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Anticancer, Antioxidant and Antimicrobial Evaluation of Cr (III) and Rh(III) Complexes Derived from A New Mannich Base

Alya'a J. Ahmed * Mahasin F. Alias
Department of Chemistry, College of Science for Women, University of Baghdad, Baghdad, Iraq

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Abstract

The Mannich base ligand was synthesized in an ethanol medium through a condensation reaction of 2-mercaptobenzimidazole and ciprofloxacin at room temperature. Subsequently, several metal complexes of this ligand were prepared. To characterize both the base ligand and the metal complexes, various techniques were employed, including elemental analysis, FT-IR spectroscopy, UV-Vis spectroscopy, molar conductivity measurements, magnetic moment determination, and melting point analysis. The results were shown that the metal complexes formed have the formula $[Cr(L)_2Cl_2]\ Cl.H_2O$ and $[Rh(L)_2(H_2O)_2]\ Cl_3.H_2O$, where L= mannich base ligand. Based on spectroscopic analytical, coordination with metal ions involves the 'N' donor atom of mannich base and 'N' atom of piprizaing ring, and two complexes are A six-coordinated octahedral structure is suggested. Molar conductivity of these complexes showed that they were electrolytic in nature. In this study, the anticancer and antioxidant potential of the Mannich base ligand and its metal complexes were investigated against MDA-MB-231 cell lines and using the DPPH free radical scavenging assay. Moreover, the *in vitro* efficacy of the ligand and its complexes against Gram-negative bacteria *(E. coli)* and Gram-positive bacteria *(Staphylococcus aureus)*, as well as the fungal strain Candida albicans, was evaluated using the disc diffusion method. The results indicated that Cr (III) and Rh(III) complexes demonstrated the highest levels of cytotoxicity against MDA-MB-231 cell lines, enhances antioxidant and antimicrobial activity more than the free ligand. These findings suggest that these metal complexes may have promising applications in the development of novel anticancer, antioxidant and antimicrobial agents.

Keywords: Antimicrobial, Ciprofloxacin, Mannich base complexes, Free radicals.

تقييم مضادات السرطان ، مضادات الأكسدة ومضادات الميكروبات لمعقدات الكروم الثلاثي و الروديوم الثلاثي المشتقة من قاعدة مانخ جديدة

علياء جبار احمد* محاسن فيصل الياس
قسم الكيمياء ، كلية العلوم للبنات ، جامعة بغداد ، بغداد ، العراق .

الخلاصة

تم تحضير ليكاند قاعدة مانخ في وسط إيثانول من خلال تفاعل تكثيف لـ 2-مركابتوبنزيميدازول وسيبروفلوكساسين في درجة حرارة الغرفة. بعد ذلك ، تم تحضير معقدات فلزية من هذا الليكاند. لتوصيف كل من الليكاند والمعقدات الفلزية، تم استخدام تقنيات مختلفة ، بما في ذلك التحليلالدقيق للعناصر ، والتحليل

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*Email: alaia.jabbar1105a@csw.uobaghdad.edu.iq

الطيفي للأشعة تحت الحمراء ، والتحليل الطيفي للأشعة المرئية وفوق البنفسجية ، وقياسات الموصلية المولية ، وتحديد العزم المغناطيسي ، وتحليل درجة الانصهار.

أظهرت النتائج أن المعقدات المتكونة لها الصيغة أظهرت النتائج أن المعقدات المتكونة لها الصيغة $[Cr (L)_2Cl_2]Cl.H_2O$ و $[Rh (L)_2 (H_2O)_2] Cl_3.H_2O$ ، حيث L = ليجند قاعدة مانخ .استنادًا إلى نتائج التحليل الطيفي، يكون التنسيق مع ايونات المعادن من خلال ذرة نتروجين مانحة لقاعدة مانخ و ذرة نتروجين مانحة لحلقة البايبرازين و الشكل ثماني السطوح. اظهرت الموصلية المولارية ان المعقدات ذات طبيعة الكتروليتية. في هذه الدراسة ، تم فحص الإمكانات المضادة للسرطان ومضادات الأكسدة لليكاند قاعدة مانخ و معقداته ضد خطوط الخلايا MDA-MB-231 . وباستخدام DPPH .علاوة على ذلك ، تم تقييم فعالية اللكاند ومعقداته في المختبر ضد البكتيريا سالبة الجرام *(E. coli)* والبكتيريا موجبة الجرام *(Staphylococcus aureus)* ، وكذلك السلالة الفطرية *Candida albicans* ، باستخدام طريقة الانتشار القرصي. أشارت النتائج إلى أن معقدات (III) Cr و (III) Rh قد أظهرت أعلى مستويات السمية الخلوية ضد خطوط الخلايا MDA-MB-231 ، وتعزز نشاطها كمضادات للاكسدة و كمضادات للميكروبات اكثر من الليكاند الحر.تشير هذه النتائج إلى أن هذه المعقدات قد يكون لها تطبيقات واعدة في تطوير عوامل جديدة مضادة للسرطان ومضادات الأكسدة ومضادات الميكروبات.

1. Introduction

Mannich bases are produced through the condensation reaction of active hydrogen-containing compounds, aldehydes, and secondary amines, resulting in three distinct products. The formation of these Mannich base products is influenced by the nucleophilicity of the substrate and the pH of the reaction medium [1].In the realm of chemical studies, metal complexes of Mannich bases have garnered considerable attention due to their remarkable ligand selectivity and sensitivity to various metal ions [2].Due to their versatility, Mannich bases and their metal complexes have garnered a great deal of interest in chemistry studies, [4-5]. The biological and pharmacological uses of Mannich base complexes are numerous [5]. Due to their selectivity and sensitivity to physiologically significant metal ions, Mannich base metal ion complexes have been the subject of substantial research in recent years [6], [7]. Recently, there has been significant interest in exploring novel ligand systems to chelate amino and hydroxy compounds with transition metals. Among these, metal complexes of Mannich bases have demonstrated exceptional catalytic activity in various organic transformations, including oxidation, reduction, and cross-coupling reactions [8]. Fluoroquinolones, a class of synthetic antibacterial agents, have also been extensively investigated as potential bidentate ligands capable of binding to metal ions through carboxylate and carbonyl oxygen atoms. As a result, stable six-membered chelates with slightly distorted octahedral morphologies have been observed in complexes with Co(II), Ni(II), Zn(II), Cd(II), and Mn(II), while Cu(II) forms a different type of complex [9-10]. Although coordination of fluoroquinolones to metal ions via the piperazine nitrogen atom is less frequent, it seems that most metal ions prefer this location. Fluoroquinolones were recently characterized as ligands in the production of platinum complexes [11].This investigation provides insight on the fluoroquinolones' mode of action and could lead the development for the production of new antibiotics. Bacterial infections are frequently treated with the drug ciprofloxacin (CFL). It is a fluoroquinolone antibiotic used orally [12].Metal complexes, like the anticancer medication cisplatin, have been extensively investigated in medicinal chemistry, but the antibiotic industry has mostly ignored them. Metal complexes, on the other hand, have distinctive modes of action and can be found in a variety of 3D geometries, which makes them attractive starting points for the creation of novel drugs [13]. For instance, the platinum in anticancer drugs like carboplatin and oxaliplatin is associated with organic molecules like cyclobutane dicarboxylic acid, 1,2-diaminocyclohexane, and oxalic acid [14]. Metal complexes have traditionally only had a minor function in pharmaceutical chemistry, despite its potential [15]. Inversely, the fluorinated quinolone of a more recent generation known as ciprofloxacin (CIP) has structural similarities with nalidixic acid [16]. Ciprofloxacin is a widely prescribed antibiotic drug employed to treat diverse bacterial infections [17]. By combining the unique properties of metal complexes with the broad-spectrum efficacy of fluoroquinolones like ciprofloxacin, it may be possible to develop novel antibacterial agents that can combat drug-resistant bacterial infections. Further research in this area could lead to the discovery of new and effective treatments for bacterial infections. In the present study, a systematic description of new mannich base ligands 6-(4-((1H-benzo[d]imidazol2ythio)methyl)piperazin-1-yl)-4-cyclopropyl-7-fluoro-1-oxo 1,4Edihydronaphthalene-2- carboxylic acid) with Cr(III), Rh(III) metal complexes have been synthesized and characterized successfully and their anticancer, antioxidant and antimicrobial activities were evaluated.

2. Experimental

2.1. Materials and Instruments

Ciprofloxacin (99.5%), 2-mercaptobenzimidazole, formaldehyde, metal chlorides (analytical grade), and solvents were acquired from Merck (Schnelldorf, Germany). X-ray diffraction (XRD) measurements were conducted using a Rigaku XRD-6000 diffractometer, utilizing Cu K radiation ($\lambda = 0.154184$ nm) at 40 kV and 30 mA, with a scanning rate of 0.02°/s, covering a 2θ range of 10° to 80°. The metal content was determined using an AA-6880 Shimadzu atomic absorption flame spectrophotometer (Shimadzu Corporation; Tokyo, Japan). The UV-Vis spectra in ethanol were measured using a Shimadzu UV-1601 spectrophotometer (Shimadzu Company; Tokyo, Japan). The Fourier transform infrared (FTIR) spectra were recorded using the FT-IR 8300 Shimadzu spectrophotometer (Shimadzu Corporation; Tokyo, Japan). Mass spectra were obtained using Direct Probe. Melting points were examined in open glass capillaries. Using EA-034.mth, the elemental analyses (C.H.N.S.) were obtained. Measurements of conductivity were performed using a Corning conductivity meter 220, and they were done in an ethanol solvent with a concentration of (10^{-3} M)

2.2. Synthesis of mannich base ligand (L)

General procedure for the preparation of ligand (CPFX, 1.65 gm 0.005 mol) and 2-mercaptobenzimidazole (0.75 gm, 0.005 mol) in EtOH (25 mL). A solution (CH_2O) was applied and heated to reflux for 6 hours and cooled to room temperature. The precipitate was filtered and recrystallized from (ethanol in water) to give the title compound [18].

2.3. Preparation of metal Complexes

The synthesis of metal complexes employing a Mannich base ligand (L) and two distinct metal ions, Cr(III) and Rh(III), involves the following procedure: 2 mmol of the Mannich base ligand is dissolved in 10 mL of absolute ethanol, and subsequently, 1 mmol of the metal ion is added (0.158 gm of $CrCl_3.6H_2O$ or 0.22 gm of $RhCl_3.H_2O$). The resulting mixture is refluxed for 2 hours, during which time the color of the solution changes. This change in color is likely due to the formation of the metal complex. After refluxing, the solvent is evaporated to yield a precipitate. This precipitate is then recrystallized from ethanol to purify the complex, and finally dried to give the pure metal complex [19].

2.4. Cytotoxic studies-MTT assay

The cytotoxicity of free ligand and its metal complexes were studied against MDA-MB-231 cell lines by *in vitro* MTT cytotoxicity assay. Cell lines were evaluated 24 hours after being exposed to the compounds at various concentrations. The MTT testing results for the ligand and its metal complexes were presented, and all the compounds were subjected to characterization using spectroscopic, analytical, and physical methods, as detailed in Table 5. The evaluation involved comparing various concentrations (400, 200, 100, 50 μg/ml) to an untreated negative control culture medium.

2.5. Antioxidant activity

The stable free-radical DPPH had a scavenging effect on radicals, and the method was evaluated for the antioxidant efficacy of the vitamin C. 1 ml of the normal or diluted solution (100, 50, 25, 12.5, 6.25 ppm) was added to 1 ml of the DPPH solution in a test tube. After incubation at 37°C for one hour, the absorbance of each solvent was measured at 517 nm using a spectrophotometer. The potential to scavenge DPPH was assessed through triplicate experiments using the following equation [20].

$$I\% = \frac{Abs\ blank - Abs\ sample}{Abs\ blank} \times 100 \qquad \ldots\ldots(1)$$

Where the Abs sample is the test compound and Abs blank is the absorbance of the control reaction, which contains all of the reagents but not the test compound. Plotting the proportion of DPPH-scavenging activity versus sample concentration was done to calculate the IC 50, which is the absorbance of values for each synthesized compound.

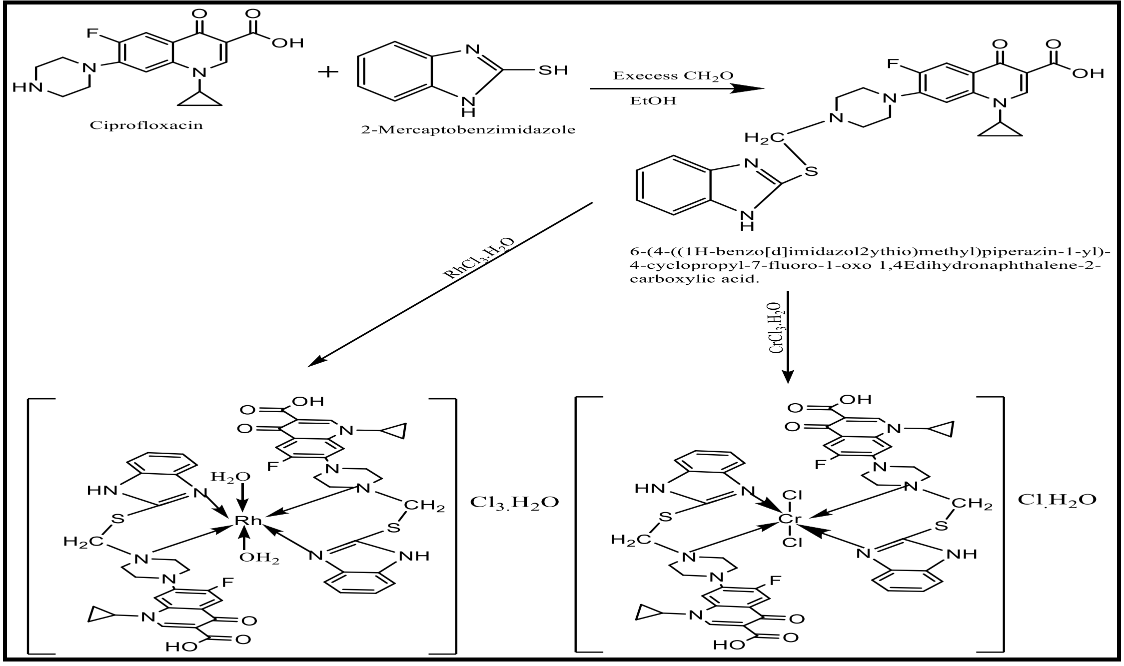
2.6. Antimicrobial activity studied

The agar well diffusion technique was employed to evaluate the antibacterial activity of the ligand and their complexes [21]. A bacterial suspension at a standard concentration of 0.5 McFarland was evenly spread on Muller Hinton agar medium. Wells were then created using a sterile cork borer. Each antibiotic sample, weighing 10 mg, was placed into the wells, and the plates were incubated at 37°C for 24 hours. The area surrounding the wells, where bacterial growth was inhibited, was measured using a caliper. Overall, this study offers significant new understandings into the antibacterial properties of different compounds and their potential as agents to combat bacterial infection**.**

3. Results and Discussion

3.1. Synthesis and Characterization of the ligand and Metal Complexes

Table 1 show some physical data and some analytical data for the ligand and synthesized complexes.



Scheme 1: Synthesis of ligand (L) and their complex.

Table 1: Color, melting point, yield, and elemental composition of ligand and metal complexes.

Com.	suggested formula	Color	melting point ℃	Yield %	elemental analysis Found (cal.)				
					C	H	N	S	M
L	$C_{25}H_{24}FN_5O_3S$	Pale Yellow	174-176	88.5	60.84 (60.89)	4.90 (4.97)	14.19 (14.25)	6.50 (6.55)	**---**
LCr(III)	$[CrL_2Cl_2]\ Cl.H_2O$	Green	188-190	65.35	51.71 (51.80)	4.17 (4.21)	12.06 (12.14)	5.52 (50.60)	**4.48 (4.56)**
LRh(III)	$[RhL_2(H_2O)_2]$ $Cl_3.H_2O$	Brown	280-282	59.7	48.10 (48.18)	4.20 (4.27)	11.22 (11.30)	5.14 (5.21)	**8.24 (8.32)**

3.2. Spectral Analysis

3.2.1.FT-IR Analysis

The FT-IR spectrum of the Mannich base ligand L (2-mercaptobenzimidazole) derived from ciprofloxacin, as shown in Figure 1, appears complex due to the presence of numerous groups with overlapping regions. However, specific bands have been selected to demonstrate the complexation. The most noteworthy IR bands of both the free ligand and its metal complexes are listed in Table 2. The spectrum of ligand Figure 1 shows stretching frequency of ν(CH_2-N) , ν(C=N), at 2964 , 2839,1552, cm^{-1} respectively, the other bands appeared at 3383,1708,1627,1051,1361,1271,1137,738 cm^{-1} are assigned to stretching frequency of ν OH of COOH group, ν COOH, ν C=O, ν NCS, ν NCN, ν CNC, ν CSC, and ν CS respectively . The FTIR spectrum of the complexes Figure 2 show distinguish frequencies at 1707 cm^{-1} and 1627 cm^{-1} which are assigned to ν(C=O) of carboxylic and carbonyl groups respectively. In comparison to the free ligand, these vibration bands occur at the same frequencies (1708 cm^{-1} and 1627 cm^{-1}) [22].These results confirmed that the oxygen atoms of carboxylic and carbonyl groups didn't participate in the coordination towards metal ions. Infrared spectra of complexes for ligand, also the ν NH, bands did not change in intensity and position when comparing the same bands of the ligand, this proves the amine does not coordinate. In Infrared spectra of ligand ,the absorption band at the bands at (2964)(2839)cm^{-1}which were attributed to the mannich of the ligand mentioned previously were shifted to higher wave numbers in both complexes about (10-13)and (13-14) cm^{-1}, while the band at 1552 which to the ν(C=N) of imidazole ring shifted to lower wave number in both complexes about (46-44)cm^{-1} as shown in Table 2. This indicates that the ligand acts as a neutral bidentate through the N atom of the mannich base and through the N atom of the imidazol ring. The weak absorption bands presence at frequencies below 500 cm^{-1} are assigned to the coordination bond ν(M-N) [23]. The complexes spectra exhibited new weak band at frequency 325 cm^{-1} assigned to stretching frequency of (Cr-Cl) [24],for Cr(III) complex. Table 2 shows the bending vibration of δH_2O and ω H_2O band coordination with central metal ion (aqua) in Rh(III) complex. In two complexes appeared bands varying between (3487-3383) cm^{-1} which refered to stretching band of H_2O uncoordination [25].

3.2.2.Electronic Spectra

Electronic spectral studies of the ligand and both complexes were carried out in ethanol. The electronic spectrum of Mannich base ligand Figure 3 generally exhibited three main bands. The first and second absorption bands appeared at (31545,33444) cm^{-1} due to interaligand (n → π*) transition of the carbonyl and -N=C- groups of imidazole in addition to the pyrazine ring· The third absorption band located at (40485) cm^{-1} attributed to (π → π*) electronic transition of the aromatic rings [26].

[CrL] The electronic spectrum of Cr(III) complex derived from the ligand ,Figure 4 showed band at 16722 cm^{-1} which due to $^{4}A_{2}g\rightarrow{}^{4}T_{2}g$. The complexes displayed a band at 35842 cm^{-1} due to $^{4}A_{2}g\rightarrow{}^{4}T_{1}g(p)$ in an octahedral geometry[27], so the second transition calculated theoretically from Tanabe–Sugano diagram for d^{3} system and found to be 26009 cm^{-1} which due to $^{4}A_{2}g\rightarrow{}^{4}T_{1}g_{(F)}$. The values of racah parameters (10Dq, B`, and β) have been calculated to be (16359 cm^{-1},779 , and 0.84) respectively. The μ effect value of this complex equal to 3,33 BM. Conductivity measurement showed that the complex was to be ionic. From the analysis of data and spectroscopy techniques and from all results, the distorted octahedral prepared for this complex can be suggested. [RhL] three main absorption bands were observed in the electronic spectra of the Rh(III) complex in ethanol solution Figure 5, and these bands were assigned to the transitions $^{1}A_{1}g \rightarrow {}^{3}T_{1}g$, $^{3}T_{2}g$, $^{1}A_{1}g \rightarrow {}^{1}T_{2}g$ and $^{1}A_{1}g \rightarrow {}^{1}T_{1}g$ respectively, in an octahedral geometry [28].Table 3 shows the magnetic moment for the solid complex exhibits diamagnetic behaviour . Measurements of conductivity indicated that the compound will exhibit ionic behaviour. The prepared distorted octahedral for this complex can be suggested based on data analysis, spectroscopy methods, and all results.

Table 2: Selected FTIR absorption bands of metal complexes

Comp.	ν COOH	ν C=O	νC-N ν N-C-N	νCH_2-N	νN-H piprazing ring	νC=N	νM-N	Others
L	1707	1627	1271 1107	2964 2837	-----	1552	----	----
CrL	1708	1620	1271 1101	2978 2852	2617	1508	420	ν OH(H_2O) = 3383 ν Cr-Cl = 325
RhL	1707	1625	1271 1111	2974 2853	2619	1504	440	ν OH(H_2O) = 3383 ω H_2O = 1080 δH_2O = 900.76

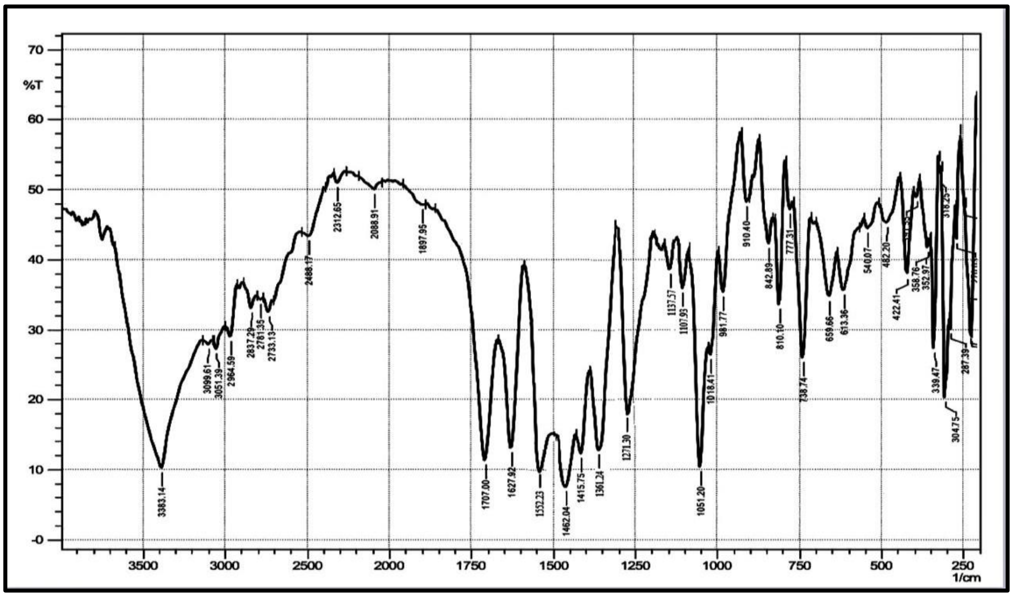


Figure 1: FTIR spectrum of ligand.

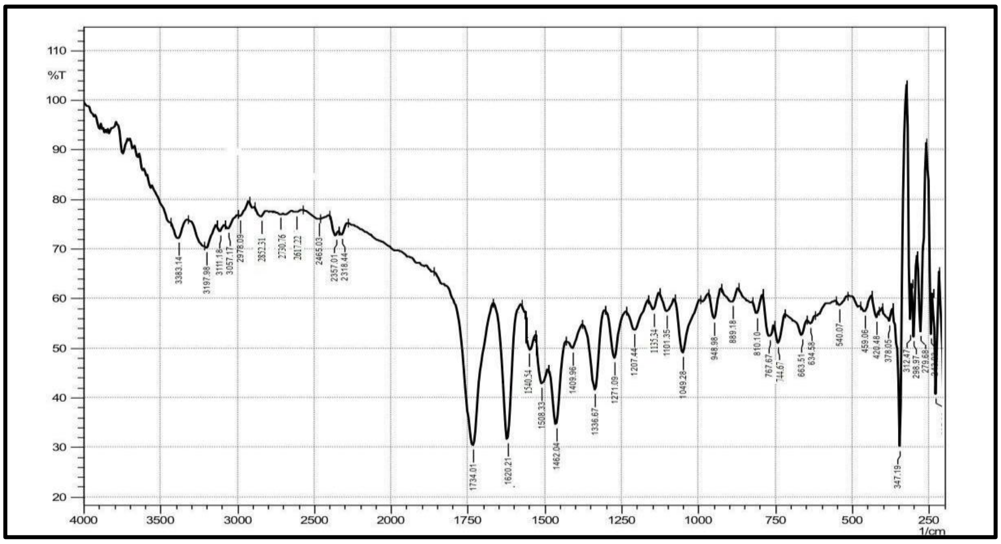
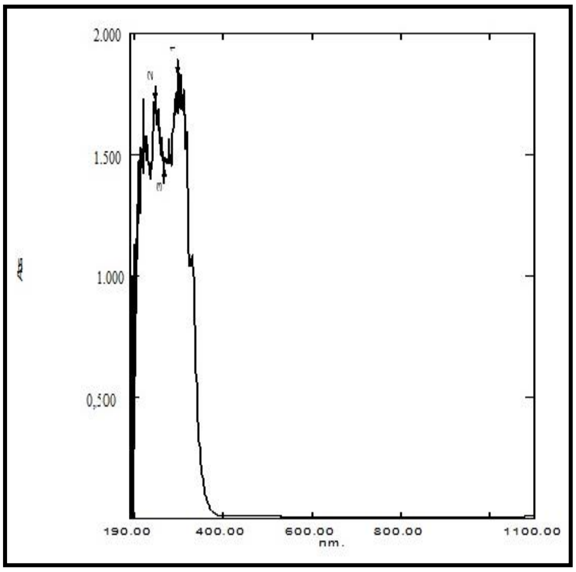


Figure 2: FTIR spectrum of Cr(III) complex.



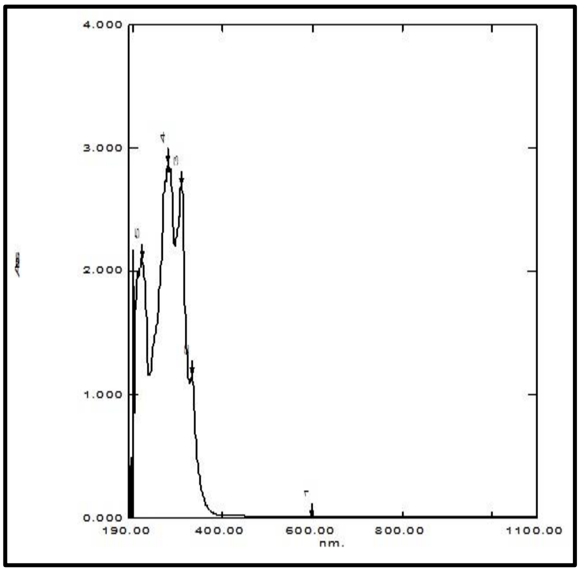


Figure 3: UV-VIS spectrum of L

Figure 4 : UV-VIS spectrum of Cr(III) complex

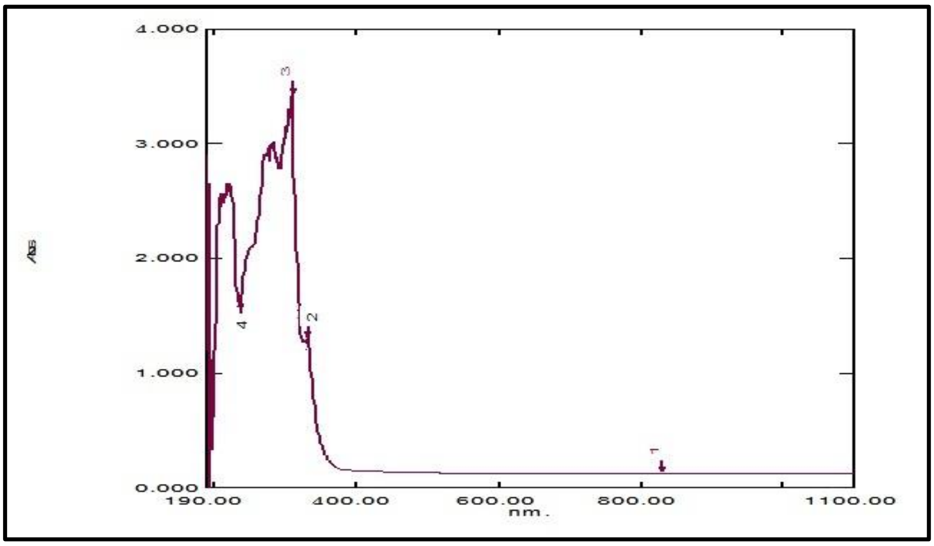


Figure 5: UV-VIS spectrum of Rh(III) complex.

Table 3: Electronic transitions, conductivity, and suggested geometry of metal complexes.

Comp.	Absorption cm^{-1}	Assignments	B°	B'	B	15 B'	10Dq	μ eff B.M	ϵ max $Mol^{-1}.L^{-1}$	Suggested Geometry
L	31545,33444 40485	$n \rightarrow \pi *$ $\pi \rightarrow \pi *$	---	---	---		---	---	---	**---**
Cr(III)	16722 26009(Cal.) 35842 37735	$^4A_2g \rightarrow {}^4T_2g$ $^4A_2g \rightarrow {}^4T_1g_{(F)}$ $^4A_2g \rightarrow {}^4T_2g(p)$ L→Cr CT.	918	779	0.84	11685	16359	3.30	52	**Distorted Octahedral**
Rh(III)	11025 31746 35714	$^1A_1g \rightarrow {}^3T_1g, {}^3T_2g$ $^1A_1g \rightarrow {}^1T_1g$ $^1A_1g \rightarrow {}^1T_1g$	---	---	---	---	---	0.00	95	**Distorted Octahedral**

3.2.3 . Mass Spectroscopy

The mass spectrum is a technique used to calculate the molecular weight of the prepared compounds and determine the fragmentation belongs to the compounds under study. In Figure 6, the mass spectrum of the prepared ligand showed agreement with the proposed structural formula $C_{25}H_{24}FN_5O_3S$. Notably, various bands were recorded for each ligand in their spectrum, and one of them corresponded to the molecular ion, observed at 494.4 m/z for the ligand. Additional distinct peaks revealed in the mass spectra for each ligand were resulting from the successive fragmentation

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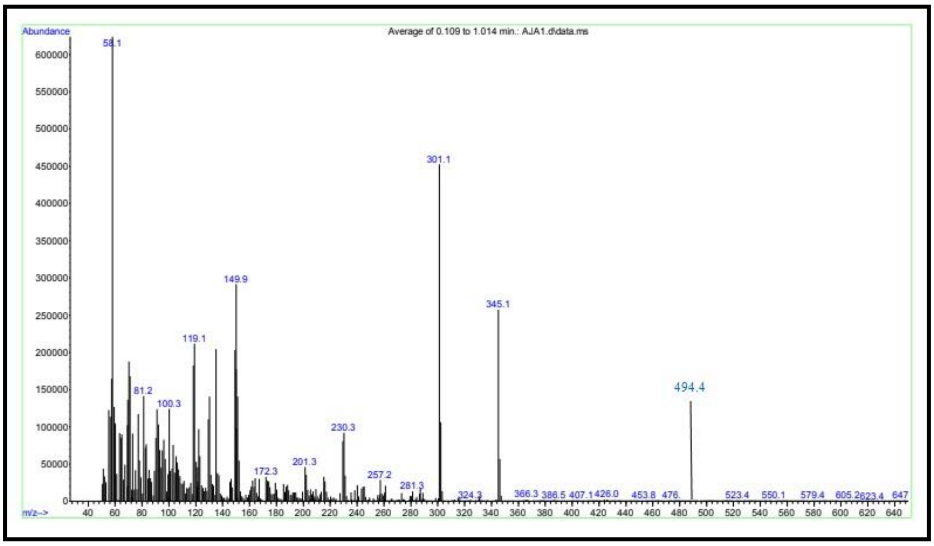

Figure 6: mass spectrum of ligand.

3.2.4.X-Ray Diffraction Spectra.

In this study, XRD data was utilized to not only confirm the formation of different phases but also to calculate the particle size of each specimen. By analyzing the main peaks of each sample, the Debye- Scherrer equation was employed to determine the average particle size [29].

$$\mathbf{D=k\lambda/\beta cos\theta} \qquad …….(2)$$

Where D=crystallite site,k=shape factor =0.9, θ =diffraction angle at maximum peak intensity. β=full width at half maximum of diffraction angle in radians. λ= x-ray wavelength. Certain prominent peaks in X-ray diffraction were investigated and the corresponding d-values were compared to the standard. The X-ray diffraction of a synthetic Cr(III) complex is shown in Figure 7. At 2θ values, the Cr(III) complex displays the X-ray diffraction patterns (11.49, 13.82, 16.15, 17.25, 22.46, 26.70, 28.38, and 32.52°). Figure 8 displays the X-ray diffractogram of the Rh complex. The graph presents a comparison of the index 2 values for each peak. Upon examination, it becomes evident that there is a favorable agreement between the 2θ and d values. The face-centered structure of the Rh complex is specified using the standard JCPDS values (JCPDS No. 5-685) [30], which were used to index the diffraction peaks at 2θ values (15.80, 16.64, 19.50, 25.45, and 27.31°).

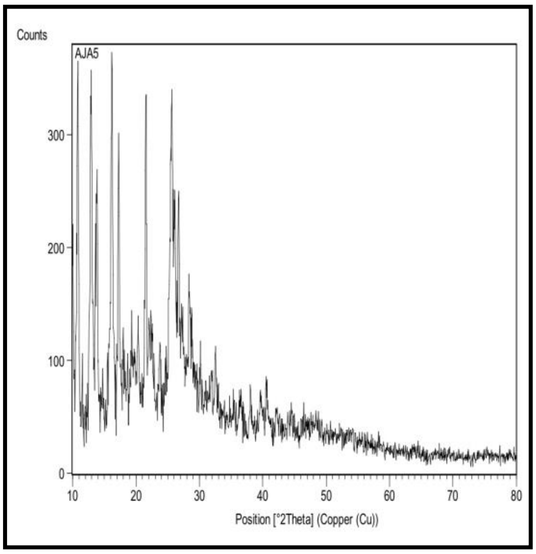

Figure 7: X-ray diffraction of Cr (III) Complex

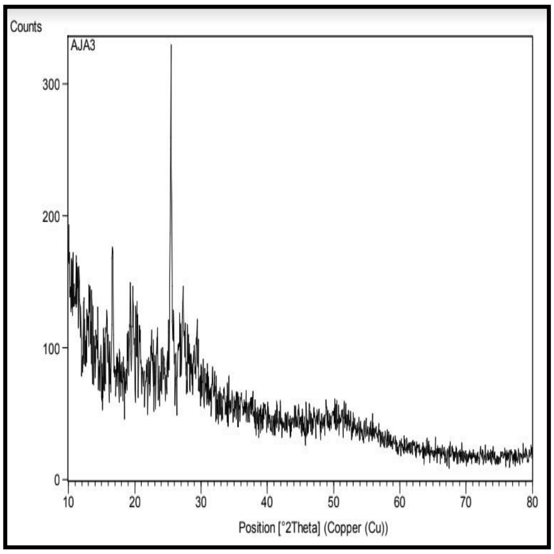

Figure 8: X-ray diffraction of Rh (III) Complex

3.3. Biological Activity

3.3.1. Invitro Anticancer activities

Malignant diseases like cancer are characterized by aberrant cell proliferation. Chemotherapy and surgical intervention are the two most popular treatments for this disease, both of which have negative side effects. Despite their usage in cancer treatment, platinum-based compounds like cis-platin have limited effectiveness. To explore potential alternatives, in vitro experiments were conducted to test chromium and rhodium complexes against cancer MDA-MB-231 cell lines.

Mannich base ligand and their complexes were tested for cytotoxicity in the 50–400 (g/ml) concentration range. Figure 9, which lists the LSD values for each complexes, reveal the results .The chromium(III) complex showed the highest cytotoxicity effect with an LSD value of 7.95, followed by the rhodium(III) complex with an LSD value of 7.88,. The free ligand showed the lower cytotoxicity effect with a value of 7.16. The cytotoxicity tests demonstrated that all the examined complexes exhibited potential against MDA-MB-231 cancer cells. These findings suggest that these complexes hold promise as effective anticancer agents and warrant further investigation and study.

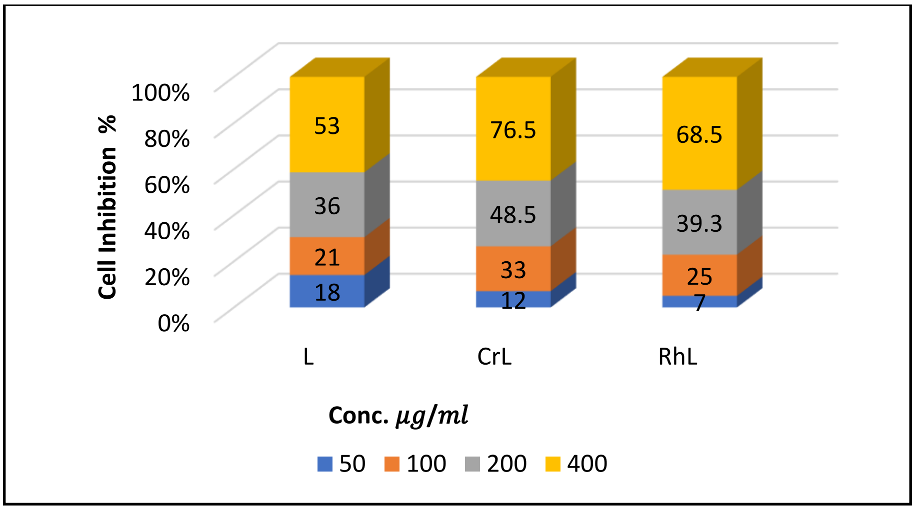

Figure 9: The percentage inhibition in (400,200,100,50 $\mu g/ml$) after exposure to ligand and its complex at 24 hrs.

3.3.2. Antioxidant activity

The use of antioxidants is a simple way to protect biomolecules from oxidative stress. Various assays are available to measure antioxidant capacity *invitro*. DPPH test commonly used for natural and synthetic biologically active compounds. This assay allows a quick comparison of the free radical scavenging abilities of new derivatives. In this study, DPPH scavenging of Mannich base ligands and their complexes was tested and compared with the activity of the parent compound. The results indicate that the scavenging activity of these compounds was dependent on their concentration. Among the compounds tested, the Rh(III) complex showed high activity. The IC50 value of this complex was 63.35 µg/ml, lower than that of the standard compound ascorbic acid (10.21 µg/ml), and the Cr(III) complex exhibited moderate activity. The IC50 value of the conjugate was 97.6 µg/ml, and the ligand showed a mild level of antioxidant activity. Additionally, all compounds exhibited lower activity when compared to the standard ascorbic acid. The IC50 values of all the substances were assessed and compared with the reference standards listed in Figure 10. The graph presented in Figure 10 illustrates the comparison of the antioxidant activity of the compounds with that of ascorbic acid, the established standard.

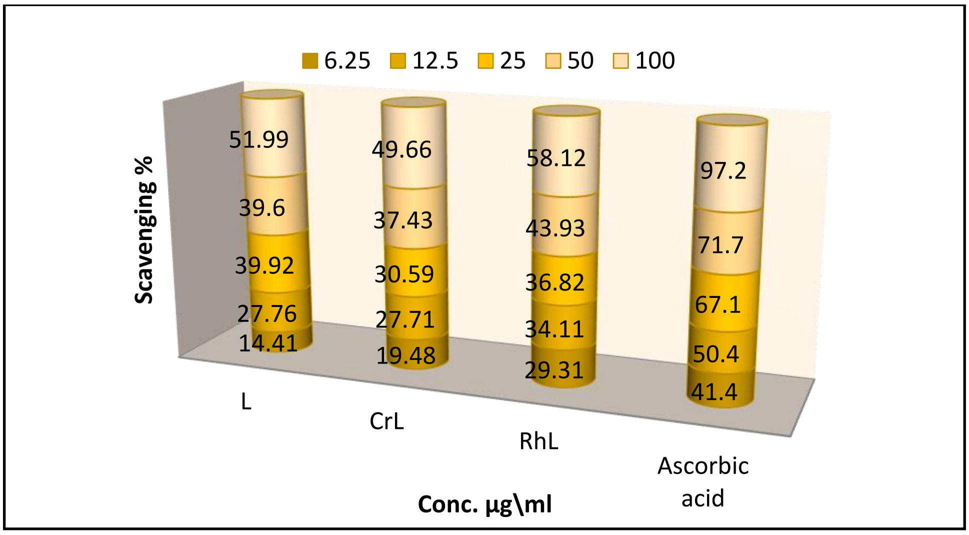

Figure 10: Scavenging activity of ligand and its complexes.

3.3.3. Antimicrobial activity

The *in-vitro* susceptibility of certain strains of bacterium such as *S. aureus*, *E. coli*, and antifungal against one species *candida albicans* toward ciprofloxacin, mannich base ligand and their metal complexes was studied. The investigated compounds, i.e. ligand and their metal complexes, were dissolved (30 µg) in DMF (0.01 ml). The results obtained are shown in Figure 11. Ciprofloxacin has promising antibacterial action in our studies, which may be attributable to its broad spectrum of activity against both gram-negative and gram-positive aerobic bacteria [31-32]. It has been proposed that metal complex intermediates are involved in the mechanism of ciprofloxacin action [33], which is the destruction of the activities of two important enzymes: DNA gyrase, which is essential for DNA replication, and topoisomerase IV, which is a member of the type II topoisomerase family and causes cell death [34]by forming a ternary drug-topoisomerase-DNA complex. Chelation reduces the polarity of the metal ion significantly by causing an overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. As a result, π-electrons become more delocalized over the entire chelate ring, leading to enhanced lipophilicity in the complexes. This increased lipophilicity facilitates the penetration of the complexes into lipid membranes, thereby obstructing the metal binding sites in microorganism enzymes. The complexes further disrupt the respiration process of the cell, consequently inhibiting protein synthesis and restricting the further growth of microorganisms. The chelation of the investigated complex increased the bacteriostatic effectiveness of ciprofloxacin, resulting in interesting bioactivities. At a minimum inhibitory concentration (MIC) of 100 mg/ml, the Rh(III) complex had the greatest significant antibacterial activity against all chosen microbial . This demonstrates that the Rh(III) complex is a powerful antibacterial compound with strong microbial growth inhibition properties. Rh(III) complex were more active than Cr(III) complex, on the other hand. This could be as a result of chromium light and small metal ion sizes. The metal ion's small size could result in less chelation with the ligand and reduced antibacterial action. The activity of metal chelates is significantly influenced by chelate theory. Metal ions are bound to several donor groups during chelation to produce stable complexes. This complex can have unique chemical properties that differ from those of the metal ion or ligand alone. These properties may affect the ability of the complex to interact with its bacterial targets and inhibit their growth. Overall, the activity of metal chelates against microorganisms is a complex process that depends on several factors including oxidation state of metal ions, lipophilicity, and chelation theory. Further research on these factors may aid in the development of new and more effective antimicrobial agents.

Ciprofloxacin and its complexes demonstrate notable antifungal activity, particularly at higher concentrations of 100 µg/ml. The rhodium (III) complex exhibits significant biological activity across all concentrations, while the chromium (III) complex shows a lower inhibition zone when compared to the rhodium (III) complex.

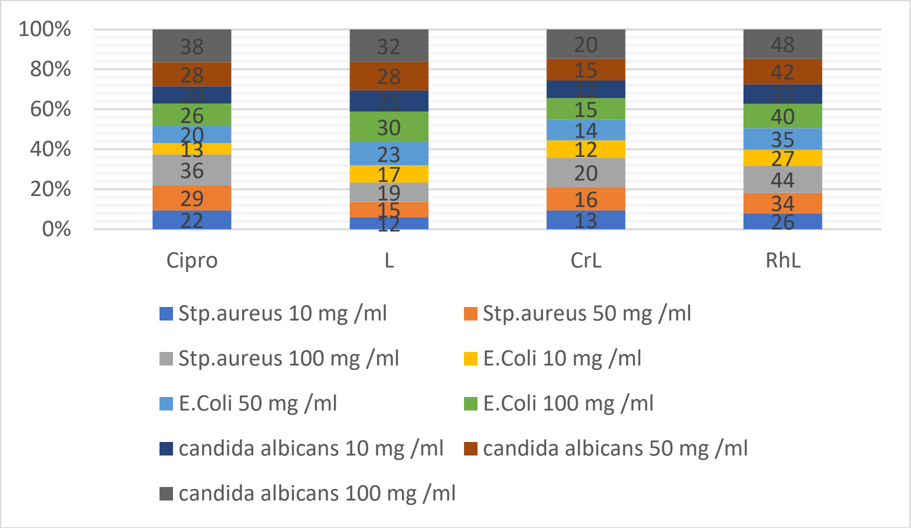

Figure 11: Antimicrobial activity of CIP, L and their metal complexes against three types of selected microorganism.

Conclusion

In conclusion, studies have been conducted on the anticancer, antibacterial, and antioxidant effects of new mannich base ligand and their Cr (III) and Rh (III) complexes. All synthesized metal complexes displayed moderate cytotoxicity against MDA-MB-231 cancer cell lines. According to in vitro biological investigations, the Cr (III) complex exhibited the most promising anticancer efficacy against the MDA-MB-231 cell line, with an LSD value of 7.95. The Rh(III) complex, when compared with standard drug, demonstrated the best biological activity against G^+,G^- bacteria and fungi among all the complexes, demonstrating its promising therapeutic potential. Experimental results showed that the complexes have a significant DPPH scavenging activity and might be effective antioxidants.

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