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SYNTHESIS, STRUCTURAL AND ANTIBACTERIAL STUDIES OF NOVEL MANNICH BASE LIGAND AND THEIR METAL COMPLEXES

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ABSTRACT

The condensation reaction of 1,3-cyclohexanedione, salicylaldehyde, and urea in the presence of ethanol as a solvent system yielded a novel mannich base, 1-((2,6-dioxocyclohexyl)(2-hydroxyphenyl)methyl)urea (**1**). UV-visible, FT-IR, ^{1}H & ^{13}C NMR, and elemental studies were used to determine the structure of ligand (**1**). IR and UV-visible spectra, molar conductivity, and EPR measurement were used to describe the structures of metal complexes (**1a-1e**) of the ligand (**1**). The geometries of all the complexes were octahedral. The antibacterial effect of ligand (**1**) and metal complexes (**1a-1e**) against *Pseudomonas aeruginosa, Escherichia coli*, *Staphylococcus aureus* and *Klebsiella pneumaniae* was investigated using the disc diffusion method. The metal complexes (**1a-1e**) were more significant than **Ciprofloxacin**, which served as a control.

Keywords: Antibacterial activity, Mannich base, *Staphylococcus aureus,* Transition metal complexes

INTRODUCTION

An acidic proton put adjacent to a carbonyl assembly is amino alkylated through ammonia and formaldehyde or some primary or secondary amine in the Mannich reaction.

A β-amino carbonyl molecule is the end result. Mannich processes were also proposed for reactions involving aromatic aldehydes and imides. A survey of the literature on Mannich reactions reveals a large amount of information on the biochemical, pharmacological, and toxicological properties of Mannich bases **[1-3]**.

Transition metals are necessary for the proper operational of alive things and consequently have a lot of promise as medicines **[4-7]**. The coordination behavior of nitrogen contributor Ligands is a fascinating subject to study. The complexes produced by 3d metals through bidentate Ligands utilizing together the nitrogen atoms of the ligands have received a lot of interest in this field. Metal chelates, instead of organic molecules, enhanced the action of many medicines **[8-13]**. To our knowledge, no research on this kind of metal complexes using the Mannich base Ligand has been undertaken.

In this paper, we describe the production of a novel Mannich base obtained from 1,3-cyclohexanedione, salicylaldehyde, urea, and metal complexes with Cu(II), Ni(II), Fe(II), Cr(II), and Mn(II) as part of our ongoing study. All of the metal complexes were described via the proper skills. Antibacterial activity was tested on all of the metal complexes.

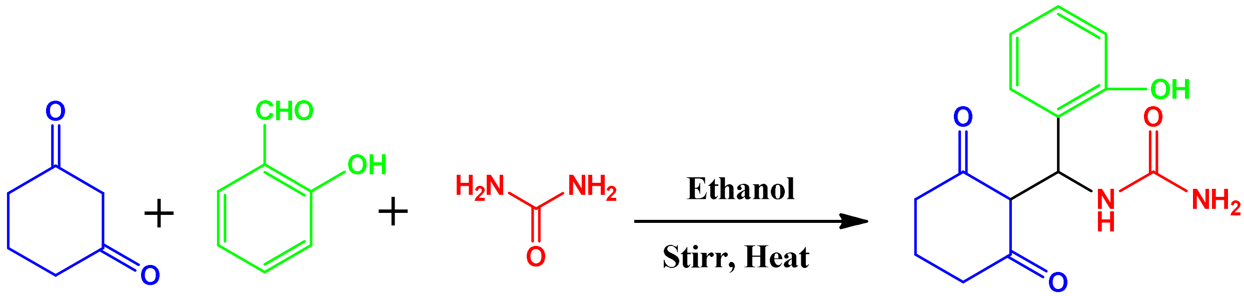
MATERIALS AND METHODS

Chemicals and reagents

Merck and Sigma-Aldrich provided all of the chemicals, which were utilized without additional cleanup. The solvents have been purified and distilled before use. Melting points were documented in open capillary tubes and were not adjusted. The UV-Visible spectrum was obtained using a Shimadzu UV - 1280 (200-800 nm) spectrophotometer. The FT-IR spectrum (KBr) are obtained using a Shimadzu 8201pc (4000-400 cm-1) spectrophotometer.

Synthesis of Ligand (1)

In a 100mL RB flask, 1,3-cyclohexanedione (5.60 g, 0.05 mol), salicylaldehyde (6.1 mL, 0.05 mol), and urea (0.05 mol, 3.0 g) were dissolved in 20mL ethanol. The contents of the flask are thoroughly stirred after 30 minutes of heating using a magnetic stirrer. Afterwards, a white dust-like residue developed. It has been dried and filtered. To produce pure product, the final prepared sample was recrystallized in hot ethanol. In **Scheme 1**, the production of ligand 2 is shown.



Scheme 1: Synthesis of ligand 1

1-((2,6-dioxocyclohexyl) (2-hydroxyphenyl)methyl)urea (1)

Dust white solid; mw: 276.29; mp:146°C; IR (KBr cm^{-1}) ν_{max}: 3436.28 (-OH), 3076.46 (-NH), 2924.22 (-CH), 1641.32 (-C=O), 1236.77 (-C-N-C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.08 (s, 1H, NH), 7.12-6.90 (m, 4H, Ph-OH), 5.55 (s, 2H,NH_2), 5.32 (d, *J* = 1.7 Hz, 1H, CH-Ph), 5.30 (s, 1H, OH), 4.22 (d, *J* = 6.9 Hz, 1H, CHD), 2.40-1.91 (m, 6H, CHD); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 208.83 (2C, C=O), 162.72 (1C, C=O), 154.0 (1C, C-OH), 130.19, 128.12, 126.50, 121.13, 115.75 (5C, Ar ring), 72.10 (1C, CH), 56.01 (1C, CH), 40.89 (2C, CH_2), 16.57 (1C, CH_2); EI-MS: m/z 277.11 (M^+,16%); Elemental analysis: Anal. Calcd. for $C_{14}H_{16}N_2O_4$: C, 60.86; H, 5.84; N, 10.14;%. Found: C, 60.84; H, 5.86; N, 10.12;%.

Antibacterial activity

Antibacterial assessments of the ligand (**1**) and its complexes (**1a-1e**) were experienced in vitro against the bacteria *Escherichia coli*, *Klebsiella pneumaniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* via Kirby Bauer Disc diffusion technique **[14]**. The antibacterial activity of ciprofloxacin was utilized as a reference. The bacterial cultures were cultivated on petri dishes on nutrient agar medium. The compounds were synthesized in DMSO and immersed in a 5 mm diameter, 1 mm thick filter paper disc. After 24 hours, the width of the inhibitory zone **[15, 16]** surrounding each disc was evaluated for antibacterial activity, and the discs remained put on the already implanted plates and incubated at 37°C. Minimum inhibitory concentrations (MIC) were used to reflect the antibacterial action of ligand (**1**) and its metal complexes (**1a-1e**).

RESULTS AND DISCUSSION

Metal complexes (1a-1e) with ligand (1)

Physical properties

Table 1 shows the physical possessions of the ligand (**1**) as well as the complexes (**1a-1e**) generated from it.

Solubility

The solubility of the ligand (**1**) and the complexes (**1a-1e**) in various solvents was investigated, and the findings are shown in **Table 2**. The metal complexes (**1a-1e**) derived from ligand (**1**) dissolve more readily in aprotic solvents than in protic solvents, according to solubility experiments **(Table 2)**.

Conductivity measurements

Various solvents, including water, ethanol, chloroform, and DMSO, were used to test the solubility of the newly synthesized metal complexes. The Equiptronics digital conductivity meter (Model EQ-660) was used to determine molar conductance in DMSO, with the cell constant calibrated using 0.1M KCl solution. The electrical conductivity of a 10^{-3} M solutions of respective complexes (2a-2e) in DMSO was determined, revealing the complexes' neutral (non-electrolytic) character. The molar conductance of the mixed ligand complexes (**1a-1e**) of ligand (**1**) ranges from 17 to 26 $\Omega^{-1}mol^{-1}cm^2$. The chloride ions were shown to be coupled to metal ions via conductivity tests, suggesting that they function as ligands rather than ions. Combinations for the produced complexes were allocated based on the metal – ligand ratio (1:2) and the characteristics of the electrolytes as determined by conductance measurements, which aids in describing the structure of the complex. The Conductance properties of metal complexes (**1a-1e**) with ligand (**1**) Were shown in **Table 3**.

NMR Spectra of ligand (1)

The hydrogens of the aromatic rings show a multiplet at 7.12-6.90 ppm in the ^1H NMR spectrum of the Mannich base ligand (**1**) during investigation **(Figure 1)**. The methylene hydrogens linked to the salicylaldehyde and amine hydrogens of the urea show as a peak at 4.22 ppm, whereas the aromatic –OH occurs at 5.30 ppm. The ligand's creation is also determined by the change in a signal equivalent to the secondary amine $–NH_2$ proton of because it was removed in the Mannich process. The carbons of the aromatic rings had peaks at 130.19-115.75 ppm in the ^{13}C NMR spectra of the Mannich base ligand (**1**) in investigation **(Figure 2)**. The presence of a peak at 56.01 ppm shows that the methylene carbon is linked to the salicylaldehyde and urea's amine hydrogens. Furthermore, the carbonyl carbons of the 1,3-cyclohexanedione and urea constituent are represented by the peaks at 208.83 and 162.72 ppm, respectively.

IR Spectra

The existence of a strong band at 3436.28 and 3076.43 cm^{-1}, which would be attributable to the vO-H and N-H groups, is a significant finding in the ligand spectrum **(Figure 3)**. The bands attributable to O-H and N-H moved towards lower frequency in all the complexes **(Figure 4-8)**, suggesting that oxygen and nitrogen were engaged in coordination between metal ions. In copper complex (**1a**), the M-N bond is represented by the new peak appeared at 855.81 cm^{-1}. The M-O bond is represented by the new peak at 758.11 cm^{-1}. At 493.66 cm^{-1}, there are new bands, which corresponds to the M-Cl bond. The IR Spectral data of complexes (**1a-1e**) and the ligand (**1**) were displayed in **Table 4**.

UV-Visible Spectra

The ligand and complex UV-Visible spectra were measured in the region of 100-1100 nm. The UV spectra of ligand (**1**) primarily revealed two strong maximum bands at 375nm and 200nm, which correspond to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions, correspondingly **(Figure 9)**. The Cu (II) complex being investigation has a wide band in the wavelength range of 262 nm, indicating octahedral geometry. Broad peaks at 262 nm and 290 nm were seen in the Ni (II) complex, confirming its octahedral geometry. At 263nm, the location of bands detected for Cr (II) complex displays wide signals, indicating that it has an octahedral geometry. At 258 nm, the Fe (II) complex produced wide signals, confirming its octahedral geometry. The Mn (II) complex emitted wide signals at 262 nm, indicating that it is octahedral. **Figure 10-14** shows the UV spectrums of metal complexes (**1a-1e**).

EPR spectra

The type of metal ligand binding interactions and the arrangement of paired and unpaired electrons may be learned through EPR spectrum analysis. Cu (II) complexes have a unique character in coordination chemistry, with geometries such as tetrahedral, square planar, octahedral, and square pyramidal that may be distinguished by EPR spectroscopy. $g_\parallel$, $g_\perp$, gavg and G are EPR characteristics that indicate whether the compound is octahedral or tetrahedral. The following criterion confirms the existence of an unpaired electron in the dx^2-y^2 orbital: $g_\parallel > g_\perp > 2.0023$. For the copper complex, the measured $g_\parallel$ and $g_\perp$ values are 2.1462 and 2.0131, respectively. The ionic nature is shown by a $g_\parallel$ value more than 2.3, while the covalent nature is indicated by a $g_\parallel$ value less than 2.3. We can see that the g value (2.1462) is smaller than 2.3, indicating that the compound is covalent. According to Hathaway, G values less than four indicate a significant exchange contact amongst metal centers, whereas G values higher than four indicate a minimal exchange interaction. The G value is 4.76 in this case, thus the exchange interaction is insignificant. The Cu (II) complex exhibits deformed octahedral geometry, according to the EPR characteristics. The EPR spectra of copper complex (**1a**) was shown in **Figure 15**.

Structure of the Complexes

We recommend the following structure for the complexes (**1a-1e**) produced utilizing the Mannich base ligand (**1**) based on the preceding findings.

Biological screening

Antibacterial activity

The antibacterial assessment of the synthesized ligand (**1**) and complexes (**1a-1e**) were tested. The ligand (**1**) had low activity compared to the corresponding complexes (**1a-1e**). The investigation was done in a controlled environment. In the (**1a-1e**), series, the complex **1b** was only highly active, with an MIC of 2 µg/mL in *S. aureus*. Obviously, the copper complex **1a** was more active against *K. pneumoniae*, with MIC of 4 µg/mL, than the control **Ciprofloxacin**, which had MIC of 8 µg/mL. The manganese complex **1e** was more active against *E. coli*, with MIC of 4 µg/mL, than the control **Ciprofloxacin**, which had MIC of 6 µg/mL. In comparison to complexes (**1a-1e**), complex **1a** (Cu II), complex **1b** (Ni II), and complex **1e** (Mn II), has exceptional activity. Table 5 summarizes the findings.

Table 1: Physical possessions of complexes (1a-1e) and its ligand (1)

Compound	Color	Melting point (^{o}C)
Ligand (1)	Dust white	146
Copper complex (1a)	Blue	162
Nickel complex (1b)	Pale green	142
Iron complex (1c)	Brown	154
Chromium complex (1d)	Green	160
Manganese complex (1e)	White	172

Table 2: Solubility test results

Compound	Water	Ethanol	Chloroform	DMSO
Ligand (1)	Insoluble	Insoluble	Sparingly soluble	Soluble
Copper complex (1a)	Insoluble	Insoluble	Insoluble	Soluble
Nickel complex (1b)	Insoluble	Insoluble	Insoluble	Soluble
Iron complex (1c)	Insoluble	Insoluble	Insoluble	Soluble
Chromium complex (1d)	Insoluble	Insoluble	Insoluble	Soluble
Manganese complex (1e)	Insoluble	Insoluble	Insoluble	Soluble

Table 3: Conductance properties of metal complexes (1a-1e) with ligand (1)

S. No.	Compounds	Conductance ($\Omega^{-1}mol^{-1}cm^2$)
1.	Copper complex (1a)	17
2.	Nickel complex (1b)	26
3.	Iron complex (1c)	23
4.	Chromium complex (1d)	20
5.	Manganese complex (1e)	21

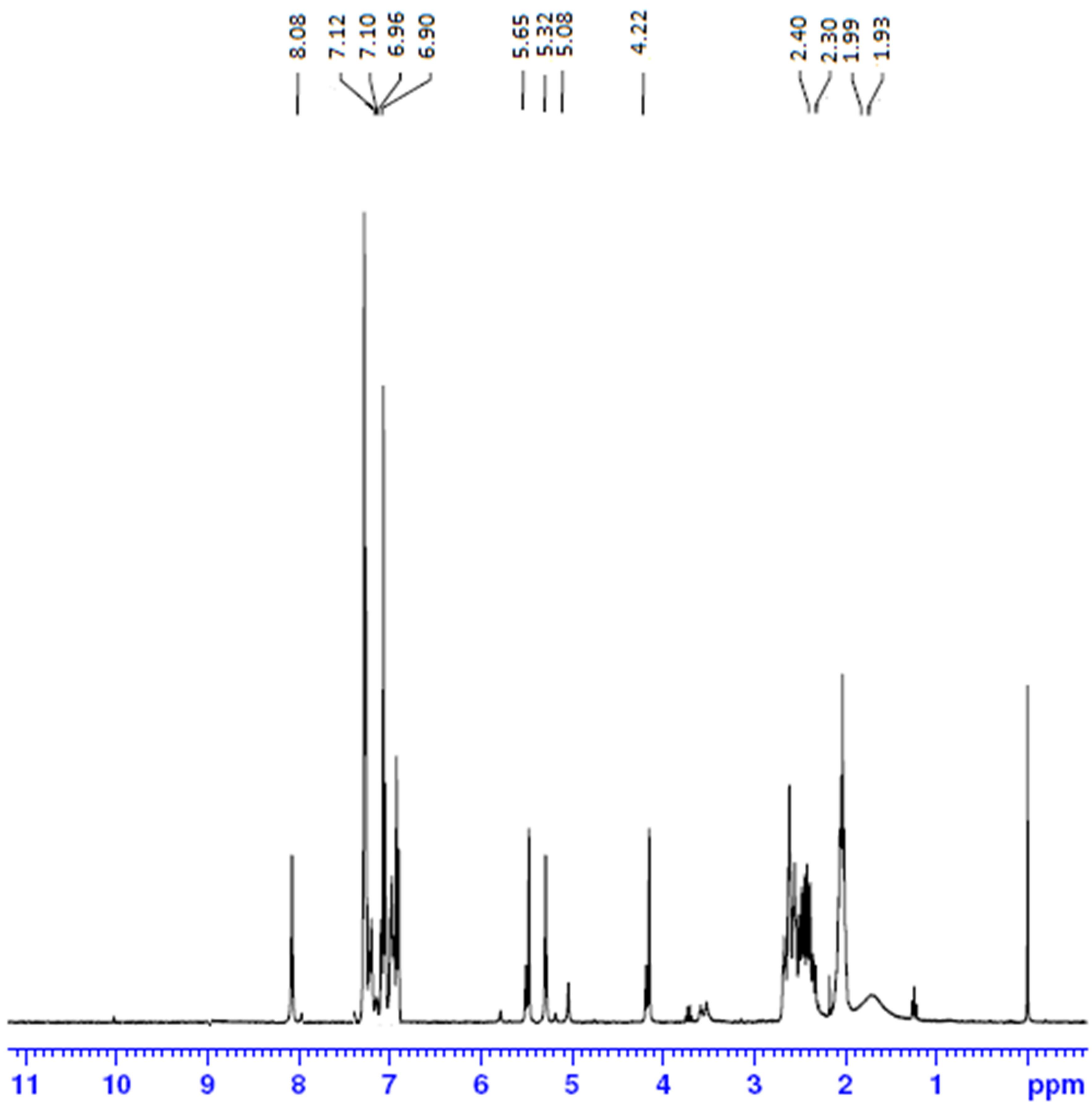


Figure 1: Ligand (1)-^{1}H-NMR spectrum

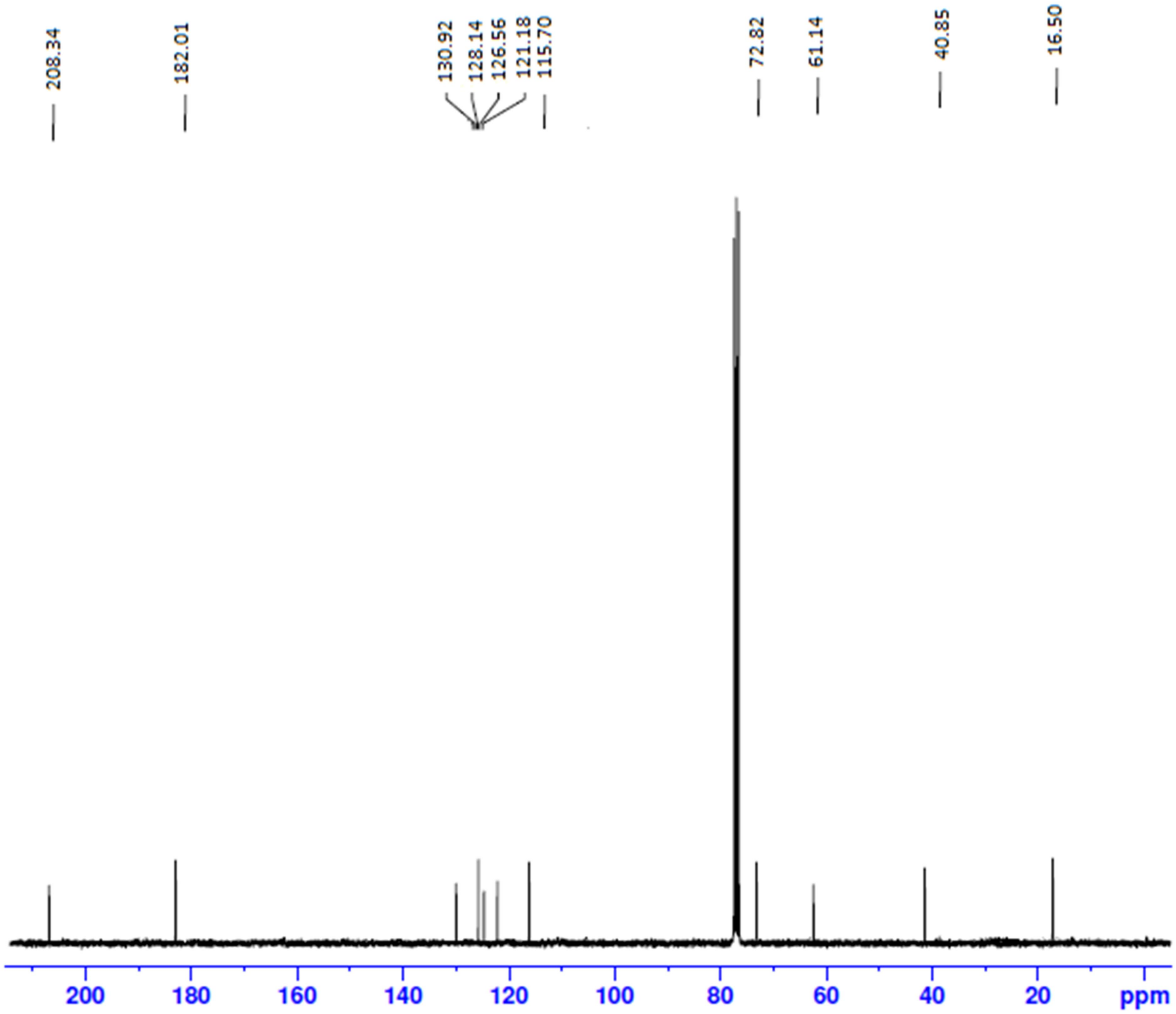


Figure 2: Ligand (1)-^{13}C-NMR spectrum

Table 4: IR Spectral data of complexes (1a-1e) and the ligand (1)

Compound	IR stretching frequency (cm^{-1})				
	-OH	-N-H	M-N	M-O	M-Cl
Ligand (1)	3436.28	3076.46	-	-	-
Copper complex (1a)	3450.43	2950.60	855.81	758.11	493.66
Nickel complex (1b)	3449.65	2950.60	851.78	771.18	493.95
Iron complex (1c)	3440.23	3077.30	854.63	774.91	494.62
Chromium complex (1d)	3450.41	2950.95	854.18	774.95	494.63
Manganese complex (1e)	3429.49	3077.24	853.62	759.76	493.88

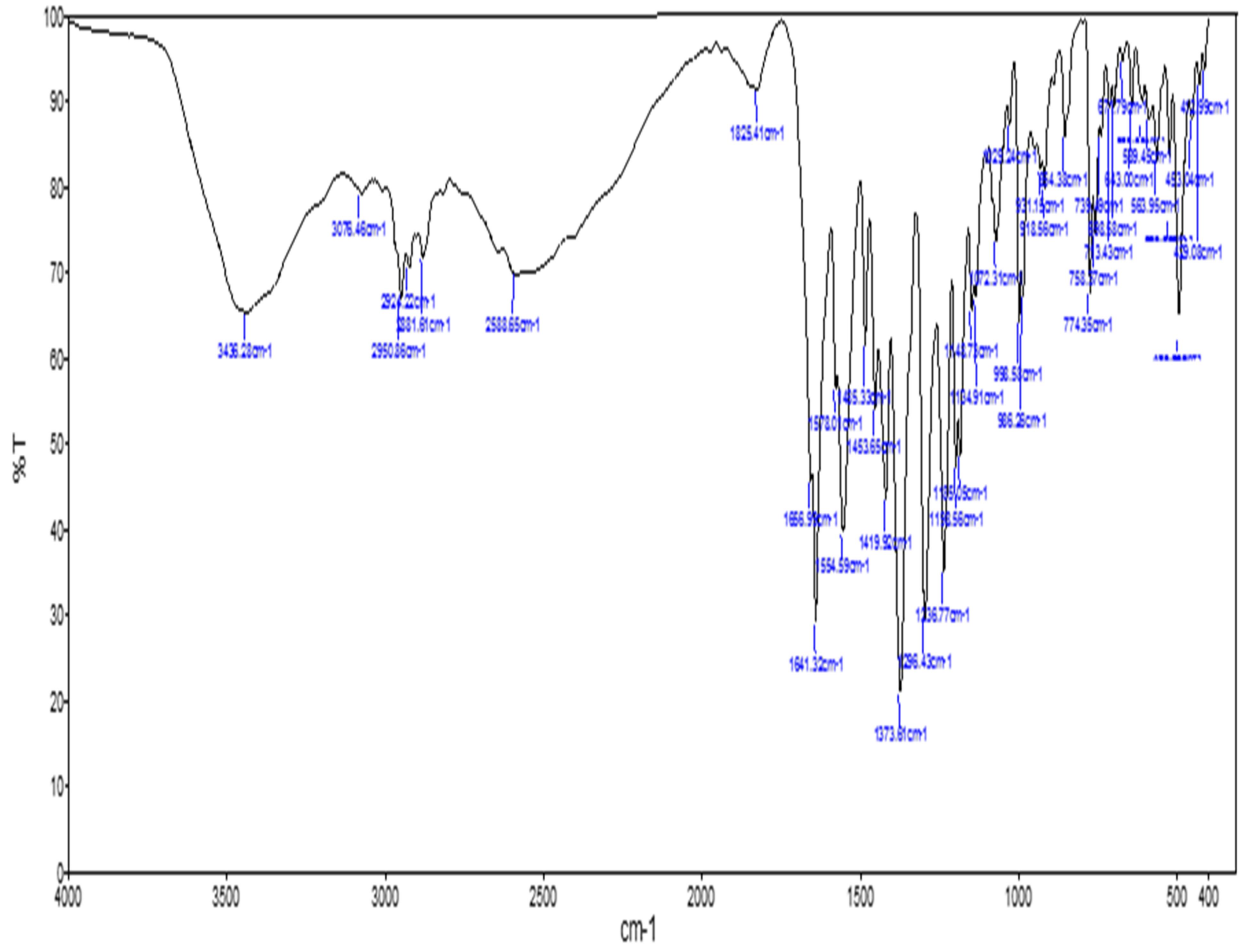


Figure 3: Ligand (1)-FT-IR spectra

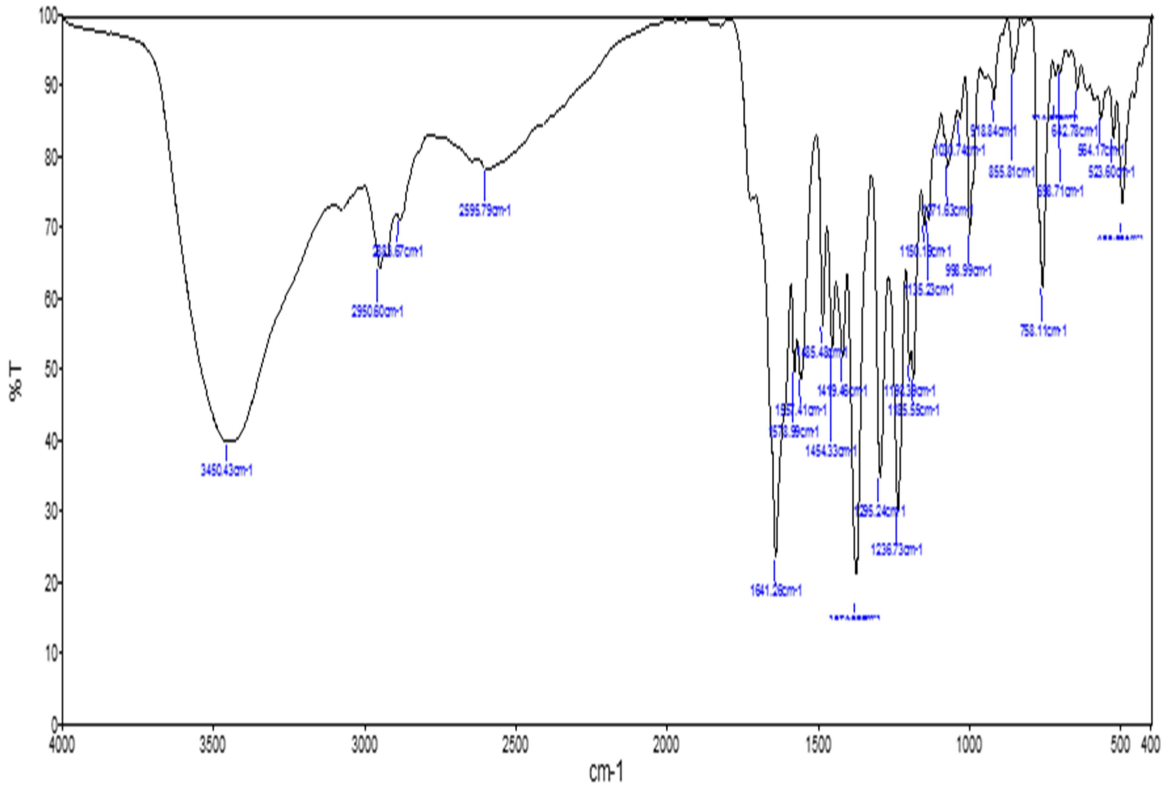


Figure 4: Copper complex (1a)-FT-IR spectra

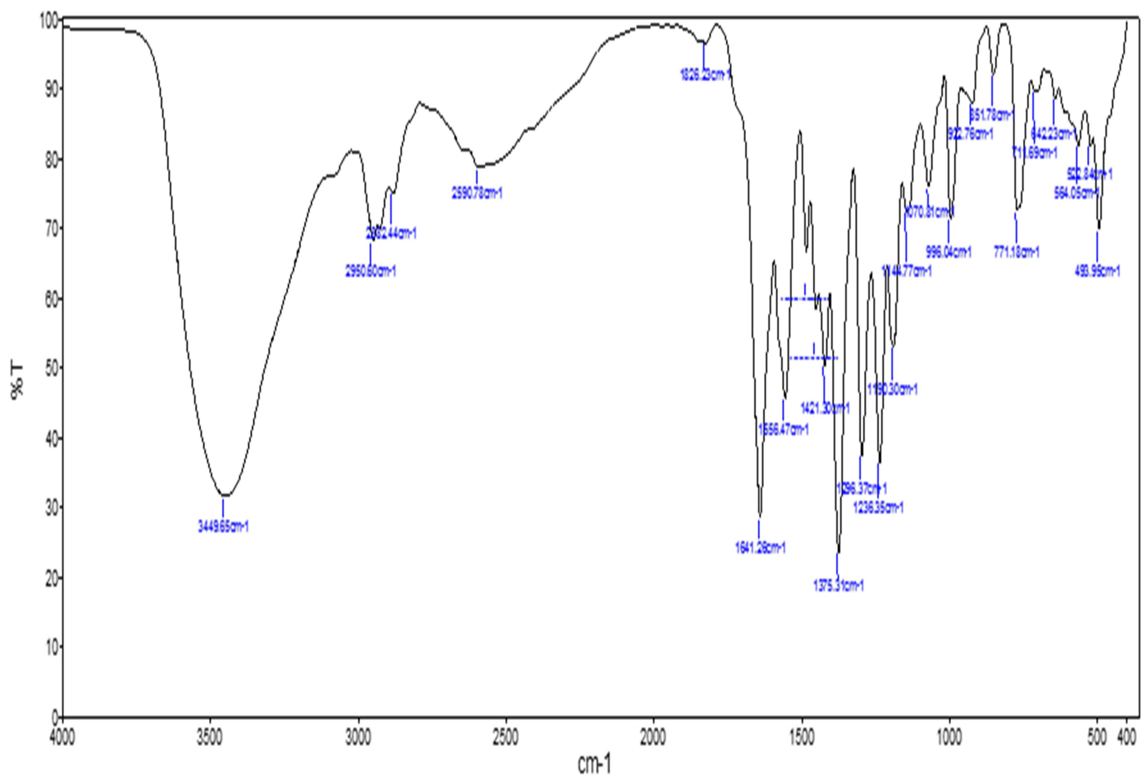


Figure 5: Nickel complex (1b)-FT-IR spectra

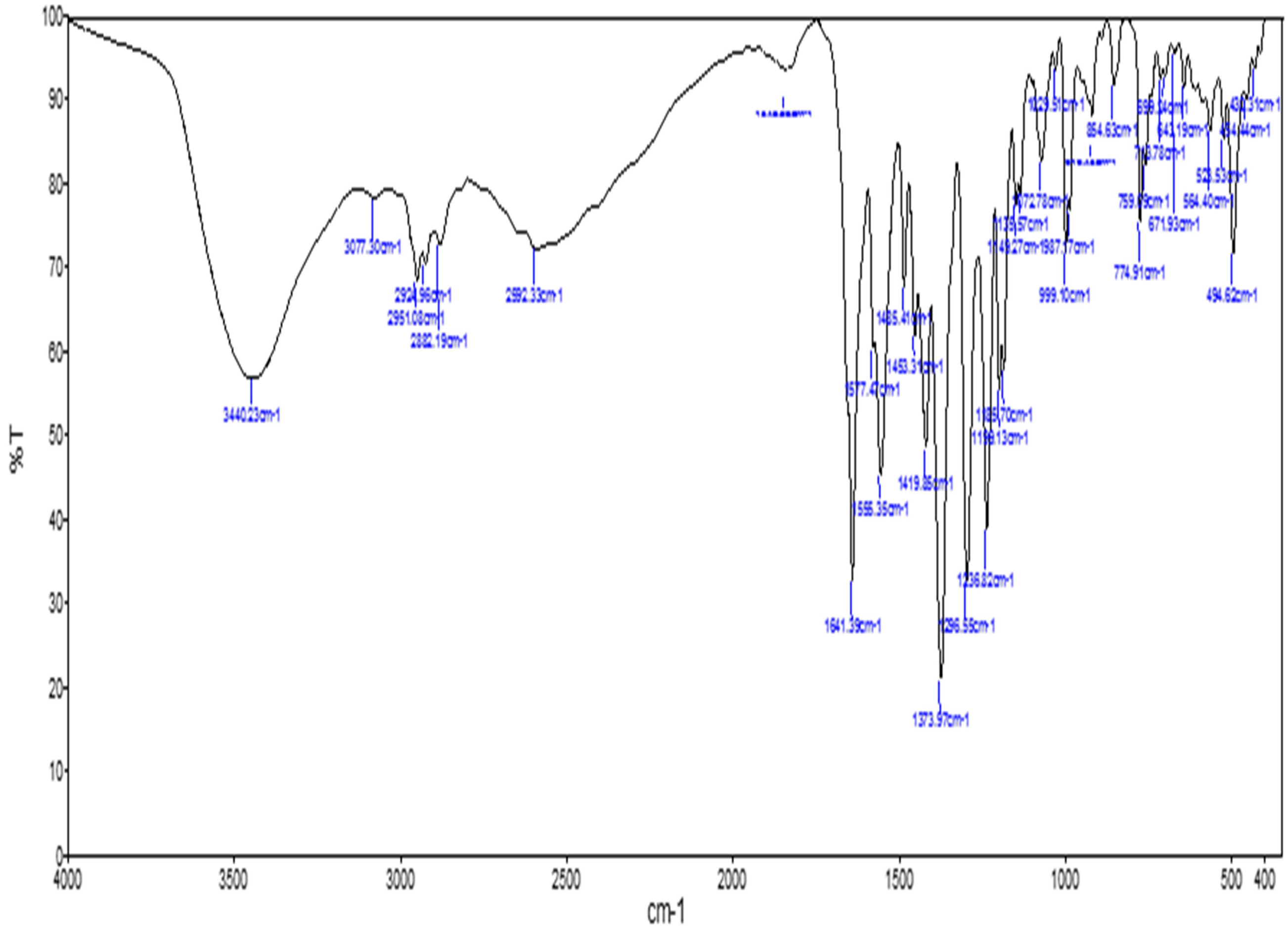


Figure 6: Iron complex (1c)-FT-IR spectra

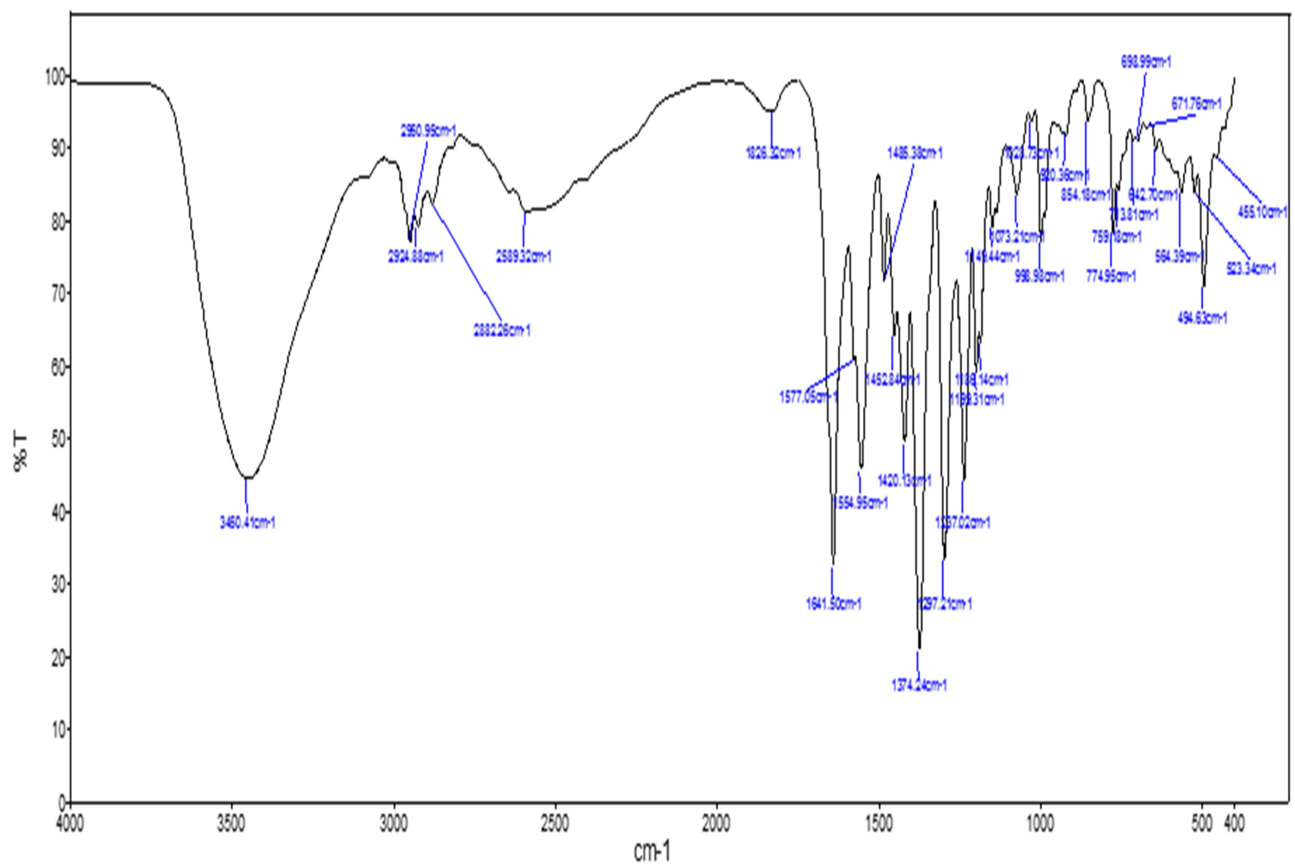


Figure 7: Chromium complex (1d)-FT-IR spectra

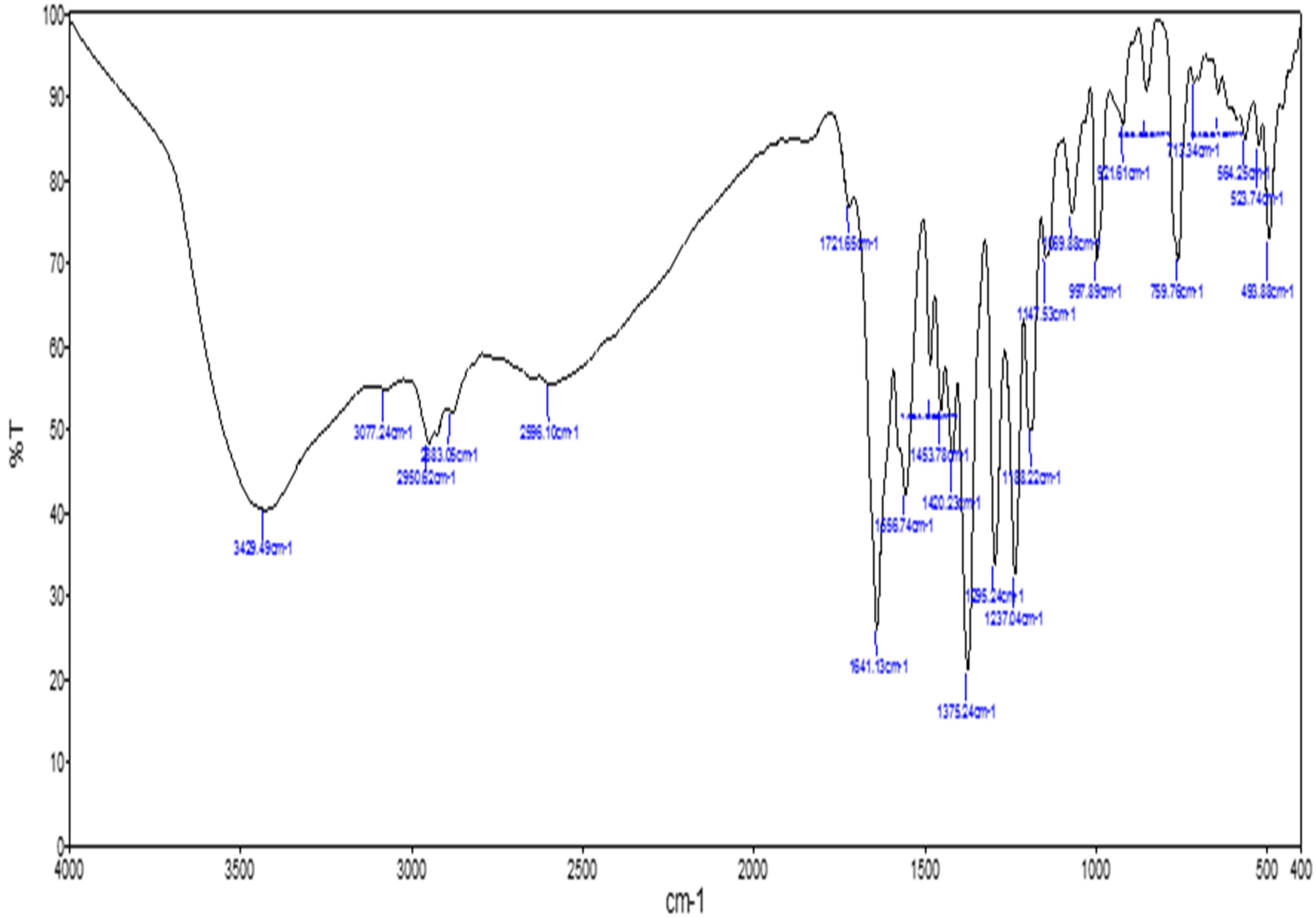


Figure 8: Manganese complex (1e)-FT-IR spectra

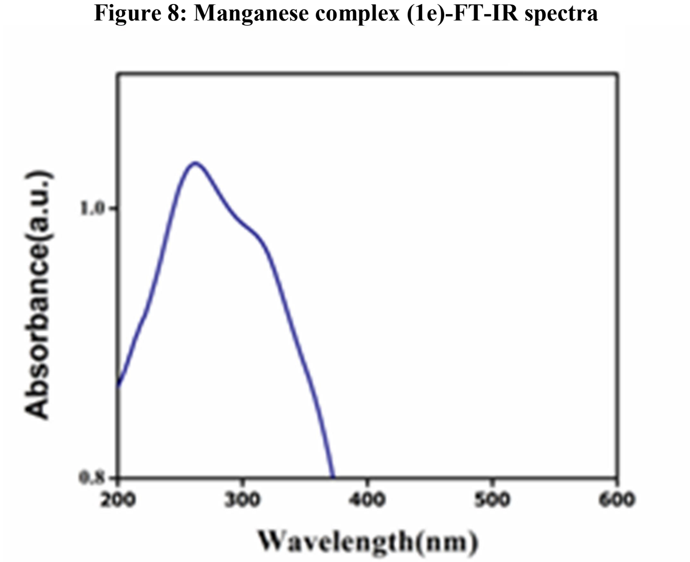


Figure 9: Ligand 1 UV-Spectra

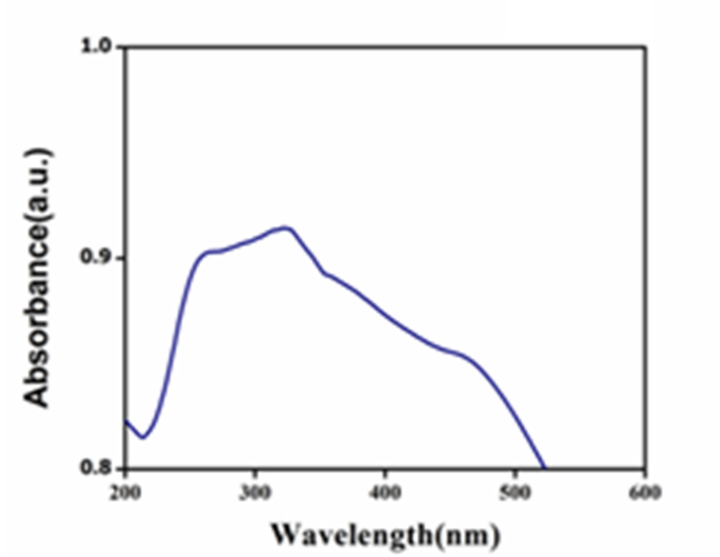


Figure 10: Copper complex (1a) UV-Spectra

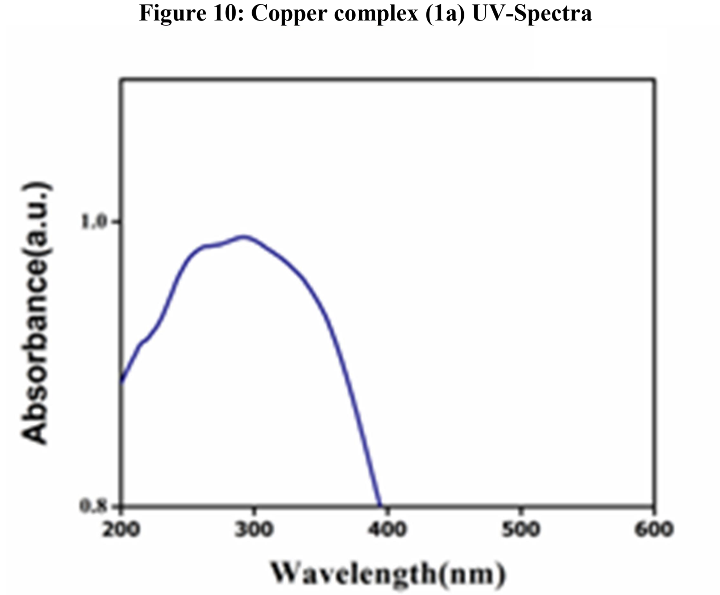


Figure 11: Nickel complex (1b) UV-Spectra

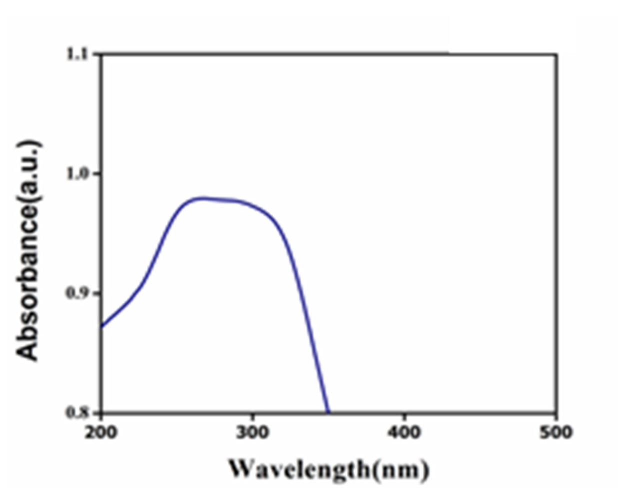


Figure 12: Iron complex (1c) UV-Spectra

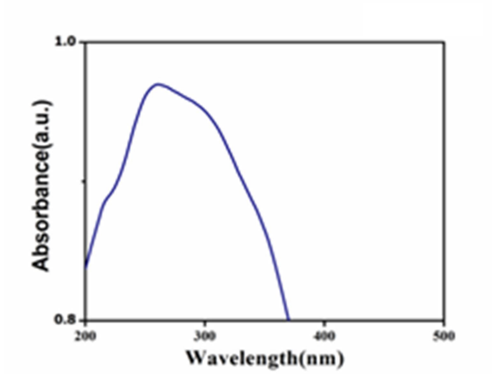


Figure 13: Chromium complex (1d) UV-Spectra

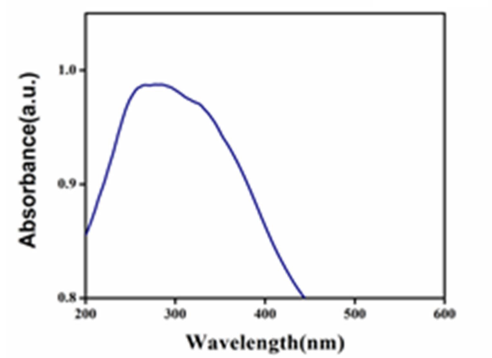


Figure 14: Manganese complex (1e) UV-Spectra

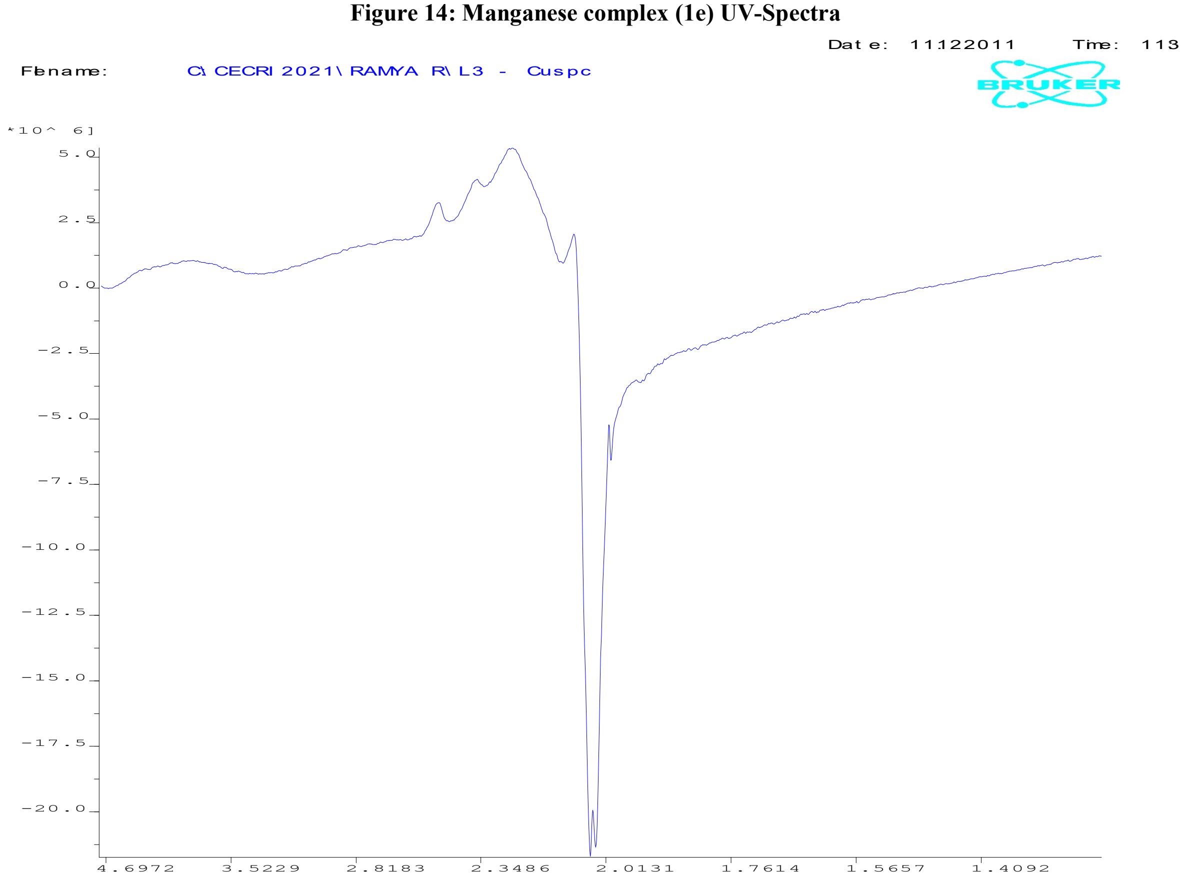
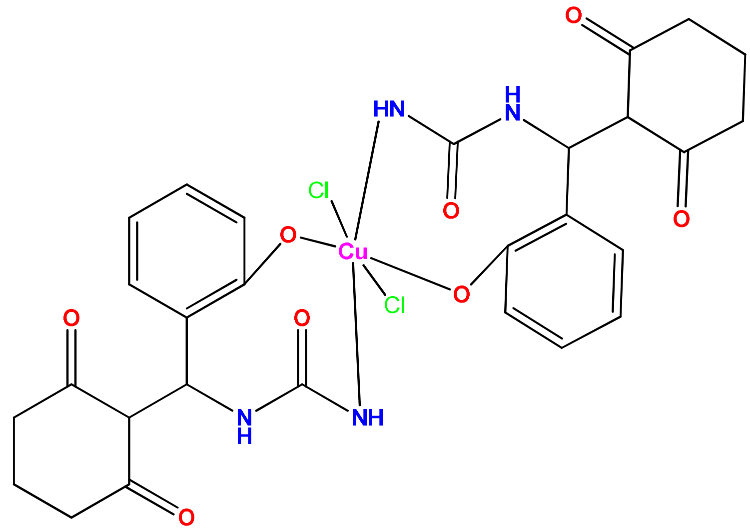
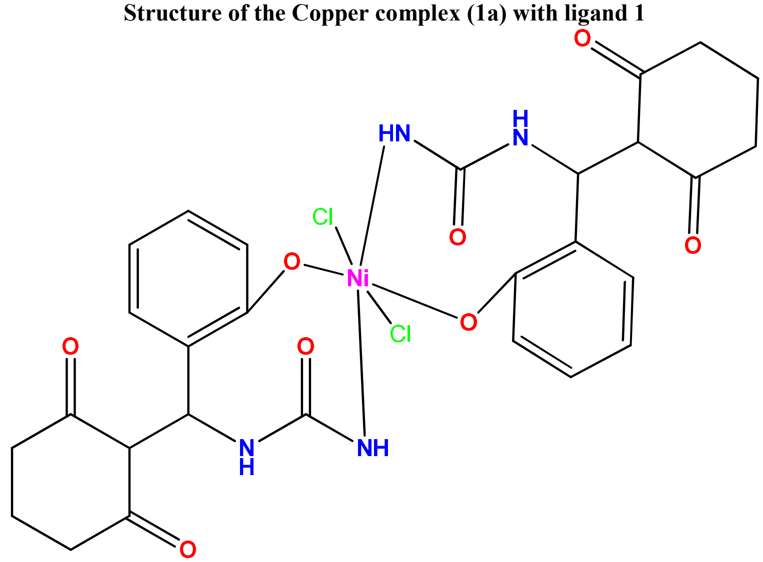


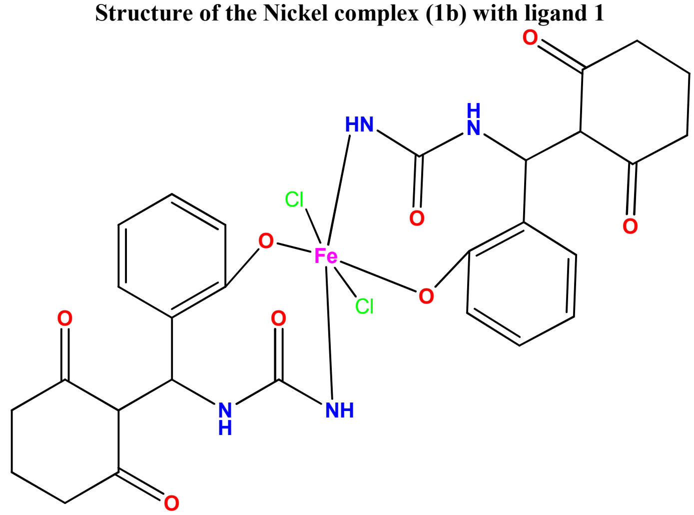
Figure 15: Copper complex (2a) EPR spectra



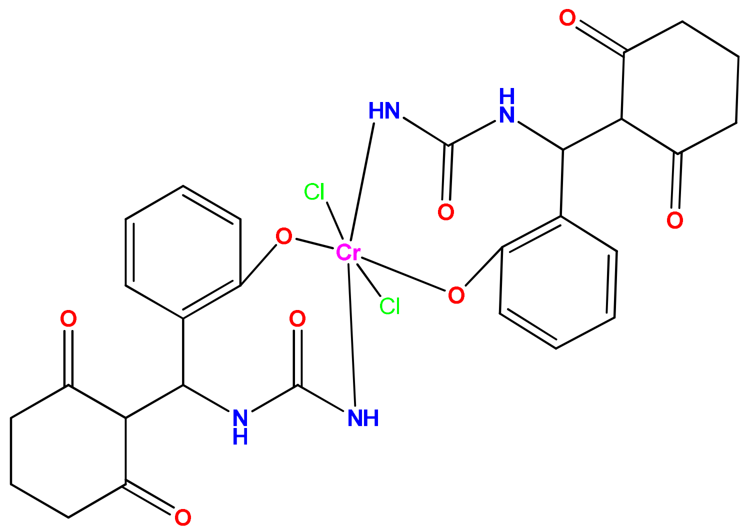
Structure of the Copper complex (1a) with ligand 1



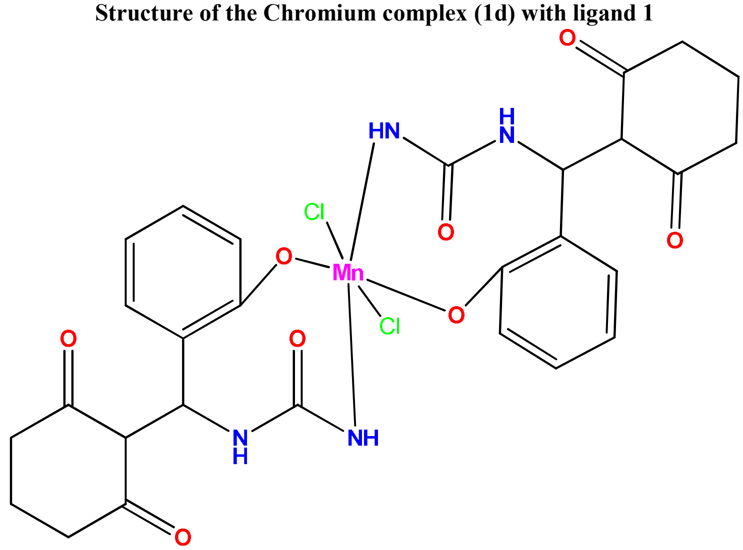
Structure of the Nickel complex (1b) with ligand 1



Structure of the Iron complex (1c) with ligand 1



Structure of the Chromium complex (1d) with ligand 1



Structure of the Manganese complex (1e) with ligand 1

Table 5: Antibacterial assessment of ligand (1) and its complexes (1a-1e)

Compounds	Minimum inhibitory concentration (MIC) in µg/mL			
	S. aureus	*K. pneumaniae*	*E. coli*	*P. aeruginosa*
1	32	16	16	32
1a	8	4	8	8
1b	2	10	10	4
1c	12	14	12	8
1d	6	12	8	4
1e	10	28	4	6
Ciprofloxacin	4	8	6	2

CONCLUSION

The coordination behavior of a Mannich base ligand synthesized from 1,3-cyclohexanedione, salicylaldehyde, and urea is reported in this article. Using the aforementioned Mannich base ligand, Cu(II), Ni(II), Fe(II), Cr(II), and Mn(II) complexes have been produced and described utilizing analytical and spectroscopic measurements. The Mannich base functions as a neutral bidentate ligand by coordinating to the metal ion via its urea nitrogen and oxygen of salicylaldehyde. The complexes are all octahedral in shape. Antibacterial assessment of the ligand and its metal complexes was tested. The ligand (**1**) and its metal complexes (**1a-1e**) were revealed to exhibit significant antibacterial action against a variety of disease-causing bacteria. The metal complexes revealed to be a more efficient bactericide than ciprofloxacin.

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Conflicts of interest

The authors declare no conflicts of interest.

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