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Fluorescence study, Raman spectroscopy, crystal structure, antimicrobial and antioxidant activities of cobalt (III) complex with a tridentate coumarin ligand

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Abstract: Cobalt (III) complex diamine chloride was obtained from the reaction of Co $(OAc)_2$, $4H_2O$; ethylene diamine and 3-acetyl-4-hydroxy-chromene- 2-one. The obtained product was characterized by Raman scattering, fluorescence and UV-visible spectroscopy. The room temperature structure was determined by single-crystal X-ray diffraction. The purity of the product was checked by X-ray powder diffraction analysis. Rietveld refinement using powder XRD confirms structure model determined by single-crystal X-ray diffraction (space group Pcca, a = 20.599(2) Å, b = 7.888(4) Å, c = 18.3820(1) Å and Z = 4). The metal complex was checked for its antimicrobial activity using well diffusion method and shows significant results by comparison with commercial antibiotics. Antioxidant tests indicate that the complex is an effective DPPH radical scavenger with an IC50 value of 26.32 µM.

Keywords: X-ray powder diffraction; Fluorescence; Raman spectroscopy; Biological activities; Cobalt complex.

Introduction

During the past two decades, considerable attention has been paid to the chemistry of the metal complexes of Schiff bases containing nitrogen and other donors[1-3]. Schiff bases offer a versatile and flexible series of ligands capable to bind with various metal ions to give complexes with suitable properties for theoretical and/or practical applications. Since the publication of Schiff base complexes, a large number of polydentate Schiff base compounds have been structurally characterized and extensively investigated. This may be attributed to their stability, biological activity and potential applications in many fields such as oxidation catalysis, electrochemistry, etc.; various studies have shown a relationship between the metal ions and their metal complexes as antitumor and antibacterial agents, which is a subject of great interest. The inorganic pharmacology started to be an important field with more inorganic compounds being used in therapy as antibacterial, antiviral and anticancer drugs[4-6].

Recently, there has been increasing interest in the synthesis and characterization of unsymmetrical Schiff base ligands and their metal complexes. This is due partly to the belief that the systematic investigation of these complexes may shed light on the nature of complexes of biological interest[7]. Cobalt was accepted as an essential metal element widely distributed in the biological systems such as cells and body, and thus the interaction of DNA

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with cobalt complex has attracted much attention [8-9]. The biological role of cobalt is mainly focused on its presence in the active center of vitamin B12, which regulates indirectly the synthesis of DNA. Additionally, cobalt is involved in the coenzyme of vitamin B12 used as a supplement of the vitamin[10].

Various cobalt complexes structurally characterized showing antitumor[11], antimicrobial[12], antifungal[13], antiviral[14] and antioxidant[15] activities have been reported. Although the non-substituted parent coumarin (2-oxo-2H-chromene) exhibits zero or very weak fluorescence, the properly substituted derivatives yield an intense fluorescence, and these are widely used in different branches of chemistry, biology, medicine and physics[16].

These derivatives are an important part of fluorescence probes, sensors and switches, as reviewed by Prasanna de Silva[17].

The chelating ability of coumarin derivatives has been studied to suggest their use as chelating agents[18]. One of the most interesting spectroscopic futures of these organometallic complexes is the intramolecular electron transfer which can take place during the excitations[19].

In continuation of our previous research, where we particularly have been interested in the preparation of some cobalt (III) octahedral complexes[20-22] and the study of some coumarin derivatives[23, 24-27], we report in this article, fluorescence study, Raman spectroscopy, crystal structure of cobalt (III) complex with a tridentate coumarin ligand. We also report some of its biological activities.

Experimental Section

All the chemicals were of reagent grade and used without further purification. DPPH: 2, 2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazine. Electronic spectra were recorded in the range 200 - 900 nm on a Varian spectrophotometer 50 scan. The melting point was measured by Kofler Bench. Raman spectroscopy measurement was made using a Horiba HR 800 monochromator. The spectrometer had a wave number resolution better than 3 cm^{-1}, and equipped with a microscope (Olympus BX41), with the Helium ion laser 633 nm emission lines at a power of 15 mW and with a CCD detector.

Fluorescence spectrum was obtained with a SHIMADZU RF-5301 Pc. X-ray powder diffraction patterns were collected on Siemens D 5000 diffractometer using Co Kα radiation (λ = 1.790 Å) at room temperature in the range 8 - 60° (2θ) with the step 0.03°.

Single crystal was characterized by a Bruker Smart Apex CCD diffractometer using graphite monochromated Mo - Kα radiation (λ = 0.71073 Å) at room temperature. The programs used for structure solution and refinement were SHELXS-97and SHELXL-97 respectively[28].

Synthesis of $[Co(C_{13}N_2O_3H_{13})_2]\ ClO_4,\ C_2N_2H_8,\ 2H_2O$

The complex was prepared by refluxing a mixture of the ethylene diamine (0.164 mL, 0.0025 mol) added to a methanolic solution (20 mL) of 3-acetyl-4-hydroxy-chromene- 2-one (0.5 g, 0.0025 mol) for 30 min and the $Co(OAC)_2.4H_2O$ (0.3 g, 0.0012 mol) in 10 mL methanol. The mixture was stirred for 3 h. An aqueous solution of sodium perchlorate was added, the deposited red product was collected by gravity filtration. Single crystals, suitable for X-ray data collection, were obtained by slow evaporation of the CH_3COCH_3 / $C_2N_2H_8$ solution. The crystals were filtered and washed with acetone.

Biological activity of the complex

In vitro antibacterial activity

The complex synthesized was screened for its antibacterial activity by well diffusion method[29] using five bacteria: *Escherichia coli* ATCC10536, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538, *Bacillus cereus* ATCC 11778 and *Listeria innocua* CIP8011T. Microorganisms were provided from the culture collection of the Laboratory of Microbial Technology and Ecology (Institut National des Sciences Appliquées et de Technologie, Tunisia) and UPR- 000263 JE 1 (Ecole Nationale des Ingénieurs en Techniques Agricoles de Bordeaux, France). Each tested microorganism was set up 16h before the assays in order to reach the logarithmic phase of growth. Molten agar (5 mL) containing 0.1 mL of each microorganism suspension was spread over the surface of agar plates containing Muller Hilton Agar and left to solidify. The test compound was prepared at a concentration of 200 µg/mL. Solvent control that is, DMSO was also maintained throughout the experiment simultaneously. Commercially available drug disc, chloramphenicol was used as positive reference standard. Aliquot (100 µL) of the compound was dispensed in wells of uniform diameter (6 mm), and plates were incubated overnight at 37°C. Inhibition of growth was determined by an area of inhibition surrounding each agar well. Growth inhibition was compared with the drug.

Antifungal Activity

Five phytopathogenic fungi were obtained from the culture collection of the National Institute of Agronomic Research of Tunisia (INRAT). Cultures of each fungus were maintained on agar potato dextrose (PDA) and were stored at 4°C in 1 mL of 25% glycerol at 20°C. Fungal species used in this study were: *Fusarium avenaceum*, *Fusarium culmorum*, *Fusarium oxysporum*, *Bipolaris sorokiniana* and *Botritys cinemara*.

A disk of about 5mm in diameter, cut in the periphery of a culture of 7 days, was inoculated into the center of each PDA plate (90 mm diameter) and incubated in the dark at 24°C for 7 days. PDA plates treated with Tween 20 (0.1%) without the synthesized compounds were used as negative control. Complex is dissolved in 1mL DMSO 20 (1% v / v) and added to 20 mL PDA at 50 °C to obtain the final concentration (5 µg / mL). The solution was suitably diluted with sterile water to get the level of concentration of 250 µg / mL.

The antifungal activity was studied by an in vitro test that produces a contact inhibition of hyphal growth[30].

DPPH assay

The method used by Takao, Watanabe, Yagi, and Sakata[31], was adopted with suitable modifications. DPPH (2.4 mg) was dissolved in methanol (100 mL) to obtain a concentration of 24 µg / mL. Serial dilutions were carried out with stock solutions (4 mM) of the complex in methanol to obtain concentrations of 20, 40, 60, 80, 100, 120, 140 µM. Diluted solutions (2 mL each) were mixed with DPPH (2 mL) and allowed to stand for 30 min for any reaction to occur. The absorbance was recorded at 515 nm using the Varian spectrophotometer 50 with scan range in the 200 - 900 nm.

The experiment was performed in triplicate and the average absorbance was noted for each concentration. The IC_{50} value, which is the concentration of the test compound that reduces of the initial free radical concentration, was calculated in µM. Ascorbic acid was used as

reference standards, at the same concentrations in methanol as were used for the tested compounds. Control sample was prepared containing the same volume without test compounds and reference compounds. The radical-scavenging activity of the tested samples, expressed as percentage inhibition of DPPH, was calculated according to the formula:

$$IC(\%) = [(A_o - A_T) / A_0] \, 100$$

Where At is the absorbance value of the tested sample and A_0 is the absorbance value of blank sample, at a particular time. Percentage inhibition after 30 min was plotted against concentration, and the equation for the line was used to obtain the IC50 value. A lower IC_{50} value indicates greater antioxidant activity.

Results and Discussion

The complex $[Co(C_{13}N_2O_3H1_3)_2]ClO_4$, $C_2N_2H_8,2H_2O$ is stable in air and remarkably soluble in DMSO, partially soluble in water and acetone, slightly soluble in ethanol, methanol, ethyl acetate, and chloroform. It decomposes at about 320°C in air by Kofler bench.

Crystal structure

Crystallographic data and details of structure refinement for $[Co(C_{13}N_2O_3H_{13})_2]ClO_4$, $C_2N_2H_8,2H_2O$ are listed in Table 1 and some selected bond lengths and angles are represented in Table 2. An anisotropic refinement by full-matrix least-squares was performed for all atoms, dealing to high values of thermal displacement parameters for C19 and C20 atoms, indicating that they are statistically disordered. The structure of complex is shown in Figure1. It consists of isolated $[Co(III)(C_{13}H_{13}N_2O_3)_2]^+$ complex cation with distorted octahedral geometry, ClO^-_4 counter anion, ethylene diamine solvent and water molecules. The perchlorate anion is present in the lattice to give a charge balance to this cationic complex counterpart. The space group *Pcca* was the best one to describe the symmetry of the crystal.

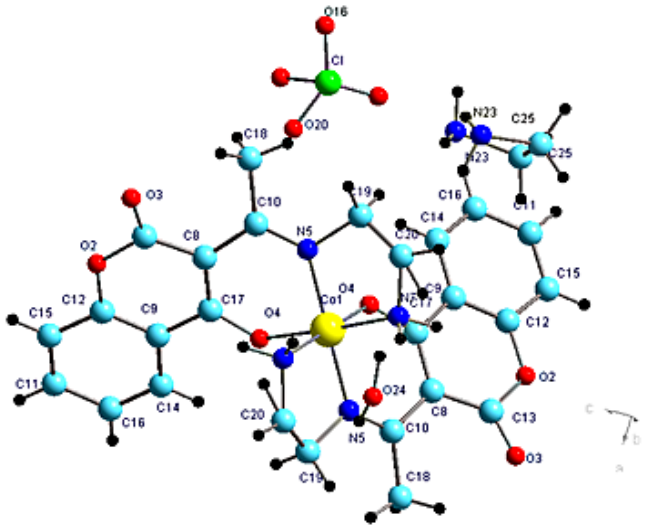

Figure 1. The structure and labeling scheme of complex cation, $[Co(L)_2]^+$, ClO_4^- anion, ethylene diamine solvent and water molecules.

Table 1. Crystal data and structure refinement for cobalt complex.

Empirical formula	$C_{28}Cl\ Co\ H_{38}\ N_6\ O_{12}$
Molecular weight	3279.84 g.mol^{-1}
Diffraction measurement device	Bruker Smart Apex CCD area-detector
Temperature	293 (K)
Wavelength (A°)	0.71073 Å
Crystal system	Orthorhombic
Space group	'P c c a'
a (A°)	20.599(2)
b (A°)	7.888(4)
c (A°)	18.382(1)
Volume (A$^{\circ\ 3)}$	2986.8
Z	4
Dcalc (Mg/m^3)	1.8
F(000)	1552
Crystal size (mm^3)	0.22 *0.12 * 0.06
Absorption coefficient (mm^{-1})	0.742
Theta range for data collection	1.98-28.9°
Index ranges	-23< h <+27, -9 < k <+10, -24< l < +2025 l < +25
Reflections collected number	3905
Reflections number with [I >2 σ(I)]	2407
R_{int}	0.004
Refinement method	full-matrix least-Squares on F^2
Final R indices [I > 2 σ(I)]	R1 = 0.08, wR1 = 0.24
Goodness-of-fit on F^2	1.079

Recently we have published [22] the structure of the same cobalt complex at low temperature (T= 120 K). The two structures mainly differ by solvent contents. The title compound crystallizes with ethylene diamine as solvent molecule in the structure; however, the one studied at low temperature, crystallizes with acetone in the structure. The Co-O (enol) distances [1.900(3) A˚] and the average Co-N (1) [1.918(4) A˚] and Co-N(2) [1.946(4) A˚] are in good agreement with those reported for the structure at 120 K[22] and for similar complexes in the literature[32-34]. The bond angles of the adjacent coordinated atoms to Co(III) atom are all close to 90°; example: O(4)-Co-N(5) [91.43(14)°], N(5)-Co-N(7) [86.18(17)°] and O(4)-Co-O(4) [86.67(19)°]. The values of the N–Co–N, N–Co–O and O–Co–O bond angles are typical for a distorted octahedral coordination environment[35].

The crystal structure cohesion is stabilized by N---H···O hydrogen bonds between neighboring ligands, and O---H···O links between water molecules and the ligands on the one hand and counter-anions on the other. (See Table 3 for the hydrogen bond)

Table 2: Selected bond distances (A˚) and angles (°).

Bond lengths (A°)			
Co - O(4)	1.902(3)	Cl - O(13)	1.201(13)
Co - N(5)	1.911(4)	Cl - O(16)	1.387(13)
Co - N(7)	1.937(4)	C(20) - N(7)	1.414(9)
C(8) - C(17)	1.402(6)	O(2) - C(12)	1.368(6)
C(10) - N(5)	1.291(6)	O(2) - C(13)	1.374(6)
Selected angles (°).			
angles 5 (°)			
O(4) – Co - O(4) O1 C1 C2	86. 77(19)	C(20) - N(7)-Co	109.3(4)
O(4) - Co- N(5)	91.70(15)	C(19) - N(5)-Co	111.3 (3)
O(4) – Co - N(7)	90.59(17)	C(10) - N(5) -Co	128.4(3)
N (7) – Co - N(7)	86.30(17)	N(7) - Co - N(7)	93.0(3)
N(5) – Co - N(7)	86.3(17)	C(12) - C(9) - C(14)	118.6(4)
O(13) - Cl - O(16) - O(16)	125.7(9) 353534(17)	N(7) - C(20) - C(19)	119.4(6)
O(13)-Cl-O(20)	110.3(10)	C(8) - C(17) - C(9)	119.5(4)

Table 3. Hydrogen bonds for the title compound (Å and °).

D-H···*A*	*d*(*D*···*H*)	*d*(*H*···*A*)	*d*(*D*···*A*)	<(DHA)
N7-H8…O2[a]	0.93	2.40	3.186 (5)	141.8
N7-H8….O3[a]	0.93	2.22	3.107 (5)	157
N7-H7….O24	0.74	2.52	3.225(12)	160

[a]1-x,1-y,-z

X-ray powder diffraction structural analysis

The observed, calculated and difference profiles for the Rietveld refinement of [Co ($C_{13}N_2O_3H_{13}$)$_2$] ClO_4, $C_2N_2H_8$, $2H_2O$ complex are shown in Figure 2. X-ray powder diffraction analysis indicates that the complex is a single phase and no diffraction peaks due to impurities of products or raw materials are detected.

Table 4. Summary of the vesting conditions and the results of refinement of X-ray diffraction

Formula	$C_{28}ClCoH_{38}N_6O_{11}$
M_r	3279.84 g.mol^{-1}
Crystal system	Orthorhombic
Space Group	Pcca
a (Å)	20.607(2)
b (Å)	7.8942(4)
c (Å)	18.4200(11)
Z	4
V (Å^3)	2996.4(4)
T (K)	293
Peak shape function	Thompson-Cox-Hastings pseudo-Voigt Axial

	divergence asymmetry
Wavelength radiation	1.7891 / 1.7932 Å (Co Kα1/ CoKα2)
2θ range used for refinement, step width(°)	8 -60, 0.03
Rwp(%)	18.62
Rp(%)	13.70
Rexp(%)	6.18

The model obtained from single-crystal structure determination was used for further Rietveld analysis. Successive refinements show a good agreement between the observed values and calculated ones with *Pcca* space group. In order to do not affect the final molecular shape, we decide to fix all the atomic positions during the Rietveld refinement. Consequently, this led to a significantly less improved profile fit (Rp = 13.70%, Rwp = 18.62%). This situation is typical for home lab powder diffraction data, where the resolution of the experiment is usually not sufficient for calculation of detailed crystal structure of organic compounds. Refinements of the physically sensible isotropic temperature factors were unstable, but better results were obtained by refinement of the overall isotropic displacement factor. Finally, refinement of the preferred orientation correction in the [301]-direction led to a significant decrease of $\chi 2$, and a considerable improvement in the visual quality of the fit. The X-ray powder pattern of this material has been accepted by the editors of the ICDD and will appear in the earliest possible set of the powder diffraction file.

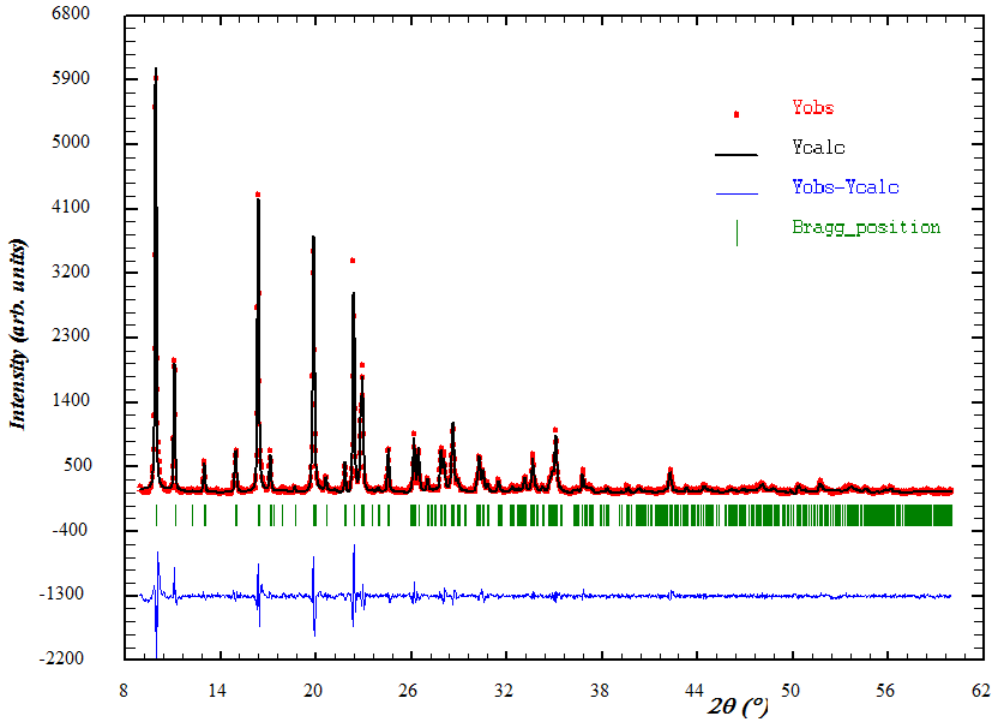

Figure 2. Observed, calculated and difference X-ray diffraction patterns of $[Co(C_{13}N_2O_3H_{13})_2]\ ClO_4,\ C_2N_2H_8,\ 2H_2O$.

Fluorescence measurements

In the difficulty of preparing of the free tridentate ligand, we choose to study the fluorescence proprieties of the cobalt complex by comparison with those of another ligand, dihydroxycoumarine Schiff-base [23], which is a tetradentate (ONNO) ligand.

The fluorescence spectra of the tetradentate (ONNO) ligand and the cobalt complex in acetone solution at room temperature are shown in Figure 3. For the ligand, the spectrum was recorded with an excitation wavelength of 350 nm and exhibits a strong fluorescence emission in the blue-green region (409 nm). The spectrum of the cobalt complex, excited with 387 nm wavelength radiation also, exhibits a broad emission band at 432 nm and a tail extending up to 700 nm.

The emission maximum wavelength of the Schiff base in the complex is red-shifted about 21 nm in comparison with the free ligand. It is evident, from the fluorescence spectra that fluorescence emission intensity of Schiff base decreased dramatically from the free ligand to cobalt complex formation. A similar emission pattern, characteristic of coumarinyl salen cobalt complexes [36], carboxaldehyde Schiff bases cobalt complexes[37] and amino acid Schiff bases zirconium complexes[38] was also recently observed.

The decreased fluorescence intensity with formation of metal complex is due to the decrease in electron density on the Schiff base[39] and it seems that an intramolecular photoinduced electron transfer (PeT) from the coumarin moiety to the cobalt cation is established[36]. The luminescence properties of cobalt complex may be derived primarily from organic ligand.

Here the Co (III) electron/energy transfer interaction with the d–d* electronic transition, results in quenching of fluorescence.

The absorption spectrum of the metal complex shows a band at 346 nm and other wide band around 400 to 600 nm[22] , the fluorescence spectrum is approximately the mirror image of the absorption spectrum but shifted to longer wavelengths 414 to 614 nm. For many of the common fluorophores, the vibrational energy level spacing is similar for the ground and excited states, which results in a fluorescence spectrum that strongly resembles the mirror image of the absorption spectrum. This is due to the fact that the same transitions are most favorable for both absorption and emission.

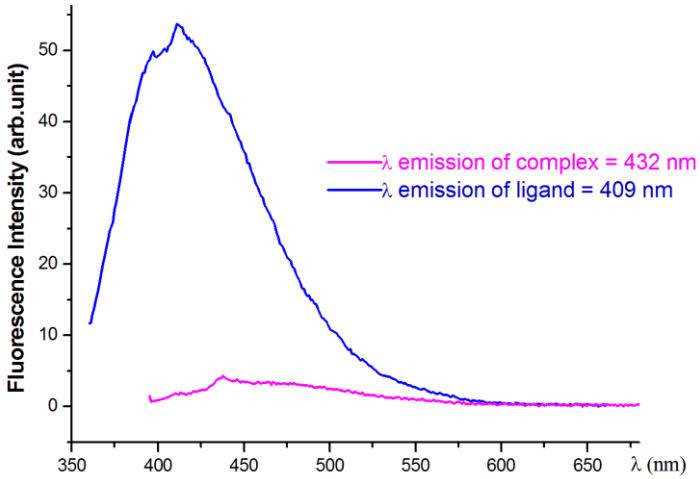

Figure 3: Fluorescence spectra of Schiff base and its metal complex.

Raman Spectroscopy

The Raman spectrum at room temperature (293 K) was used to analyze the different observed bonds of the complex.

The Raman bands associated with the (ν C = N) and (ν C-O) are observed at 1608 cm^{-1} and 1328 cm^{-1} respectively by analogy with the copper complex[40]. The band at 1623 cm^{-1} can be assigned to (ν C = O) (pyrone)[41]. The characteristic vibrational frequency of the perchlorate[42] ClO_4^- is usually observed at about 931 cm^{-1}. Stretching vibrational modes of the ν(NH_2) and CH_2 deformation vibrations of the free ethylene diamine are situated as expected[43] in the high frequency 1581 cm^{-1} and 1212 cm^{-1} respectively, whereas these of the coumarin ethylene diamine ligand are lowered to 1487 cm^{-1} and 1226 cm^{-1} respectively by complexation of the NH_2 groups to cobalt. This observation confirms the structure determined by X ray diffraction suggesting ethylene diamine as a solvent part constituting the structure.

The latter region corresponding to the frequency range 2800 - 3000 cm^{-1} is due to patterns of C-H stretching. The Raman band associated with the (ν Co-O) was located at 530 cm^{-1} by analogy with [44], another vibration stretching (ν Co-N) was located[45] at 300 cm^{-1}.

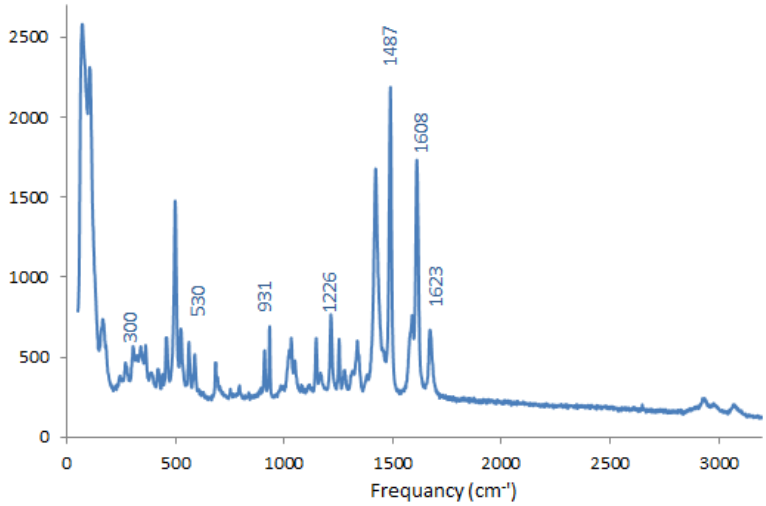

Figure 4. Experimental Raman spectrum of Cobalt (III) complex.

Antimicrobial Discussion

The experimental results show that the complex has the highest activity against *Staphylococcus aureus* (13 mm) and *Bacillus* (14 mm) show 2 mg / mL concentration. This complex has been shown good antifongic activity against *Fusarium oxysporium* and *Fusarium avenareum* by the disc method.

This good antimicrobial activity can be explained by Overtone Concept and chelation theory[46]. The theory states that chelation tends to make the Schiff bases act as bacteriostatic agents more powerful and effective to inhibit the growth of bacteria and fungi. The theory suggested that the polarity of the metal ion is reduced to a greater extent, due to the superposition of the orbital of the ligand and sharing partial positive charge of the metal ion with donor groups. In addition, the π-electron delocalization over the whole chelate ring is increased and the lipophilicity of the complexes is improved. It has been suggested that the ligands with nitrogen donor systems and oxygen inhibit the enzyme activity, since the enzymes which require these groups for their activity seems particularly more sensitive to deactivation by coordination of metal ions.

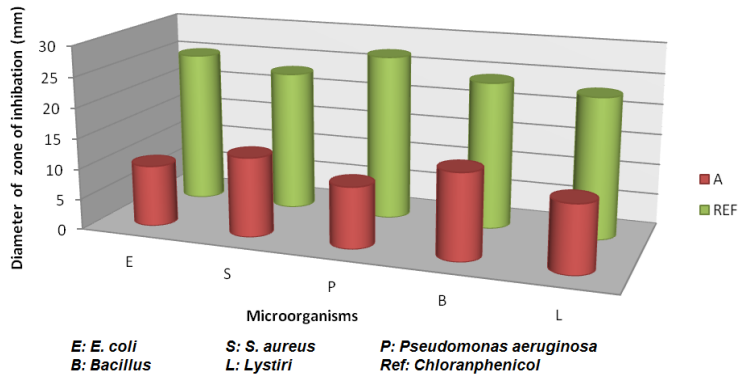

Figure 5: Antibacterial activity spectrum of the complex (A).

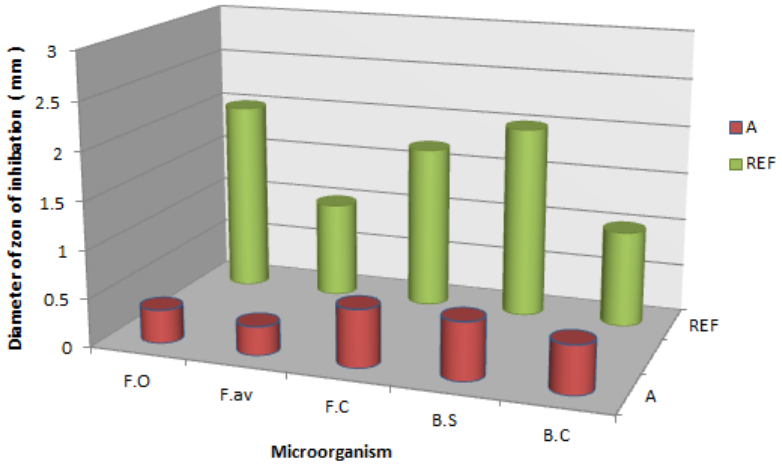

Figure 6. Graphical representation of antifungal data of the complex (A).

Minimum Inhibitory Concentration

The minimum inhibitory concentration was determined using the tube dilution method by preparing different concentrations of metal complex solutions (50 µg / mL, 100 µg / mL, 150 µg / mL, 200 µg / mL)[47]. Cleaned test tubes were taken and different concentrations of metal complex such as 50 µg / mL, 100 µg / mL, 150 µg / mL, 200 µg / mL were prepared and made the volume of medium up to 2 mL with nutrient broth and prepared the control with 2 mL of nutrient broth without any metal complex and sterilized the medium at 121°C temperature at 15 lbs pressure for 15 minutes in autoclave. After sterilization, the medium was allowed to cool and 0.2 mL of overnight cultures of each organism was dispensed into sterile medium and incubated for 24 hours. The activity was measured by turbidity in the broth.

MIC is the lowest concentration of an antimicrobial compound that will inhibit the visible growth of a microorganism after overnight incubation. It was found that the concentration plays a vital role in increasing the degree of inhibition, when the concentration increases, the activity improves.

The effect of complexation on MIC values was determined against Gram-positive bacteria (Table 5). The complex is considered more active against *Bacillus* and *Staphylococcus aureus* with a MIC value 100 µg / mL.

Table 5. Minimum Inhibitory Concentration assay of metal complex (A) against bacterial pathogens

Compound code	Name of bacterial Pathogens	Observation of Growth			
		50µg / mL	100 µg / mL	150 µg / mL	200 µg / mL
A	*Staphylococcus*	+	-	-	-
A	*aureus Bacillus*	+	-	-	-

Measurement of antioxidant activity

DPPH is a stable free radical containing an odd electron in its structure and usually used for detection of the radical scavenging activity in chemical analysis[48]. The DPPH radical is a stable organic free radical with an adsorption band at 517 nm. It loses this adsorption when accepting an electron or a free radical species. The graph (Figure 7) was plotted with percentage scavenging effects on the y-axis and concentration (µg / mL) on the x-axis.

The scavenging ability of the metal complex was compared with ascorbic acid as a standard. The metal complex showed good activities as a radical scavenger compared with ascorbic acid[49,50]. Antioxidant activity against this compound the stable free radical DPPH shows that this species is a good source that could help increase the overall antioxidant capacity of an organism. The IC_{50} of the compound was 26.32 µM, which was raised slightly higher than IC_{50} of ascorbic acid (19.82 µM). The complex showed good antioxidant activity compared to ascorbic acid.

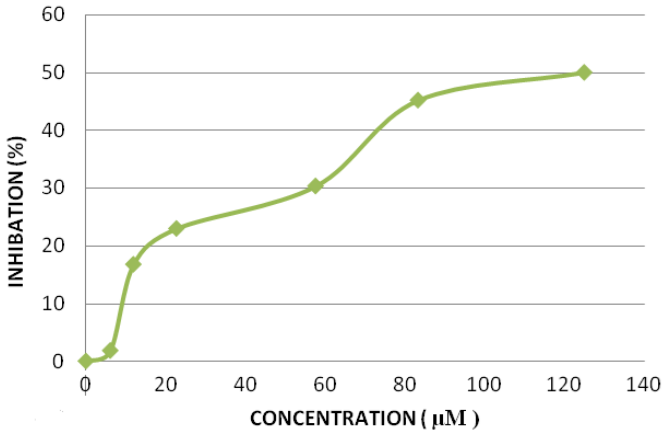

Figure 7. DPPH radical scavenging capacity of the complex.

Conclusion

The synthesis and characterization of a new cobalt (III) complex are described in this document. The room temperature crystal structure analysis showed that the complex has an interesting model; the Co (III) atom is localized between two perpendicular tridentate Schiff bases. Powder X-ray diffraction analysis showed a high purity single phase. Rietveld refinement using powder XRD confirms the structure model determined by single-crystal X-ray diffraction. Metal-ligand coordination leads to significant changes of the fluorescence properties of the ligand, including decrease of the intensity, shift of the emission wavelength. The test results of the antimicrobial compound are used to evaluate the effectiveness of the new complex of the Schiff base Co (III) as antimicrobial agents. The complex was evaluated

in different strains of bacteria such as *Bacillus*, *Pseudomonas aeruginosa* and *S. aureus* by fungi such as *Fusarium oxysporum* and *Fusarium avenareum*.

The antioxidant activity of this compound against DPPH free radicals consistently shows that it can help increase the overall antioxidant capacity of an organism

Acknowledgements

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