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Characterization and Antimicrobial Studies of Synthesized Aluminum Acetylacetonate

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ABSTRACT

Aluminum acetylacetonate was synthesized in an aqueous ammonia solution through the reaction of acetylacetone with aluminum sulfate. Both the ligand and synthesized complex were characterized using infrared and UV-visible spectroscopy. The antimicrobial properties of the complexes were evaluated using the disc diffusion method, and the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) were also determined. The results indicated that the complexes had a high yield of 81.46%, exhibited high melting points ranging from 185 to 207°C, and had low molar conductivity of 13.87 $\Omega^{-1}cm^2mol^{-1}$. The complex was soluble in acetone, ethanol, methanol, dimethyl sulfoxide (DMSO), and dimethylformamide (DMF), but insoluble in hexane and water. In the infrared spectrum, significant changes were observed in the complex compared to the ligand, particularly a shift in the C=O peak from 1584.1 to 1520.8 cm^{-1} after coordination. Acetylacetone exhibited maximum absorption at 329 nm, while the complex showed a shift to 343 nm in its electronic spectra. The complex exhibited strong antimicrobial activity against most microbes, with exceptions including Vancomycin resist enterococci, Klebsiella pneumonia, Helicobacter pylori and Proteus mirabilis. Aluminum acetylacetonate demonstrated superior antimicrobial activity against Methicillin resist Staph aureus, Campylobacter fetus and Pseudomonas aeruginosa when compared to the control drugs (Ciprofloxacin and Fluconazole), highlighting its potential as an effective antimicrobial agent.

Keywords: Characterization, Antimicrobial activity, Aluminum, Acetylacetone and Metal complex.

INTRODUCTION

Acetylacetone is one of the fundamental and widely recognized β-diketones. It functions as a bidentate ligand, capable of bonding to a metal through both of its oxygen atoms (Sodhi and Paul, 2018). Acetylacetone possesses two carbonyl (C=O) groups separated by a single carbon atom, allowing for keto-enol tautomerism (Attia *et al*., 2019). The equilibrium between the enol forms in acetylacetone is influenced by factors such as solvent polarity and the presence of substituents (Matwijczuk *et al*., 2017). This characteristic explains its ability to form stable complexes with

metals. When exposed to a base, acetylacetone transforms into the acetylacetonate anion ($acac^-$) by losing a proton. Three acetylacetone (acac) are however required to bond to a metal center before an octahedral coordination may occur in order for the metal complex $[M(acac)_3]^{n+}$ to form (Ablyasova *et al*., 2023; Sodhi and Paul, 2018).

Metal-acetylacetonate complexes have wide-ranging applications in both scientific research and various industrial sectors. They can serve as starting materials for catalysts and other substances that modify the properties of resulting polymers (Ullah *et al*., 2021). These complexes are also employed in drug production and as additives in cosmetics to mitigate the effects of UV radiation on the skin (Kwon *et al*., 2018). Additionally, they find use in numerous medical applications, including the treatment of ulcers, asthma, diabetes, and lung diseases (Kostova, 2023). Research by Avci-Camur *et al*. (2018) has shown that metal acetylacetonates offer an alternative means of introducing metals into the aqueous synthesis of metal-organic frameworks (MOFs).

Many conventional drugs and antibiotics face resistance from microorganisms, diminishing their effectiveness (Cheesman *et al*., 2017). Several studies have reported that certain medications exhibit enhanced efficacy when administered as metal complexes (Terhemba and Aondoaver, 2021; Yiase *et al*., 2018; Ndagi *et al*., 2017). These researchers have observed that metal complexes formed by combining metal (II) ions with acetylacetone demonstrate potent activity against specific microbial strains. The complexation of metal ions with the free ligand enhances the therapeutic potential of these compounds (Awolope *et al*., 2023).

Information regarding the use of metal acetylacetonates synthesized from acetylacetone and group 3 metals as antibiotics is scarce. Therefore, this study aims to investigate the antimicrobial properties of aluminum (III) acetylacetonate against various microorganisms.

MATERIAL AND METHODS

Materials

Acetylacetone, Ammonia, Aluminum sulphate, Acetone, Ethanol, Methanol, DMSO, DMF and Hexane were purchased from a vendor in Makurdi, Benue State. All the reagents used were of analytical grade and used as received without further purification.

Method

Acetylacetone (3 mL) and 40 mL distilled water were separately measured into a conical flask. 8 mL of dilute (5 mol L^{-1}) ammonia solution was later added. In a separate beaker, aluminum sulphate (3 g) was dissolved in 30 mL of distilled water. These two solutions, the ammoniacal acetylacetone, and the aluminum sulfate, were gradually combined in the conical flask while stirring. The resulting mixture was checked for neutrality, and as it remained acidic, small amounts of ammonia solution were added to achieve neutrality. The flask was placed in a water bath and heated to 30°C until a cream-colored precipitate formed. Afterward, the solution was taken out of the ice bath, allowed to cool, and then filtered using Whatman filter paper. The filtered sample was rinsed with small quantities of cold distilled water, filtered once more, and finally dried in a vacuum desiccator (Moore, 2015). The chemical reaction involved in this process is depicted in Scheme 1.

$$Al^{3+} + 3CH_3COCH_2COCH_3 \rightarrow 3H^+ + Al(CH_3COCHCOCH_3)_3 \quad (1)$$

Melting Point Measurements

Electrothermal melting point apparatus was used to obtain the melting point of the ligand and complex. The samples were placed into individual capillary tubes and introduced into the heating block of the melting point apparatus. The samples were then subjected to heating, and the temperature at which each sample melted was recorded from the digital display.

Solubility Test

2 g of the metal complex weighed into 10 ml of individual solvents (acetone, ethanol, methanol, DMSO, DMF, hexane and water) and allowed to dissolve for 20 mins. The solubility was then noted.

Conductance Measurement

0.001 g of the sample was dissolved in a test tube containing 10 cm^3 dimethyl sulfoxide (DMSO). The conductivity cell of the Conductivity Meter (EC 215) was submerged into each solution, and the recorded results were noted after allowing the readings to reach a stable state.

Infrared and UV-Visible Studies

The infrared (IR) spectra of the samples were obtained using a Scimadzu FT-IR spectrophotometer (8400S), and KBr pellets were used for this purpose. A quantity of 2 mg of the sample was measured with addition of a drop of nujol. The mixture was then ground in a laboratory mortar. The resulting mull was applied to the spectrophotometer cell and scanned within the range of 4000-400 cm^{-1}.

The UV-Visible spectra of the samples were obtained using a UV-Visible spectrophotometer (2500PC Series) was utilized. 2 mg of the sample was measured into 10 mL DMSO. From this solution, 2 mL was withdrawn into the spectrophotometer cell. A matched cell containing only the pure solvent was used as a reference. The cell was placed in the spectrophotometer holder and scanned across the range of 200-800 nm.

Antimicrobial Studies

The antimicrobial properties of the complexes were assessed through the disc diffusion method, as described by Sharma and Chaturvedi (2014). 0.01 mg of the complex were weighed into 10 mL DMSO and allowed to dissolve. To prepare the agar medium, Mueller Hinton agar was sterilized at 120 ºC for 16 min and subsequently transferred into sterile petri dishes, cooled for some time and allowed to solidify. 0.1 mL of the standard test microorganism was added to the sterilized medium. A sterile swab was used to evenly distribute the inoculum on the medium's surface. Then, a standard cork borer with a 6 mm diameter was used to create a well in the center of each agar medium containing the inoculum. Into each well, 0.1 mL of a complex solution, prepared at a concentration of 100 µg/mL, was carefully placed. The medium was then incubated at 37°C for 24 h. Following incubation, the agar plates were examined for zones where microbial growth was inhibited. Transparent ruler was used to gauge the size of the inhibition zones, and the results were recorded in millimeters (mm).

Minimum Inhibition Concentration (MIC)

The minimum inhibitory concentration (MIC) of the complex was determined using the broth dilution method (Yiase *et al*., 2018). To prepare Mueller Hinton broth, 10 mL of the complex was transferred into test tubes. This broth was disinfected at 120 ºC and allowed to cool for 2 min. The test microorganism was introduced into the sterilized broth, and the mixture was incubated for 6 h at 37

ºC. The complex was subjected to serial dilution to create various concentrations (i.e. 100, 50, 25, 12.5 and 6.25 µg/mL). The microorganism being tested was introduced into various concentrations by adding 0.1 mL of normal saline. Subsequently, the samples were placed in an incubator at a temperature of 37°C for 24 hours. The minimum inhibitory concentration (MIC) was identified as the lowest concentration of the compound in the sterile liquid medium that did not show any cloudiness or turbidity at the end of the incubation period.

Minimum Bactericidal Concentration (MBC) / Minimum Fungal Concentration (MFC)

The Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of the compound was also determined. Mueller Hinton agar was first sterilized at 120°C for 16 minutes. Afterward, it was poured into sterile petri dishes and allowed to cool and solidify. The different dilutions of the compound, as tested in the MIC assay, were spread onto the prepared agar medium. After an incubation period of 24 h at 37°C, the agar plates were inspected to see whether there was any colony growth or not. The MBC/MFC values were identified based on the plates that showed no colony growth and contained the lowest concentration of the compound.

RESULT AND DISCUSSION

Physical Characteristics

Table 1 presents the physical characteristics of both acetylacetone and aluminum acetylacetonate. The aluminum acetylacetonate complex was white and displayed a notably high yield of 81.46%. Terhemba and Aondoaver (2021) also reported similarly high yields for Ni(II) and Cu(II) acetylacetonates, at 85% and 82%, respectively. Acetylacetone had a melting point of -19 ºC, whereas aluminum acetylacetonate had a considerably higher melting point ranging from 185 to 207 ºC. This elevated melting point in the complex suggests a strong interaction between the metal and the ligand (Kirthan *et al*., 2020). The molar conductance values for acetylacetone and aluminum acetylacetonate were 10.73 and 13.87 $\Omega^{-1}cm^2mol^{-1}$, respectively. The observation of low molar conductance values in this study indicates that there are fewer ions present, and they have limited dissociation in the solution, as pointed out by AL-Adilee *et al*. (2016). The metal complexes demonstrated solubility in acetone, ethanol, methanol, dimethyl sulfoxide (DMSO), and dimethylformamide (DMF). However, they were found to be insoluble in hexane and water.

Table 1: Physical characteristics of acetylacetone and aluminum acetylacetonate

Compound	Colour	Yield in mole (%)	Melting Point (ºC)	Molar Conductance ($\Omega^{-1}cm^2mol^{-1}$)	Solubility
Acetylacetone	-	-	19	10.73	
$Al(acac)_3$	Milk white	81.46	185-207	13.87	Soluble in acetone, ethanol, methanol, DMSO and DMF Insoluble in hexane and water

Infra-Red Spectral of the Ligand and Metal Complex

Figure 1 displays the IR spectrum of acetylacetone, revealing several distinctive bands. Notably, there

are weak bands observed at 3004.2 and 1416.4 cm^{-1}, which are associated to the stretching mode of the methyl group (-CH^3) (Yiase *et al*., 2018). The out-of-plane bending of the methyl groups is indicated by the band at 1356.8 cm^{-1}. Other bands at 1707.1, 1610.2, and 1509.6 cm^{-1} are likely associated with the C=O group, while those at 1300.8 and 1244.9 cm^{-1} correspond to the C-O group. Additionally, the spectrum exhibits bands at 1155.5 cm^{-1} (corresponding to C-C stretching) and 998.9 cm^{-1} (corresponding to C=C stretching).

Figure 2 illustrates the IR spectrum of aluminum acetylacetonate. Notably, there is an absence of any bands around the 3000 cm^{-1} region in the complex's spectrum. This absence suggests deprotonation and bond formation between the phenolic hydroxyl group of the ligand and the metal ions (Karcz *et al*., 2020). The $Al(acac)_3$ complex displays a distinctive band at 1379.1 cm^{-1}, corresponding to the stretching and out-of-plane bending of H_2O (Mohan *et al*., 2012). Bands at 2996.8, 2922.2, and 2117.1 cm^{-1} in the complex's spectra correspond to the vibrational stretching mode of the methyl group $v(CH_3)$ in the ligand. C=O group in the metal complexes is evident in the peaks at bands around 1584.1 and 1520.8 cm^{-1}. It is apparent that the absorption bands of the carbonyl group (C=O) have shifted to lower frequencies after coordination with Al(III) ions, indicating the presence of a lone-pair resonance in the enol (Sandler *et al*., 2021). This shift suggests bond formation between the C=O group of acetylacetone and the metal ions (Agharia, 2015).

Broad bands observed at 1285.9 and 1013.8 cm^{-1} in the complex's spectra correspond to the C-O group. The C-C and C=C stretching modes are evident in the peak at around 1192.7 cm^{-1} and 931.8 cm^{-1}, respectively. The strong bands observed at 771.6 and 685.8 cm^{-1} in the complex's spectra indicate a robust interaction between Metal-Oxygen in the complex (Abu-Dief *et al*., 2019). This shift in the Metal-Oxygen bond signifies that the coordination of Al (III) ions has occurred at the phenolic oxygen of acetylacetone (El-Sonbati *et al*., 2019).

Table 2: Infra-Red spectral data of acetylacetone and aluminum acetylacetonate

Acetylacetone	$Al(acac)_3$	Possible Functional Groups
3004.2, 2885.0, 2650.1, 2091.0, 1997.9, 1416.4, 1356.8,	2996.8, 2922.2, 2117.1, 1379.1	C-H
1707.1, 1610.2, 1509.6,	1584.1, 1520.8	C=O
1300.8, 1244.9	1285.9, 1013.8	C-O
1155.5	1192.7	C-C
998.9, 954.2, 913.2	931.8	C=C
777.3	771.6, 685.8	M-O

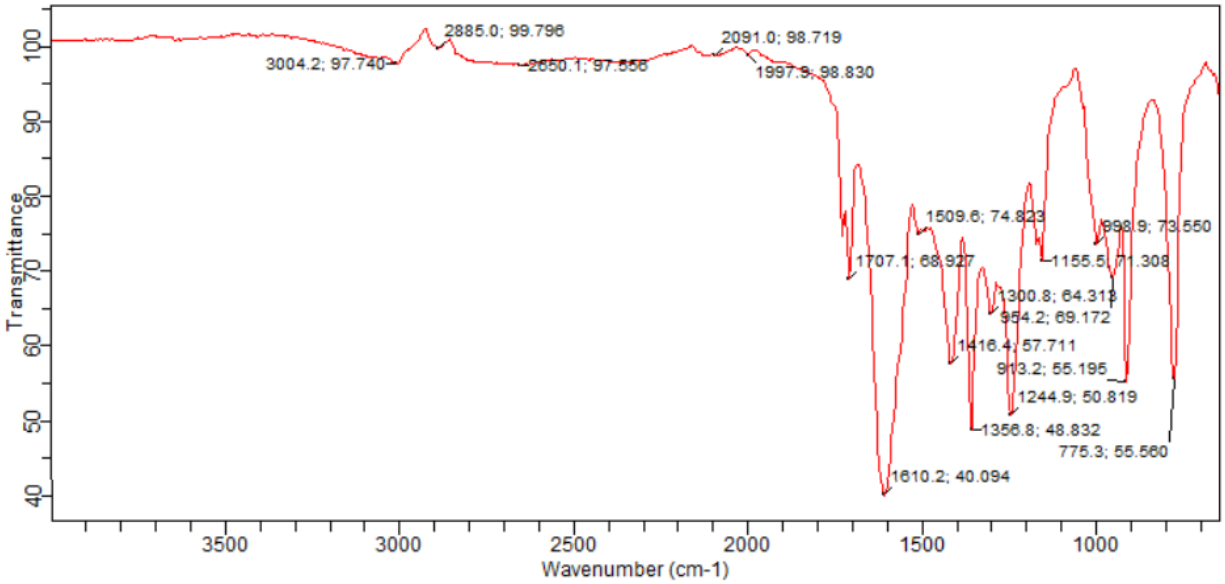

Figure 1: FTIR spectrum of acetylacetone

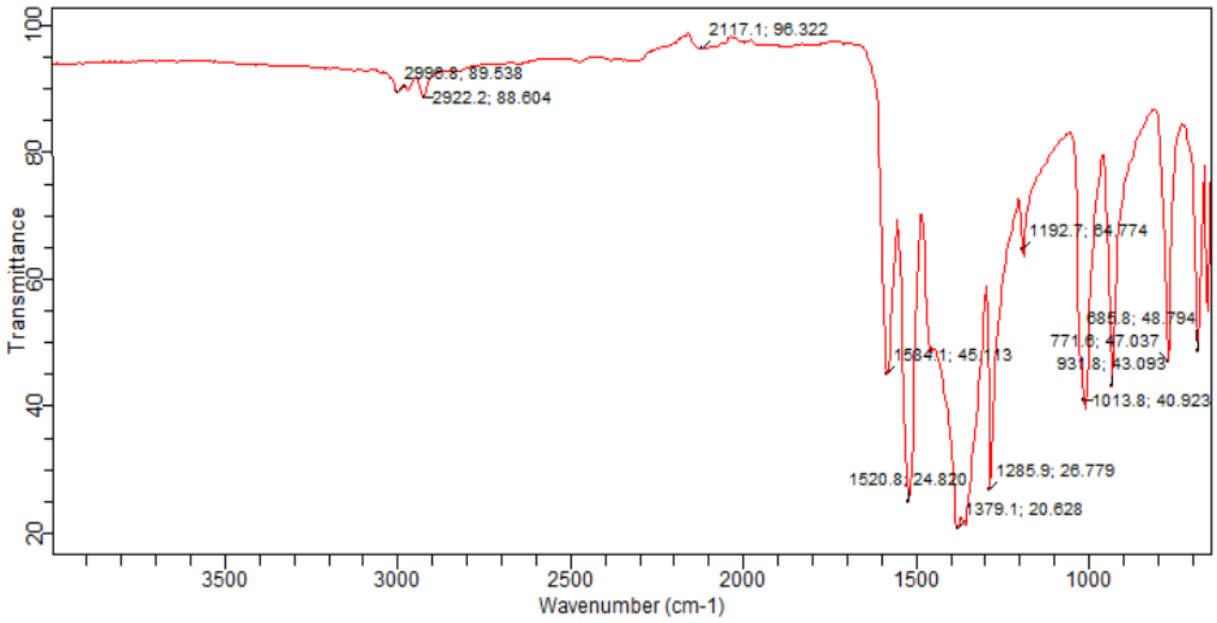

Figure 2: FTIR spectrum of aluminum acetylacetonate

Electronic Absorption Spectra of the Ligand and Metal Complex

The electronic spectra information is presented in Table 3. Acetylacetone exhibited an absorption maximum (λmax) at 329 nm (Figure 3). In the case of the Al(III) complex, this λmax shifted to 343 nm (Figure 4). Such shifts in the λmax absorption towards lower energy levels (bathochromic shift) provide compelling evidence for the interaction between acetylacetone and metal ions (Gubendran *et al*., 2018). Figure 5 shows that proposed structure of the complex.

Table 3: Electronic spectra data of acetylacetone and aluminum acetylacetonate

Compounds	λ (nm)	Assignment	Geometry
Acetylacetone	329	π – π*	-
$Al(acac)_3$	343	π – π*	Octahedral

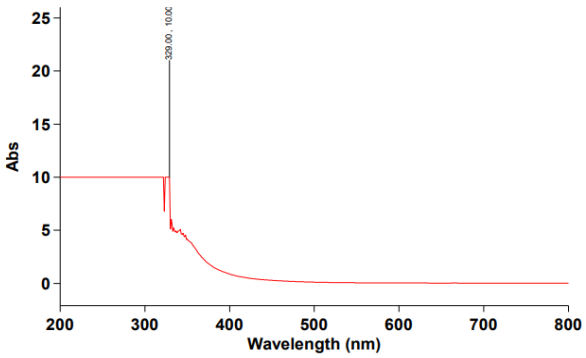

Figure 3: UV-Vis absorption spectrum of acetylacetone

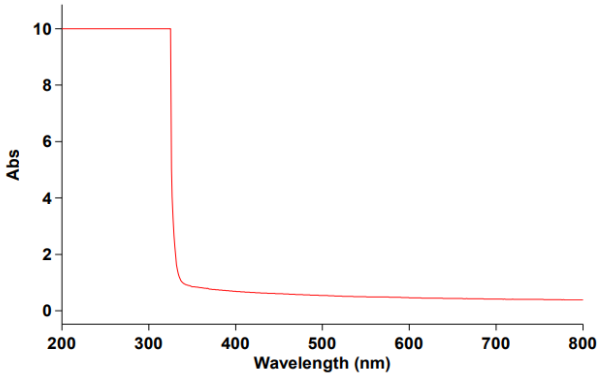

Figure 4: UV-Vis absorption spectrum of aluminum acetylacetonate

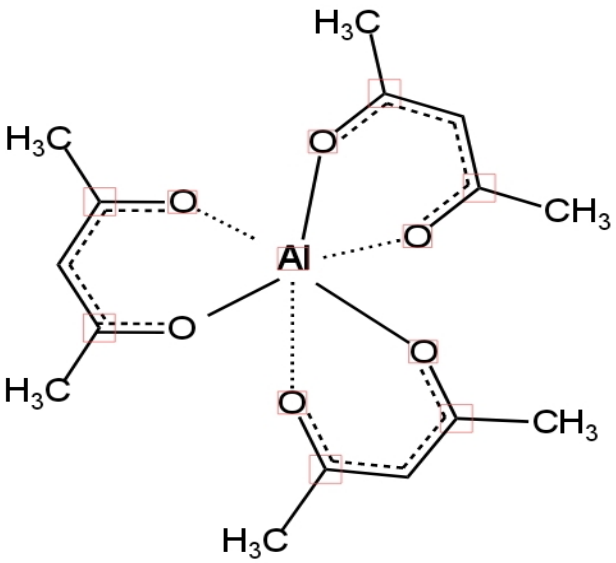

Figure 5. Proposed structure of the complex $[Al(acac)_3]$ - tris(acetylacetonato)aluminium(III)

Antimicrobial Activities of the Complex

The antimicrobial activity of the complex was assessed, and the findings are revealed in Table 4. The activity of the complex was compared to Ciprofloxacin and Fluconazole (control drugs).

The Al(III) complex exhibited activity against several microorganisms, including *Campylobacter fetus, Escherichia coli, Methicillin-resistant Staphylococcus aureus, Pseudomonas aeruginosa, Candida tropicalis, Candida Krusei,* and *Staphylococcus aureus*. However, it did not show activity against *Vancomycin-resistant enterococci, Klebsiella pneumonia, Helicobacter pylori,* and *Proteus mirabilis*. Notably, the complex demonstrated higher activity against *Methicillin-resistant Staphylococcus aureus, Campylobacter fetus,* and *Pseudomonas aeruginosa* when compared to the control drugs Ciprofloxacin and Fluconazole.

The MIC and MBC/MFC values of the synthesized complexes were determined within a concentration range of 6.25 – 100 µg/ml, as shown in Table 5. *Methicillin-resistant Staphylococcus aureus, Escherichia coli, Campylobacter fetus, Pseudomonas aeruginosa* and *Candida tropicalis* required a lower concentration of the $Al(acac)_3$ complex (MIC: 12.5 µg/ml) to inhibit their visible growth. In contrast, higher concentrations (MIC: 25 µg/ml) were needed to prevent the visible growth of *Staphylococcus aureus* and *Candida krusei*. For MBC/MFC values, *Methicillin-resistant Staphylococcus aureus, Escherichia coli, Campylobacter fetus* and *Pseudomonas aeruginosa* required a significantly higher concentration (MBC/MFC: 25 µg/ml) to prevent visible growth, while *Staphylococcus aureus, Candida tropicalis* and *Candida krusei* necessitated a higher concentration (MBC/MFC: 50 µg/ml) for the same purpose. The MBC/MFC values doubled the MIC values for the $Al(acac)_3$ complex, except for Candida tropicalis, where the concentration required to prevent visible growth was four times the MIC value (MIC: 12.5 µg/ml, MBC/MFC: 50 µg/ml). These low values indicate the high efficacy of the complex (Yeganegi *et al*., 2018).

Table 4: Zone of inhibition of aluminum acetylacetonate on organisms

Test Organisms	$Al(acac)_3$	Ciprofloxacin	Fluconazole
Methicillin resist Staph aureus	29	0	0
Vancomycin resist enterococci	0	0	0
Staphylococcus aureus	26	35	0
Escherichia coli	30	37	0
Klebsiella pneumonia	0	0	0
Helicobacter pylori	0	31	0
Campylobacter fetus	31	0	0
Proteus mirabilis	0	30	0
Pseudomonas aeruginosa	29	0	0
Candida tropicalis	28	0	32
Candida krusei	27	0	30

Table 5: Minimum Inhibitory Concentration, Minimum Bactericidal/Fungicidal Concentration of aluminum acetylacetonate on organisms

	MIC					MBC/MFC				
Test Organisms	**100µg/ml**	**50µg/ml**	**25µg/ml**	**12.5µg/ml**	**6.25µg/ml**	**100µg/ml**	**50µg/ml**	**25µg/ml**	**12.5µg/ml**	**6.25µg/ml**
Methicillin Resist Staph aureus	-	-	-	0*	+	-	-	0*	+	++
Vancomycin resist enterococci										
Staphylococcus aureus	-	-	0*	+	++	-	0*	+	++	+++
Escherichia coli	-	-	-	0*	+	-	-	0*	+	++
Klebsiella pneumonia										
Helicobacter pylori										
Campylobacter fetus	-	-	-	0*	+	-	-	0*	+	++
Proteus mirabilis										
Pseudomonas aeruginosa	-	-	-	0*	+	-	-	0*	+	++
Candida tropicalis	-	-	-	0*	+	-	0*	+	++	+++
Candida krusei	-	-	0*	+	++	-	0*	+	++	+++

KEY: - = No turbidity (no growth), 0* =MIC, += turbid (light growth), ++ = moderate turbidity, +++ = high turbidity.

CONCLUSION

Aluminum acetylacetonate was synthesized from acetylacetone and aluminum salt. The prepared complex was characterized by various physico-chemical methods as well as Infra-red and UV-visible spectroscopy. The complex was prepared in good yield and was soluble in majority of solvents. Higher melting points and lower molar conductance value were observed for the complex than the ligand indicating a strong metal-ligand interaction in the structure of the complex. Significant changes in the spectrum of the metal complex was observed showing that coordination of the Al (III) ion to the ligand took place. The complex showed good antimicrobial activity on most microbes except *Vancomycin resist enterococci, Klebsiella pneumonia, Helicobacter pylori* and *Proteus mirabilis.* When compared to the control drugs (Ciprofloxacin and Fluconazole), aluminum acetylacetonate can serve as a better antibiotic for *Methicillin resist Staph aureus, Campylobacter fetus* and *Pseudomonas aeruginosa.*

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