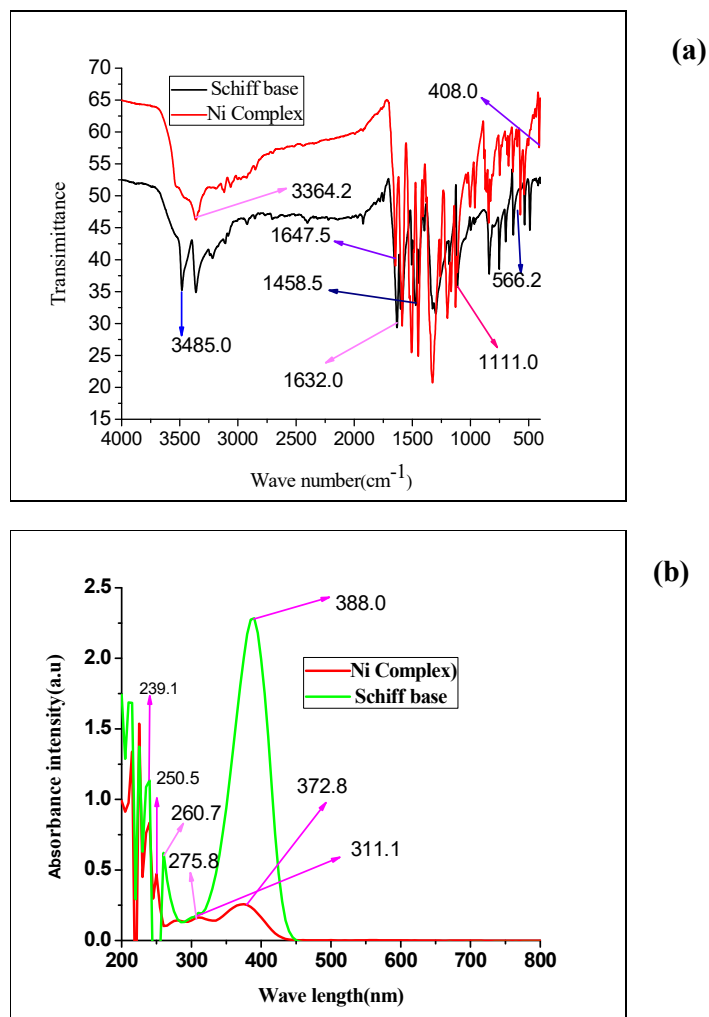


Figure 1. (a) FT-IR spectra of Schiff base ligand and their Ni(II) complex and (b) UV-Vis spectra of Schiff base ligand and their Ni(II) complex.

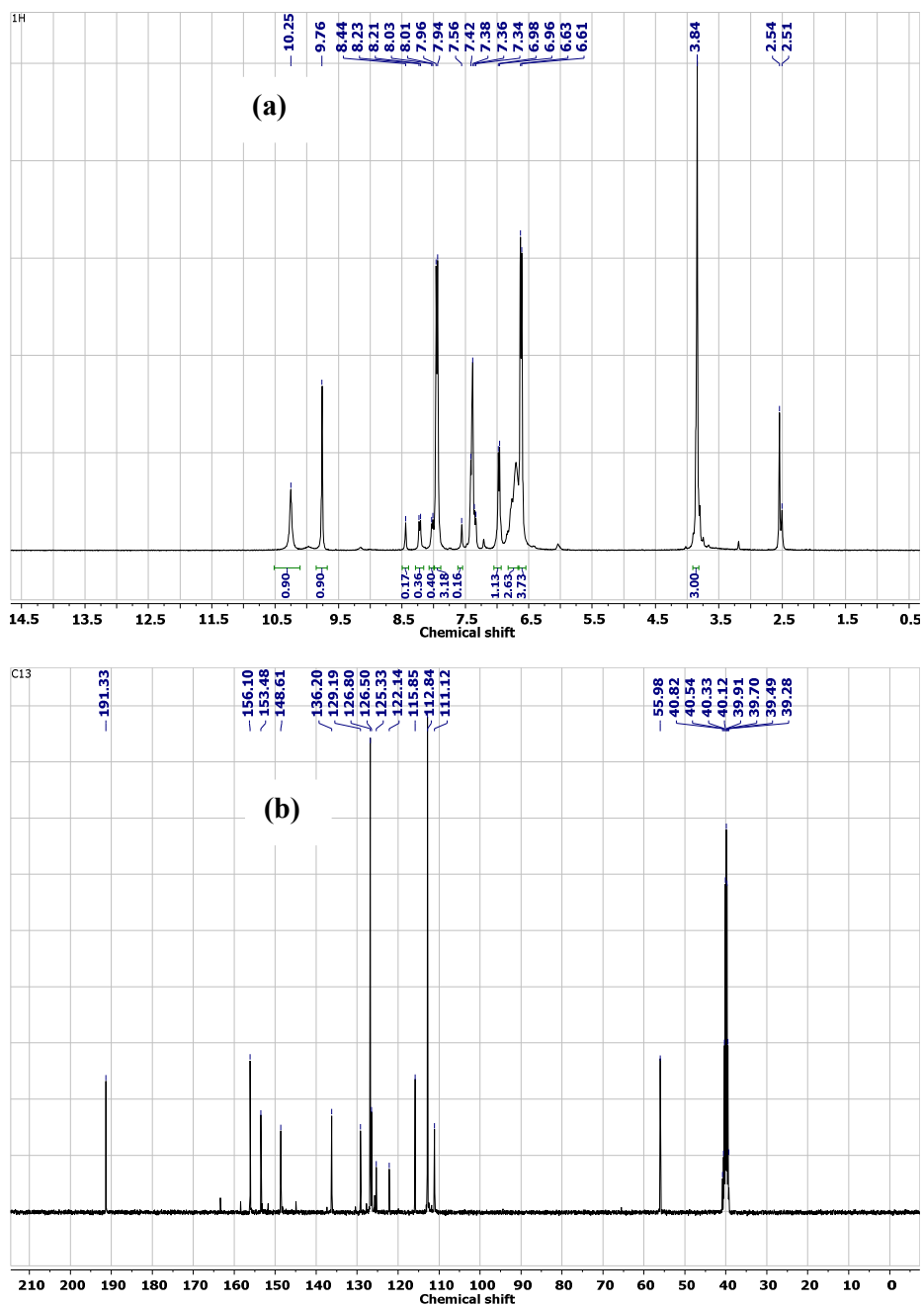


Figure 2. (a) $^1\text{H-NMR}$ spectra of Schiff base and (b) $^{13}\text{C-NMR}$ spectra of Schiff base.

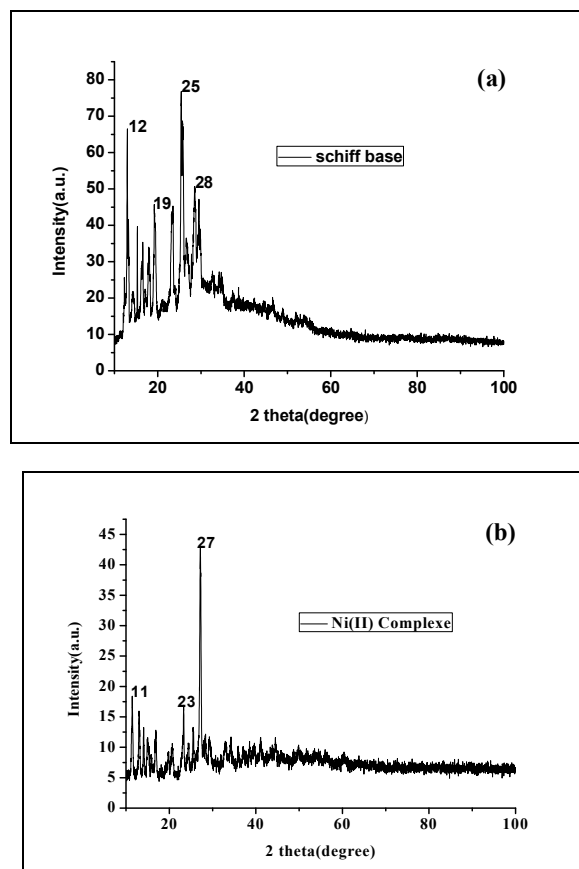


Figure 3. (a) Powder (XRD) of Schiff base ligand, 2-methoxy-4-[(4-nitrophenyl) iminomethyl] phenol (MNIMP) and (b) X-ray diffractogram of nickel(II) Schiff base complex.

UV-Vis (electronic) spectral analysis

The UV-Vis spectra ligand and the complex are shown in Figure 1b and organized in Table 2. It can be shown that the compounds peaks are found within the range of 200-800 nm. There are two absorption bands in the electronic spectrum of the ligand which could be assigned to $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions. These transitions are also found in the spectra of complex, but they appeared to be shifted towards lower and higher wavelength, confirming the coordination of ligands to the metal ions. The electronic spectra of the ligand showed three major bands at 239.1, 260.7 and 388.0 nm (Figure 1b and Table 2). The ligand transitions such as $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ are expected due to the presence of C=N, C=C and C-O groups in the ligand's structure. The band in the region of 239.1 and 260.7 nm can be recognized for possible $\pi \rightarrow \pi^*$ transitions of phenyl ring (-C=C-) and C=N chromospheres. The spectral bands which was observed at 388.0 nm assigned possibly to $n \rightarrow \pi^*$ transitions of C=N chromospheres. The complex shows three major bands at 275.8, 311.1 and 372.8 nm. However, there is a shift in the positions of the bands to a lower wavelength when compared to the ligand, from which formation of the complex can be confirmed. The expected d-d transition in the visible region for complexes of the ligand cannot be detected even

with concentrated solution. It may be lost in the low energy tail of the charge transfer transition [33].

¹H-NMR and ¹³C-NMR spectral analysis

The ¹H and ¹³C-NMR spectrum of the ligand is shown in Figure 2a and 2b, respectively. In the ¹H NMR spectrum of ligand (Figure 2a), a signal at $\delta = 9.8$ ppm were attributed to the azomethine protons of the ligand suggesting the presence of C=N linkage. The proton which present next to the imine carbon shows more downfield when compare with the other protons in the aromatic ring. The signal due to aromatic ring protons appeared as multiplet in the range $\delta = 6.5$ -8.5 ppm, whereas signals due to methoxy protons appeared as singlet at $\delta = 3.85$ ppm. Another singlet corresponding to hydroxyl proton is observed at $\delta = 10.3$ ppm. The free NH₂ protons of 4-nitroaniline usually show a broad singlet peak in a region at 4-6 ppm and thus signal are absent/disappeared in the observed spectra of Schiff bases which indicates the formation of the -C=N- bond. While the band at $\delta = 2.51$ ppm to 2.54 ppm related to H₂O in d-DMSO and in overall the ¹H-NMR confirms the presence of 12 protons (H₁₂) within SB ligand (Figure 2a and Table 3).

The ¹³C NMR spectrum of the free ligand revealed peaks at $\delta = 156.5$, 153.5 and 148.7 ppm attributed to carbon atom of azomethine (-N=CH-) group, sp² oxygenated quaternary aromatic carbons where a hydroxyl and methoxy groups are attached, respectively [34, 35]. The signal appearing $\delta = 60$ ppm belongs to quaternary aromatic carbon (C-N) group where the carbon is attached. The signal appearing at $\delta = 136$ ppm belongs to quaternary aromatic carbon (C-N) group where the nitrogen is attached and the signal appearing $\delta = 191$ ppm belongs to quaternary aromatic carbon where the nitro group is attached. Seven aromatic methenes are clearly evident between $\delta = 129$ -111 ppm. The multiplet and overlapping peak around $\delta = 40$ ppm corresponds to carbon of methoxy group (-OCH₃) in the Schiff base ligand and d₆-DMSO/TMS (Figure 2b). In general, the ¹³C-NMR confirms the presence of 14 carbons (C₁₄) within Schiff base ligand (Table 3).

Table 3. ¹H-NMR and ¹³C-NMR spectral data of MNIMP ligand.

¹ H-NMR spectral data (400 MHz, DMSO-d ₆)			¹³ C-NMR spectral data (100 MHz, DMSO-d ₆)		
Functional group assigned	Chemical shift (δ_H , ppm)	No. of proton/s	Group assigned	Chemical Shift (δ_C ppm)	No. of carbon/s
Phenolic -OH	10.3	1	Aromatic carbons where a hydroxyl groups (-C-OH) are attached	153.5	1
			Carbon atom of azomethine (-N=CH-) group	156.5	1
Azomethine -HC=N	9.8	1	Aromatic methines	129-111	7
			Aromatic carbons where a methoxy groups (-C-OCH ₃) are attached	148.7	1
Aromatic rings protons	6.5-8.5	7	Aromatic carbon (-C-N-) group where the nitrogen is attached	136	1
			Aromatic carbons where a nitro groups(-NO ₂) are attached	191	1
CH ₃ /-OCH ₃	3.85	3	Aromatic carbon (-C-N-) group where the carbon is attached	60	1
			Carbon atom of methoxy (OCH ₃)	40	1
d ₆ -DMSO	2.5	Quintet	d ₆ -DMSO	40	2 (septet)

Powder X-ray diffraction analysis

The X-ray diffractometer is used to characterize the synthesized materials' structure and crystallite size. The pure MNIMP and its complex of powder (XRD) patterns are shown in Figure 3(a) and (b). At least four distinct intense diffraction peaks are visible in the ligand MNIMP diffractogram at $2\theta = 12^\circ, 19^\circ, 25^\circ,$ and 28° with maxima at $2\theta = 28^\circ$ which shows that it is polycrystalline structure. Also the complex show the peaks at diffraction angles of $2\theta = 11^\circ, 23^\circ$ and 27° . The high intensity appears at 27° for the complex and the same was observed in the ligand at 25° indicate that the metal ion is incorporated within the ligand environment. Moreover the sharp peak for the complex confirms the crystal structure.

Scherer's equations ($d = 0.9 \lambda / \beta \cos \theta$ where, λ is the wavelength, θ is Bragg diffraction angle, β is full width at 1/2 maximum of the peak position in radian) is used to obtain the average particle size [36, 37]. Ni(II) complex and the Schiff base have an average crystallite size of 57.0 nm and 38.4 nm, respectively. As a summary based on physical and spectral data the proposed geometries of nickel(II)-MNIMP complex is octahedral geometry with crystalline structure. The ligand acts as a bi-dentate coordinated to the metal ion through oxygen of phenol and nitrogen of azomethine group (Scheme 2).

Antibacterial activity studies and minimum inhibitory concentration (MIC)

By using the disc diffusion method (utilizing DMSO as a solvent), the antimicrobial activity of the ligand, MNIMP, and its complex was examined in vitro against two pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus* and compared to the well-known standard medications: gentamicin. The antibacterial activities are classified as highly active (>14 mm), moderately active (10-14 mm) and slightly active (6-10 mm) and less than 5 mm was taken as inactive [38].

The inhibition zone diameter values of the investigated compounds are summarized in Table 4. The MIC value was defined as the lowest concentration of the compounds giving complete inhibition of visible growth. Ni(II) complex; $[\text{Ni}(\text{C}_{14}\text{H}_{12}\text{N}_2\text{O}_4)_2(\text{H}_2\text{O})_2]\text{Cl}_2$ have lower MIC values against selected bacterial strains than the free MNIMP ligand; indicating that the complex is more effective as antibacterial agent. The MIC values for the Schiff base and its complexes result are presented in Table 4 and Figure 4.

Table 4. Antibacterial activity of SB-Ni(II) complex.

Test compound	Zone of inhibition (in mm)		Minimum inhibition concentration determination				
	Selected strains		Strains	Minimum inhibition concentration			MIC
	<i>S. aureus</i>	<i>E. coli</i>		$\frac{1}{2} = 50\% = 0.5$ g/10 mL, 1 g/20 mL	$\frac{1}{4} = 25\% =$ 0.25 g/10 mL 1 g/40 mL	$\frac{1}{8} = 12.5\% =$ 0.125 g/10 mL 1 g/80 mL	
Schiff base	8	12	<i>S. aureus</i>	12 mm	-	-	0.5 g/10 mL
			<i>E. coli</i>	-	-	-	1 g/10 mL
Ni(II) complex	10	18	<i>S. aureus</i>	7 mm	6 mm	-	0.25 g/10 mL
			<i>E. coli</i>	-	-	-	1 g/10 mL
Gentamicin	23	20					

As compared with previously reported selected literatures [39-41] our SB and nickel complex shows relatively better inhibition against selected bacterial strains and restricts further growth of the microbes.

Table 5. Representative examples of organic ligand and metal complex.

Representative and proposed compounds	Zone of inhibition (mm)		References
	<i>S. aureus</i>	<i>E. coli</i>	
C ₁₄ H ₁₂ N ₂ O ₄ MNINMP SB Ligand	8	12	Present work
C ₃₈ H ₃₈ Cu ₃ N ₄ O ₈ Complex	9.9	11.3	[39]
C ₄₀ H ₄₀ Zn ₃ N ₁₀ O ₁₀ Complex	0.7	2.2	[39]
C ₃₄ H ₂₆ Cu ₂ N ₁₀ O ₂ Complex	12.7	13.7	[39]
C ₁₀ H ₁₄ N ₂ O Ligand	-	-	[40]
C ₁₂ H ₁₀ N ₄ O Ligand	-	14±0.6	[40]
(C ₁₀ H ₁₄ N ₂ O)Ni Complex	13±2	11±0.6	[40]
(C ₁₀ H ₁₄ N ₂ O)Cu Complex	12±0.5	9	[40]
[(C ₁₉ H ₁₈ N ₄ O ₈)Zn] Complex	7	10	[41]
[(C ₁₉ H ₁₈ N ₄ O ₈)Mn] Complex	8	9	[41]
[(C ₁₉ H ₁₈ N ₄ O ₈)Co] Complex	10	12	[41]
[Ni(C ₁₄ H ₁₂ N ₂ O ₄) ₂ (H ₂ O) ₂]Cl ₂ Complex	10	18	Present work

A work by Zhong-Lu Youa and Hai-Liang Zhua on SBs derived from salicylaldehyde and aminomethylpyridine and transition metal derived complexes (Cu and Zn) showed their antimicrobial activity against multidrug-resistant bacteria (MDRB) [40]. Saini and his co-workers described five complexes with Co(II), Ni(II), Cu(II), Mn(II), and Zn(II) and the complexes were tested for their antimicrobial activities against *S. aureus* and *E. coli* [41].

The selected representative complexes showed lower antimicrobial activity as compared with our work (Table 5). This enhancement in inhibiting the growth of bacteria explained on the basis of their structure and presence of good electron donating characteristics of -OCH₃ and -NH₂ group. Furthermore such increased activity of the complexes is because of the nature of the central metal ions chelate forming ability.

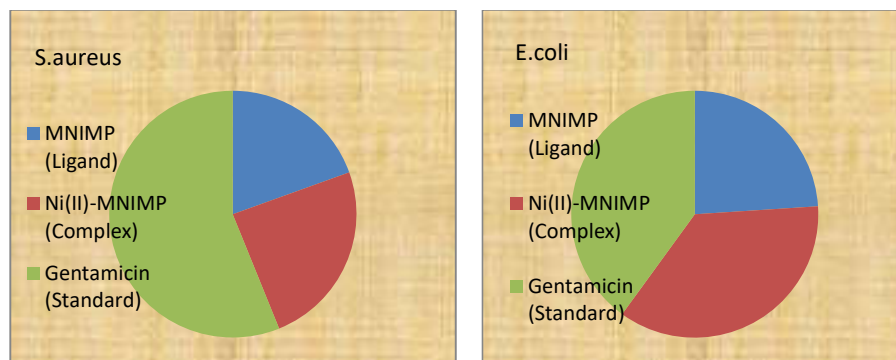


Figure 4. Comparison of antibacterial activities; gentamicin drug with nickel(II) complex.

CONCLUSION

[Ni(C₁₄H₁₂N₂O₄)₂(H₂O)₂]Cl₂ were synthesized by 1:2 M:L reaction of Ni(II) with the Schiff base (SB) ligand, 2-methoxy-4-[(4-nitrophenyl)iminomethyl]phenol (MNIMP) derived from vanillin and 4-nitroaniline via condensation reaction. The modes of bonding between ligand and metal ion and overall structure of the complex were determined and clarified through physicochemical and spectroscopic methods. FT-IR data suggested that the metal is bonded to the MNIMP Schiff

base through the phenolic oxygen and the imino-nitrogen and the ligand behaves as bi-dentate towards the metal ion and coordinated through the azomethine-N and the phenolic-O center. From magnetic susceptibility analysis and electronic spectra; the complex was suggested to have octahedral geometry. According to powder (XRD) data, the particles of the examined complex were situated in Nano scale range and crystallite phase. The melting point datum showed high thermal stability and decomposition of the investigated metal complex. The electrical conductivity study confirmed that the complex had high conducting properties. The antimicrobial activity of the ligand and its complex was examined against a pathogenic bacterium *E. coli* and *S. aureus* and the data obtained reveals that the metal ion in the complex enhances the antibacterial activity compared to the free ligand, MNIMP. The obtained result showed the suggested structure of the complex under investigation, as demonstrated in Scheme 2.

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