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Synthesis, Characterization and Antimicrobial Studies of Magnesium and Calcium Acetylacetonate Complexes

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ABSTRACT

Metal complexes have been synthesized in recent years and used as drugs for treatment purposes. In this work, acetylacetonate complexes of some Group 2 metals (magnesium and calcium) were synthesized from their salts. The complexes were characterized by solubility, melting temperature, molar conductance measurements, Infra-red and UV-visible spectroscopy. The antimicrobial activities were also evaluated using disc diffusion method. The results showed that the complexes had good yield (74.65% and 84.72%), high melting points (225 °C and 252 °C) and low molar conductance (13.48 Ω^{-1} cm²mol⁻¹ and 12.62 Ω^{-1} cm²mol⁻¹) for Mg(II) and Ca(II) complexes respectively. IR spectral result showed that the absorption bands of the carbonyl group after coordination with Mg(II) and Ca(II) ions were lowered to 1669.8 cm⁻¹, 1595.3 cm⁻¹, 1513.3 cm⁻¹; and 1796.6 cm⁻¹, which suggests reduction in bond order of C=O after chelation. The strong band observed for the ligand (329 nm) increased to 356 nm and 341 nm for Mg(II) and Ca(II) complexes respectively. Complexation of the ligand with Mg(II) and Ca(II) ions enhanced its activity as both Mg(II) and Ca(II) complexes demonstrated significant activity against 6 test organisms. It can be concluded that the complexes can act as potential antimicrobial agents and serve as precursors for chemical reactions.

Keywords: Acetylacetone, Antimicrobial activity, Characterization and Metal(II) complexes.

INTRODUCTION

Complexes serve as chemotherapeutic agents and have been synthesized in recent years due to their ability in the reduction of resistance to organisms. Certain drugs developed for the treatment of cancer, ulcers, tumors, fungal, and bacterial infections have proven to be effective but have effects on the health of users [1]. This in turn facilitated the search for substances with high selectivity for damaged cells without affecting neighbouring cells [2, 3].

Acetylacetone, the simplest β -diketone, have attracted significant interest from the fields of organic, inorganic, and physical chemistry [4, 5] due to their ability to form stable complexes

arising from their enol form [6]. Acetylacetonate complexes have been reported to be important in the treatment of cancer, ulcers, tumors, fungal, and bacterial infections [8, 9].

Previous studies have focused on the synthesis of acetylacetone with transition metals. Terhemba and Aondoaver prepared Ni(II) and Cu(II) chelates of acetylacetone and observed that the metal chelates demonstrated better activities against the tested pathogens than the free ligand due to increase in permeability of the metal chelates through the microbe's cell [10]. Yiase *et al.* prepared Mn(II) and Co(II) complexes of acetylacetone. In their work, it was reported that the antimicrobial agent of the ligand was enhanced on complexation with Mn(II) and Co(II) ions [11].

The current study aims to synthesize magnesium and calcium acetylacetonates, characterize the complexes and test for their antimicrobial activities. This study is significant because it provides a better understanding of the importance of acetylacetonates of Group 2 metals.

Acetylacetone compounds complexing with Group 2 metals have not received much attention. Group 2 metal acetylacetonates exhibit some unique properties and have various applications in different fields. Their stability, volatility, and reactivity make them attractive for a wide range of industrial and research applications [11]. The complexation of these complexes with drug molecules can alter their physicochemical properties, leading to improved therapeutic efficacy [1, 10, 11]

EXPERIMENTAL

Synthesis of Metal(II) Complexes

Synthesis of bis(acetylacetonato)magnesium(II)

The methods of Yiase *et al* [11], and Sarah [12] were adopted for the preparation of the metal(II) acetylacetonates. Acetylacetone (3 mL) and 40 mL distilled water were separately measured into a conical flask. Exactly, 8 mL of dilute (5 mol L^{-1}) ammonia solution was later added. Magnesium hydroxide (3 g) was dissolved in 30 mL distilled water contained in a beaker. The ammoniacalacetylacetone solution and magnesium hydroxide solution were then mixed in the conical flask gradually while stirring. The resulting solution was then checked for neutrality. Small portions of ammonia solution were added to the solution since it was still acidic to make it neutral. The flask was then placed in a water bath. This was allowed to heat at 30 °C until the formation of a cream-coloured precipitate was observed. The solution in the flask was removed

from the ice bath, allowed to cool and filtered using Whatman filter paper. It was later washed with small amounts of cold distilled water, filtered again and dried in a vacuum desiccator.

The equation for the reaction is given in Scheme 1:

$$Mg^{2+} + 2CH_3COCH_2COCH_3 \rightarrow 2H^+ + Mg(CH_3COCHCOCH_3)_2$$
(1)

Synthesis of bis(acetylacetonato)calcium(II)

The same procedure described in Scheme 1 was adopted for the synthesis of bis(acetylacetonato)calcium(II). This was carried out using calcium hydroxide (3 g) as the metal salt. The equation for the reaction is given in Scheme 2:

$$Ca^{2+} + 2CH_3COCH_2COCH_3 \rightarrow 2H^+ + Ca(CH_3COCHCOCH_3)_2$$
(2)

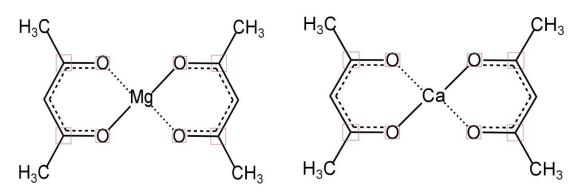


Figure 1: Structure of bis(acetylacetonato) magnesium(II)

Figure 2: Structure of bis(acetylacetonato) calcium(II)

Characterization of the Ligand and Metal Complexes

Melting Point Measurements

The melting point of the ligand and the complexes was obtained with an electrothermal melting point apparatus. Acetylacetone with the metal complexes were put in separate capillary tubes and inserted into the heating block of the melting point apparatus and heated separately. The temperature at which each of the samples melted was read from the digital screen.

Solubility Test

The solubility of the ligand and metal complexes were observed by dissolving 2 g of the metal complexes in 10 ml of the individual solvents.

Conductance Measurement

The molar conductance of the metal complexes was measured using a Conductivity meter (EC 215) [13]. This was carried out in DMSO by dissolving 0.001 g of each sample in 10 cm³ of DMSO in a test-tube, the conductivity cell was immersed into each solution, and the result was recorded after allowing the reading to stabilize.

Infrared and UV-Visible Studies

The Infrared spectra of the ligand and prepared complexes were recorded as Nujol mulls using KBr pellets with Scimadzu FT-IR spectrophotometer (8400S). Exactly 2 mg of the complex was measured. A drop of nujol was later added to the complex and ground in a laboratory mortar. The obtained mull was suspended on the spectrophotometer cell and scanned between 4000-400 cm⁻¹[11, 14]. The UV-Visible spectra of the ligand and prepared complexes were recorded in solution state in a 200 to 800 nm wavelength range with a UV-Visible spectrophotometer (2500PC Series). Exactly 2 mg of the complex measured and dissolved in 10 mL of DMSO. 2 mL of the resulting solution was withdrawn into the spectrophotometer cell. The reference cell used was the matched cell containing pure solvent. The cells were then placed in the spectrophotometer holder and scanned between 200-800 nm [11].

Antimicrobial Studies

The antimicrobial activities of the complexes were determined using disc diffusion method [15]. Pathogenic microbes were collected from the Department of Medical Microbiology at ABU Teaching Hospital in Zaria, Nigeria. To reach a concentration of 100 g/ml, 0.01 mg of the complex was weighed and dissolved in 10 mL of DMSO. This was the complex's initial concentration, which was utilized to test its antibacterial activity. Mueller Hinton agar was utilized as a growing medium for the microorganisms. The medium was sterilized at 121 °C for 15 min, put onto sterile petri dishes, and allowed to cool and solidify according to the manufacturer's instructions. The sterilized medium was seeded with 0.1 mL of the standard inoculum of the test microbe and the inoculum was spread evenly over the surface of the medium by the used of sterile swab. A standard cork borer of 6 mm in diameter was used to cut a well at the centre of each inoculated medium. About 0.1 mL of solution of the complex of 100 μ g/ml concentration was then introduced into the well on the inoculated medium. Incubation was made

at 37 °C for 24 h, after which the plates of the medium were observed for the zone of inhibition of growth. The zone was measured with a transparent ruler and the result recorded in millimetre.

Minimum Inhibition Concentration (MIC)

The minimum inhibition concentration of the complex was determined using the broth dilution method [16]. Mueller Hinton broth was made by transferring 10 mL of material into test tubes. It was sterilized at 121 °Cfor 15 min before the broths were allowed to cool. Dispensing 10 ml of the material into a sterile test tube yielded normal saline. The test microorganism was injected and incubated for 6 h at 37 °C. The complex was serially diluted twice in sterile broth to generate concentrations of 100 μ g/ml, 50 μ g/ml, 25 μ g/ml, 12.5 μ g/ml and 6.25 μ g/ml. Dissolving 0.01 mg of the compound in 10 ml of sterile broth yielded the starting concentration. The test microbe was inoculated into the varied doses with 0.1 ml of normal saline and incubated at 37 °C for 24 hours. The minimal inhibitory concentration was determined to be the lowest concentration of the complex in the sterile broth that showed no turbidity.

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

MBC/MFC assays were performed to evaluate if the test microorganisms were killed or just hindered in their development. Mueller Hinton agar was sterilized at 121 °C for 15 min before being placed onto sterile petri dishes and allowed to cool and solidify. Following that, the contents of the MIC in the successive dilutions were subcultured onto the prepared medium. After 24 hours of incubation at 37 °C, the medium plates were examined for colony growth. MBC/MFC were deduced from plates with the lowest concentration of the compound with no colony growth.

RESULTS AND DISCUSSION

Physical Characteristics of the Ligand and Metal Complexes

The physical characteristics of acetylacetone and the synthesized metal complexes are shown in Table 1. The complexes were white and powdery in nature. Their percentage yields were high i.e. 74.65% for [Mg(acac)₂] and 84.72% for [Ca(acac)₂]. The melting point of acetylacetone ligand was -19 °C. [Mg(acac)₂] and [Ca(acac)₂] had higher melting points which were 225 and 252 °C, respectively. The high melting points displayed by the complexes suggested strong metal-ligand bonds [17, 18]. The molar conductance value of acetylacetone ligand was 10.73 Ω^{-1}

¹cm²mol⁻¹. Molar conductance values of 13.48 and 12.62 Ω^{-1} cm²mol⁻¹ were determined for [Mg(acac)₂] and [Ca(acac)₂], respectively. The conductivity values were low for all the complexes and thus indicate that they are non-electrolytic in nature [19]. The metal complexes were completely soluble in acetone, ethanol, methanol and DMSO, slightly soluble in DMF and insoluble in hexane and water (Table 2).

Compound	Molecular Formular	Molecular Weight (gmol ⁻¹)	Colour	Yield in mole (%)	Melting Point (°C)	Molar Conductance $(\Omega^{-1} \text{cm}^2 \text{mol}^{-1})$
Acetylacetone	$C_5H_8O_2$	100	-	-	19	10.73
Mg(acac) ₂	$Mg[C_5H_7O_2]_2$	222	White	74.65	255	13.48
Ca(acac) ₂	$Ca[C_5H_7O_2]_2$	238	Milk white	84.72	272	12.62

Table 1: Physical Characteristics of the Ligand and Metal Complexes

Table 2: Solubility Test of the Metal Complexes

Compounds	Acetone	Ethanol	Methanol	Hexane	DMSO	DMF	Water
Mg(acac) ₂	S	S	S	IS	S	SS	IS
$Ca(acac)_2$	S	S	S	IS	S	SS	IS

Keys: S = Soluble, SS = Slightly Soluble, and IS = Insoluble

Infra-Red Spectral of the Ligand and Metal Complexes

The Infra-red spectral of acetylacetone, magnesium acetylacetonate and calcium acetylacetonate complexes are shown in Table 3. The spectrum of acetylacetone shows weak bands at 3004.2 cm⁻¹ and 1416.4 cm⁻¹ (Figure 3) which is due to symmetric stretching mode of the methyl group, v (CH₃), for keto and enol form [10]. The band at 1356.8 cm⁻¹ is attributed to the out of plane bending (wagging) of the methyl groups, ω (CH₃) for both keto and enol forms. The characteristics absorption at 2885.0 cm⁻¹, 2650.1 cm⁻¹, 2091.0 cm⁻¹, 1997.9 cm⁻¹; 1707.1 cm⁻¹, 1610.2 cm⁻¹, 1509.6 cm⁻¹ and 1300.8 cm⁻¹, 1244.9 cm⁻¹ can be assigned to the C=N, C=O and C-O groups, respectively. The vibrational bands at 954.2 cm⁻¹, 913.2 cm⁻¹ and 777.3 cm⁻¹ are attributed to the out of plane bending (wagging) of the ω (C-H) bond of the methyl groups for

both keto and enol forms. C-C and C=C stretching was observed in the peak at bands around 1155.5 cm^{-1} and 998.9 cm⁻¹ respectively. The bands at 2885.0 cm⁻¹ and 954.2 cm⁻¹ clearly show the existence of keto and enol forms of acetylacetone at equilibrium [10, 11].

Mg(acac)₂ complex showed a broad band at ~3697.5 cm⁻¹, 3391.9 cm⁻¹, 3280.1 cm⁻¹, 3078.8 cm⁻¹ and a new band at ~1401.5 cm⁻¹ (Figure 4); Ca(acac)₂ complex showed a broad band at ~3641.6 cm⁻¹ and a new band at ~1405.2 cm⁻¹ (Figure 5) that may be assigned to the stretching vibration and out of plane bending vibration of water molecules [20]. The bands observed at 2996.8 cm⁻¹, 2922.2 cm⁻¹, 2117.1 cm⁻¹ ;2988.3 cm⁻¹, 2922.2 cm⁻¹, 2117.1 cm⁻¹, 1956.9 cm⁻¹ and 2512.2 cm⁻¹, 1468.6 cm⁻¹ in the spectra of the metal complexes are assigned to vibration stretching mode of methyl group, v(CH₃), in the ligand. The presence of a carbonyl group (C=O) in the metal complexes were observed in the peak at bands around 1669.8 cm⁻¹, 1595.3 cm⁻¹, 1513.3 cm⁻¹; and 1796.6 cm⁻¹, respectively. Broad band around 1259.8 cm⁻¹, 1017.6 cm⁻¹; and 1200.2 cm⁻¹, 1013.8 cm⁻¹, on the spectra of [Mg(acac)₂] and [Ca(acac)₂] complexes respectively may be assigned a C-O group (ether or ester functional group).

C=C stretching was observed in the peak around 928.1 cm⁻¹, 875.9 cm⁻¹; 916.9 cm⁻¹, 872.2 cm⁻¹ on the spectra of $[Mg(acac)_2]$ and $[Ca(acac)_2]$ complexes respectively. The strong band observed at 764.1 cm⁻¹, 663.5 cm⁻¹ and 764.1 cm⁻¹, 711.9 cm⁻¹ in the spectra of the metal complexes showed that there is a strong interaction between Metal-Oxygen in the complex [21].

Acetylacetone	[Mg(acac) ₂]	[Ca(acac) ₂]	Possible Functional Groups
3004.2, 1416.4, 1356.8	3697.5, 3391.9, 3280.1, 3078.8, 1401.5	3641.6, 1405.2	О-Н
2885.0, 2650.1, 2091.0, 1997.9	2988.3, 2922.2, 2117.1, 1956.9	2512.2, 1468.6	С-Н
1707.1, 1610.2, 1509.6,	1669.8, 1595.3, 1513.3	1796.6	C=O
1300.8, 1244.9	1259.8, 1017.6	1200.2, 1013.8	C-0
1155.5	_	_	C-C
998.9, 954.2, 913.2	928.1, 875.9	916.9, 872.2	C=C
777.3	764.1, 663.5	764.1, 711.9	M-O

Table 3: Infra-Red Spectral Data of the Ligand and Metal Complexes

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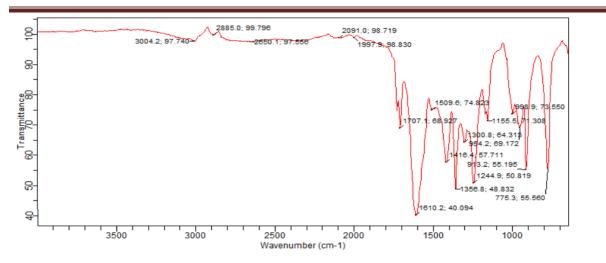


Figure 3: FTIR Spectrum of Acetylacetone ligand

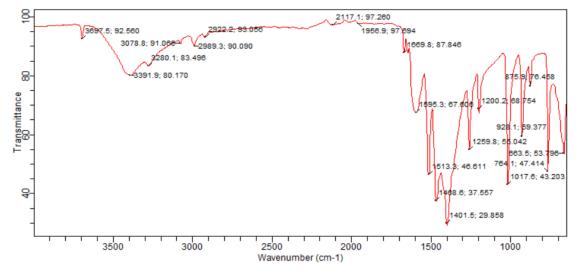


Figure 4: FTIR spectrum of magnesium acetylacetonate complex

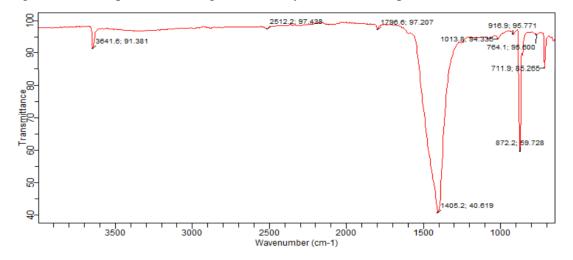


Figure 5: FTIR spectrum of calcium acetylacetonate complex

Electronic Absorption Spectra of the Ligand and Metal Complexes

The electronic absorption spectra of the ligand and synthesized complexes in DMSO were recorded from 200 to 800 nm at room temperature (Figure 6, 7 and 8). The electronic spectra data is shown in Table 4. A strong band was observed for acetylacetone ligand at 329 nm (Figure 6). Bands at 356 and 341 nm were also observed for $[Mg(acac)_2]$ and $[Ca(acac)_2]$ complexes, respectively. are consistent with the tetrahedral geometry (Figure 7 and 8). These bands were attributed to $\pi \rightarrow \pi^*$ electronic transition in the framework of the synthesized complexes. The modifications of shifts and intensity of the absorption bands indicates the coordination of the ligand to the metal ion [22].

Table 4: Electronic	Spectra Data	of the Ligand	and Metal	Complexes
	1	0		1

Compounds	λ (nm)	Assignment	Geometry
Acetylacetone	329	$\pi - \pi^*$	-
[Mg(acac) ₂]	356	$\pi - \pi^*$	Tetrahedral
[Ca(acac) ₂]	341	$\pi - \pi^*$	Tetrahedral

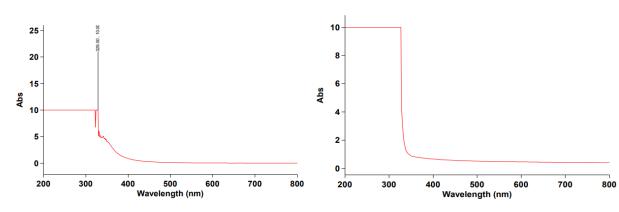
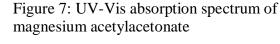
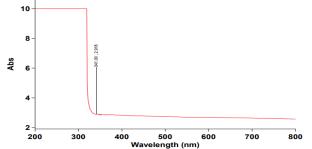
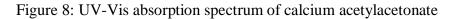


Figure 6: UV-Vis absorption spectrum of acetylacetone







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Antimicrobial Activities of the Metal Complexes

Zones of Inhibition

Results of the zones of inhibition measured are presented in Table 5. Among the metal complexes synthesized, [Ca(acac)₂] complex had the most antimicrobial activity against *Staphylococcus aureus* (30 mm), *Klebsiella pneumonia* (31 mm) and *Candida tropicalis* (30 mm). Mg(acac)₂ complex had the most antimicrobial activity against *Vancomycin resist enterococci* (29 mm). [Mg(acac)₂] complexes showed higher antimicrobial activity against *Candida krusei* (27 mm). The findings from the antimicrobial screening revealed that the activity of the complexes against some organisms were higher when compared to the control drugs used (Ciprofloxacin and Fluconazole), and therefore will be effective towards the organisms. This activity may be due to chelation of the ligand with the metal ion [23].

The metal complexes showed activity against some test organisms while no activity was recorded against some organisms. [Ca(acac)₂] complex was active against six (6) test organisms and showed no activity against *Methicillin resist Staph aureus, Vancomycin resist enterococci, Campylobacter fetus, Proteus mirabilis* and *Candida krusei*. [Mg(acac)₂] complex was active against six (6) test organisms and showed no activity against *Staphylococcus aureus, Escherichia coli, Campylobacter fetus, Proteus mirabilis* and *Pseudomonas aeruginosa*.

Minimum Inhibitory Concentration, Minimum Bactericidal/Fungicidal Concentration

The result of the Minimum Inhibitory Concentration (MIC), Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of the complexes on the test microorganisms are given in Table 6 and 7 respectively. The MIC, MBC/MFC of the complexes synthesized were studied in the concentration range of $6.25 - 100 \mu g/ml$.

For the [Ca(acac)₂] complex, *Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Candida tropicalis* required a lower concentration of Ca(acac)₂ complex (MIC: 12.5 µg/ml) to prevent their visible growth; while the growth of *Escherichia coli* and *Helicobacter pylori* can be prevented when low concentration (MIC: 25µg/ml) is required. *Staphylococcus aureus, Klebsiella pneumonia* and *Candida tropicalis* required significantly higher concentration of Ca(acac)₂ complex (MBC/MFC: 25 µg/ml) to prevent their visible growth. *Escherichia coli, Helicobacter pylori* and *Pseudomonas aeruginosa* required higher concentration (MBC/MFC: 50 µg/ml) to prevent their visible growth.

For the [Mg(acac)₂] complex, the visible growth of *Vancomycin resist enterococci* and *Helicobacter pylori* can be prevented when low concentration of the complex is required (MIC: 12.5µg/ml). *Methicillin Resist Staph aureus, Klebsiella pneumonia, Candida tropicalis* and *Candida krusei* required low concentration of the complex (MIC: 25 µg/ml) to prevent their visible growth. *Vancomycin resist enterococci* and *Helicobacter pylori* required significantly higher concentration of Mg(acac)₂ complex (MBC/MFC: 25µg/ml) to prevent their visible growth. *Methicillin resist Staph aureus, Klebsiella pneumonia, Candida tropicalis* and *Candida krusei* required higher concentration (MBC/MFC: 50µg/ml) to prevent their visible growth. The MIC values were lower than the MBC/MFC values and indicate that the complexes could be bactericidal or fungal in action.

Test Organisms	$[Ca(acac)_2]$	[Mg(acac) ₂]	Ciprofloxacin	Fluconazole
Methicillin resist Staph aureus	0	25	0	0
Vancomycin resist enterococci	0	29	0	0
Staphylococcus aureus	30	0	35	0
Escherichia coli	27	0	37	0
Klebsiella pneumonia	31	27	0	0
Helicobacter pylori	24	30	31	0
Campylobacter fetus	0	0	0	0
Proteus mirabilis	0	0	30	0
Pseudomonas aeruginosa	28	0	0	0
Candida tropicalis	30	25	0	32
Candida krusei	0	27	0	30

Table 5: Zone of Inhibition of	the Metal	Complexes	against the	Test Microorganisms

Table 6: Minimum Inhibitory Concentration of the Complexes against the Test Microorganisms

	$[Ca(acac)_2]$	$[Mg(acac)_2]$
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* + ++
Vancomycin resist enterococci		0* +
Staphylococcus aureus	0* +	
Escherichia coli	0* + ++	
Klebsiella pneumonia	0* +	0* + ++
Helicobacter pylori	0* + ++	0* +

Campylobacter fetus		
Proteus mirabilis		
Pseudomonas aeruginosa	0* +	
Candida tropicalis	0* +	0* + ++
Candida krusei		0* + ++

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

Table 7: Minimum Bactericidal/Fungicidal Concentration of the Complexes against the Test Microorganisms

	$[Ca(acac)_2]$	$[Mg(acac)_2]$
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* + ++ +++
Vancomycin resist enterococci		0* + ++
Staphylococcus aureus	0* + ++	
Escherichia coli	- 0* + ++ +++	
Klebsiella pneumonia	0* + ++	- 0* + ++ +++
Helicobacter pylori	- 0* + ++ +++	0* + ++
Campylobacter fetus		
Proteus mirabilis		
Pseudomonas aeruginosa	- 0* + ++ +++	
Candida tropicalis	0* + ++	- 0* + ++ +++
Candida krusei		- 0* + ++ +++

KEY: - =No Colony Growth, 0* =MBC/MFC, + =Scanty colonies growth, ++ =Moderate colonies growth, +++ =Heavy colonies growth.

CONCLUSION

The Mg(II) and Ca(II)complexes were synthesized from acetylacetone and corresponding metal salts (Mg(II) and Ca(II) ions). The complexes were found to have good yield, high melting points, low molar conductance (non-electrolytic in nature). The complexes were soluble in most of the solvents with the exception of hexane and water. The IR and electronic spectra data revealed that complexation shifted the bands in the spectra. The complexes revealed diverse activity against various organisms. The study proved that Ca(II)complexes had the highest antimicrobial properties. From the results, it can be concluded that the complexes are potential raw materials for synthesis of antibiotics which can be used for various medicinal applications.

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