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Riboflavin metal complexes

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ABSTARCT

This paper presents the synthesis and characterization of organic metal complexes containing Riboflavin with Cadmium and Zinc metals. Solid crystalline complexes have been studied their structure by using FT-IR and LC-MS spectra. Antimicrobial screening of gram (+ve), gram negative (-ve) and fungi for Riboflavin-Zinc complex also reported.

Keywords: Antimicrobial screening, Riboflavin (RF), Metal complexes.

INTRODUCTION

Riboflavin is a heat stable, photo labile and water soluble vitamin (B2) that functions as a prosthetic group of flavoproteins. It facilitates the transfer of electrons and hydrogen atoms towards the reduction of oxygen. Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) are the cofactor forms of RF which plays an important role for a wide range of metabolic reactions involving oxidases and dehydrogenases. The Riboflavin molecule is well investigated in photochemistry and biochemical fields and still it draws attention till to date[1-3].

Since the announcement of a tumor-inhibiting action by riboflavin[4] and later by related isoalloxazincs, little has been done to explain this activity. A number of metal salts and chelates has also been reported to be inhibitory to tumor growth which suggested the possibility that metal chelates of riboflavin might be involved in tumor inhibition or possibly in tumor growth. To test this idea, a number of metal chelates of riboflavin and that isoalloxazine reported[5] to be most active in tumor inhibition were prepared.

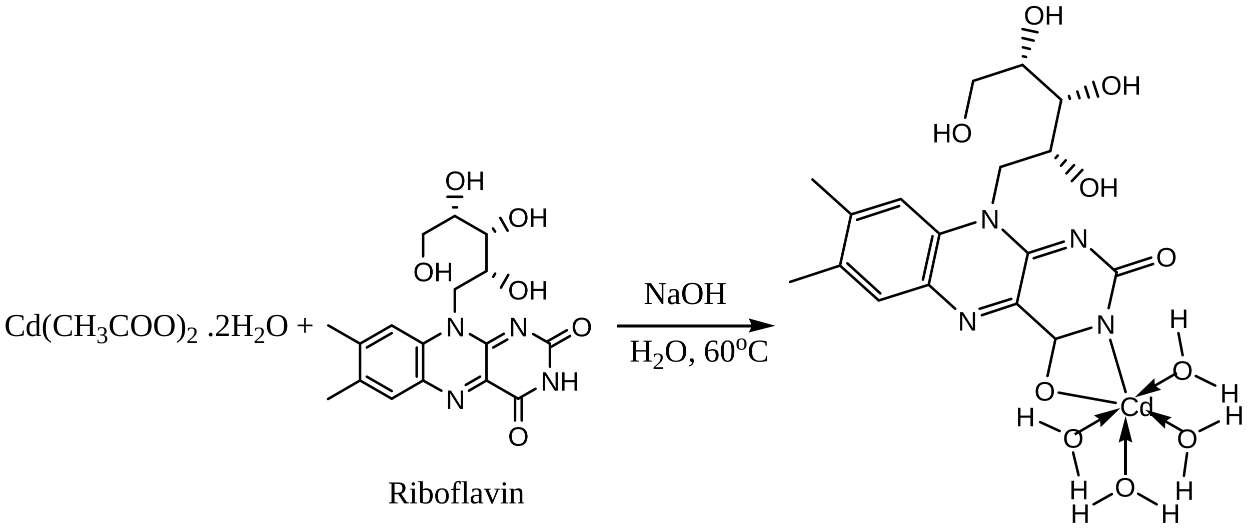
MATERIALS AND METHODS

A: REAGENTS AND INSTRUMENTS:
Riboflavin and Imidazole were purchased from molychem. All solutions and metal salts used for synthesis of metal complexes were A.R. grade and used as received for synthetic work. Elemental analysis was obtained using a FLASH EA 1112 SERIES CHNS analyzer. IR spectra were obtained with a Shimadzu FT-IR 8000 spectrometer. LC-MS Spectra were recorded on AGILANT QQQ (ESI-MS) mass spectrometer. The proposed molecular structure of the complexes were determined by using chem office 2004, 3DX prog .

B. SYNTHETIC PROCEDURE:

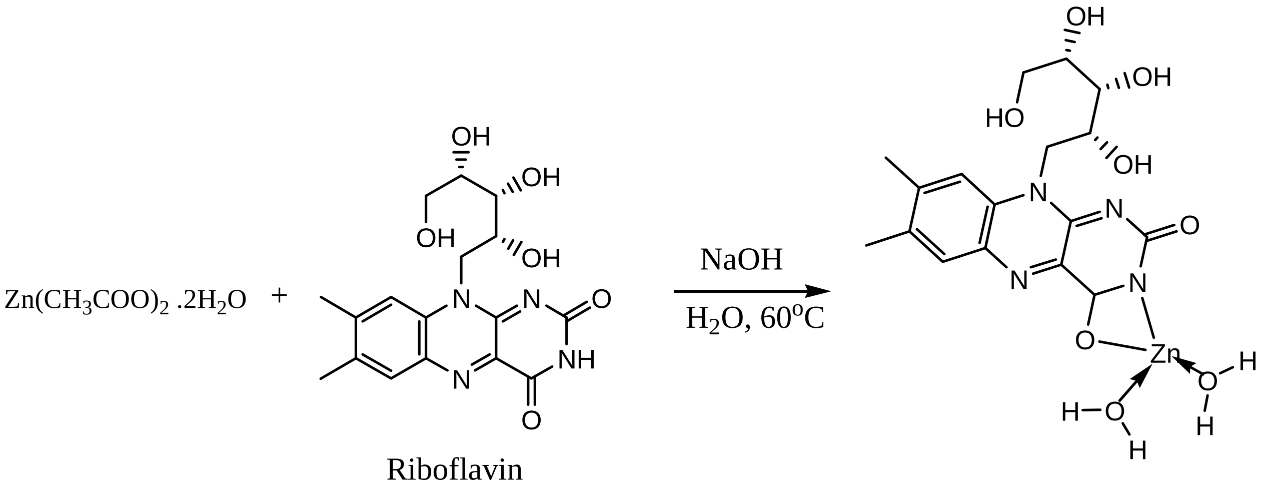
Synthesis of Cd(L)$(H_2O)_4$ (1):

A basic solution (5 ml) of riboflavin (0.188g, 0.5 mmol) in NaOH is added to an aqueous solution(5ml) of cadmium acetate dihydrate (0.113g, 0.5 mmol) under stirring conditions which resulted in orange coloured solution with some turbidity. After constant stirring at 60°C for 30 minutes, the product is filtered off. The reddish orange precipitate is collected and washed with ethanol. Yield 0.085 g (45 %). Anal. exptal. $C_{17}H_{28}N_4CdO_{10}$ (M.Wt.: 567) C, 36.41; H, 5.03; N, 9.99 Found: C, 36.21; H, 4.81; N, 9.15. Important IR absorptions (KBr disk, cm^{-1}): 3465, 2920, 1675, 1598, 1461, 1179, 1019, 848, 466. Mass peaks (m/z): 487, 507, 527, 567.



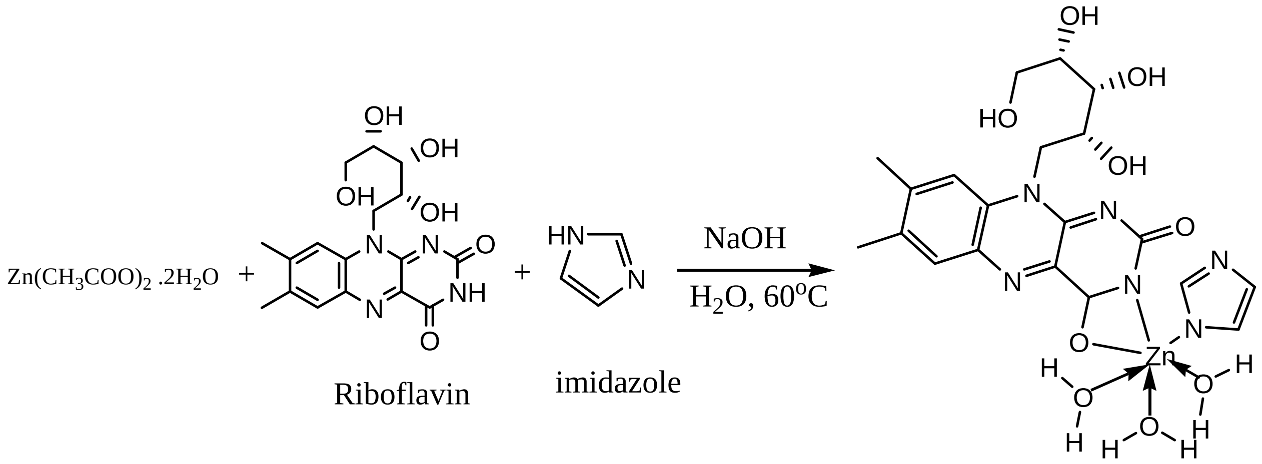
Synthesis of Zn(L)$(H_2O)_2$ (2):

A basic solution (5 ml) of riboflavin (0.188g, 0.5 mmol) in NaOH is added to an aqueous solution (5ml) of zinc acetate dihydrate (0.219g, 1.0 mmol) under stirring conditions which resulted in orange solution with some turbidity. After constant stirring at 60°C for 30 minutes, the product is filtered off. The pale orange precipitate is collected and washed with ethanol. Yield 0.11 g (50 %). Anal. exptal. $C_{17}H_{24}N_4ZnO_8$ (M.Wt.: 477.78) C, 42.74; H, 5.06; N, 11.73 Found: C, 42.12; H, 4.34; N, 11.36. Important IR absorptions (KBr disk, cm^{-1}): 3418, 2923, 1660, 1628, 1549, 1532, 1466, 1407, 904, 906, 693, 609. Mass peaks (m/z): 377, 441, 477.



Synthesis of Zn(L)(imd)(H_2O_3) (3):

A basic solution (5 ml) of riboflavin (0.187g, 1.0 mmol) in NaOH is added to an aqueous solution (5ml) of zinc acetate dihydrate (0.219g, 1.0 mmol) under stirring conditions and then aqueous solution (5 ml) of imidazole (0.068 g, 1.0 mmol) is added which resulted into orange coloured solution with some turbidity. After constant stirring at 60°C for 30 minutes, the product is filtered off. The bright orange coloured precipitate is collected and washed with ethanol. Yield 0.132 g (60.2 %). Anal. exptal. $C_{20}H_{29}N_6ZnO_9$ (M.Wt.: 561.13) C, 42.68; H, 5.19; N, 14.93 Found: C, 42.21; H, 5.11; N, 14.02. Important IR absorptions (KBr disk, cm^{-1}): 3247, 2041, 1545, 1495, 1439, 1170, 976, 962, 757, 717, 484. Mass peaks (m/z): 437, 505, 525, 561.



RESULTS AND DISCUSSION

IR Spectra:
The IR spectrum of complex 1 confirms the coordination of riboflavin to the Cd(II) metal. The IR spectra of [Cd(L)(H_2O)$_4$] contains a strong broad band observed at 3465 cm^{-1} which may be attributed to *v*(OH) of the coordinated water[6] molecules. RF–Cd also exhibits bands at 1675, 1598 and 1461 cm^{-1} that are characteristic stretching modes C=O (amide carbonyl), C=N (conjugated system) and C–N, respectively. The IR spectra of the two complexes reveal extensive broadening between the wavenumbers 3150–3600 cm^{-1} that are the characteristic O–H stretching modes originating from the aqua ligands and the four hydroxyl substituents present in RF. The NH stretch mode vibrates in the same region[7].

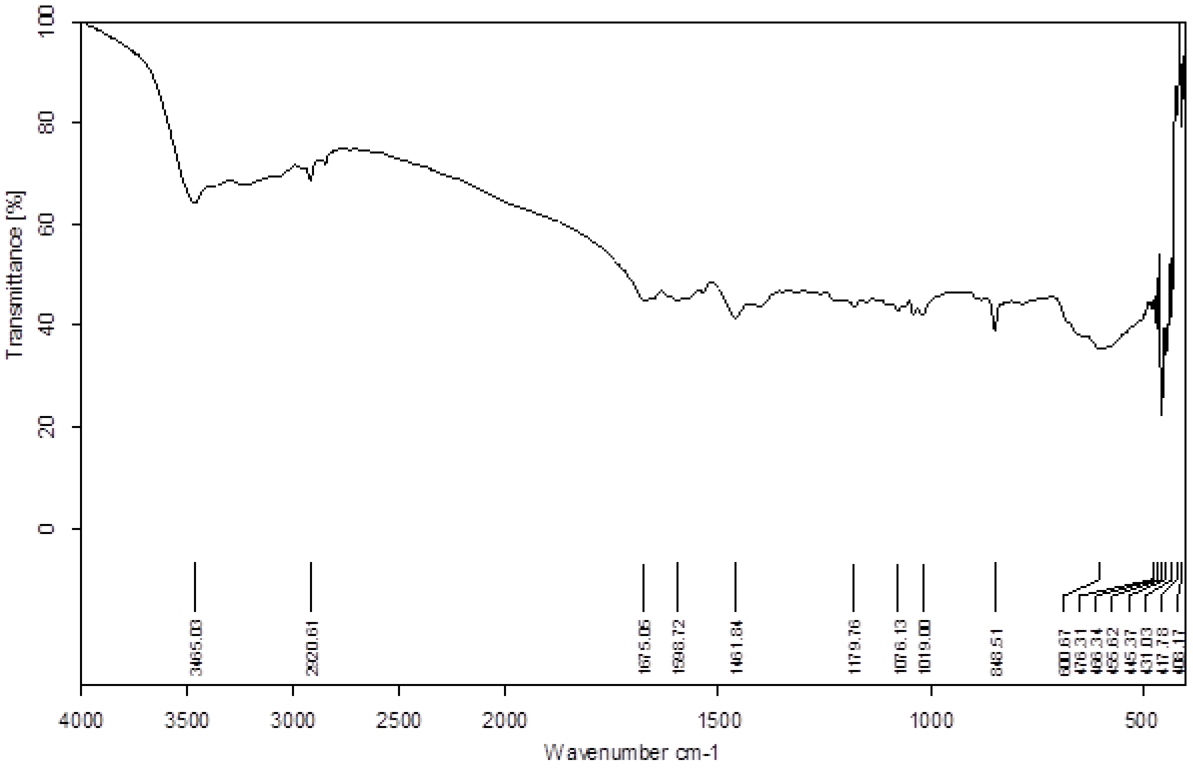


Fig1: FT-IR Spectrum of Complex 1

The IR spectrum of complex 2 confirms the coordination of riboflavin to the Zn (II) metal. The IR spectra of [Zn(L)(H_2O)$_2$] contains a strong broad band observed at 3418 cm^{-1} which may be attributed to *v*(OH) of the coordinated water[32] molecules. RF–Zn also exhibits bands at 1660, 1549 and 1407 cm^{-1} that are characteristic stretching modes C=O (amide carbonyl), C=N (conjugated system) and C–N, respectively.

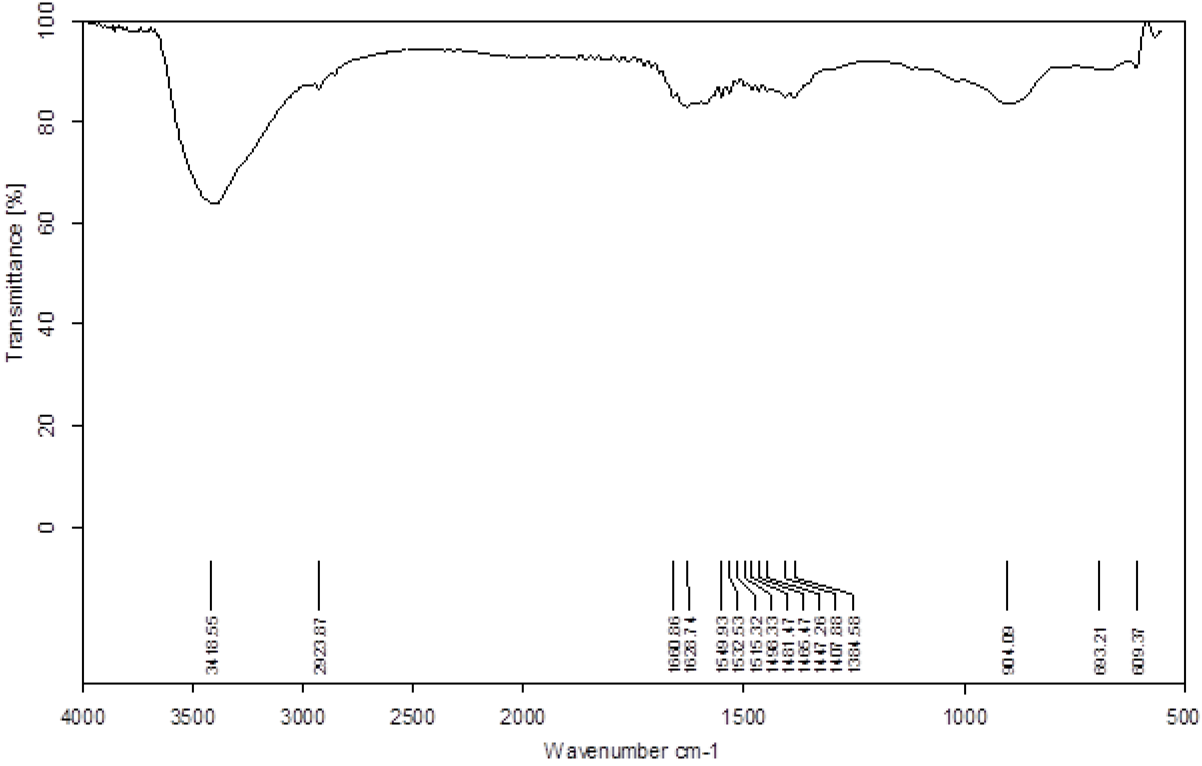


Fig 2: FT-IR spectrum of complex 2

In the IR spectrum of complex 3, The imidazole and riboflavin both are coordinated to the metal and is confirmed by presence of peak at 3247cm^{-1}(νN-H). RF–Zn also exhibits bands at 1545, 1495 and 1439 cm^{-1} that are characteristic stretching modes C=O (amide carbonyl), C=N (conjugated system) and C–N, respectively.

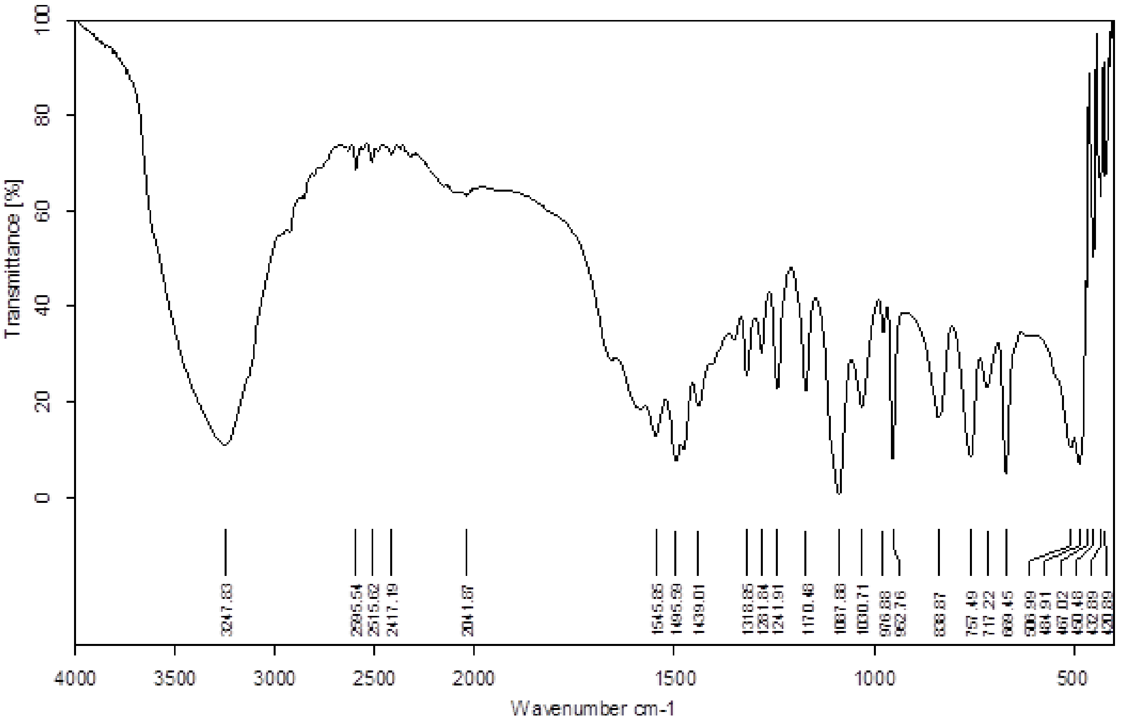


Fig 3: FT-IR Spectrum of complex 3

LC-MS Spectra:

LC-MS spectrum of Complex 1

The LC/MS studies are performed in search of the molecular weights of the complexes. LC-MS technique is a good method to analyze the complexes. The peak at 487(m/z) is complex bound to one riboflavin molecule, [Cd (ribo)] and the peak at 507(m/z) is complex bound to one riboflavin and one water molecule present in 1:1 ratio, [Cd (ribo) (H_2O)]. The peaks around 527(m/z) and 567(m/z) refer to the [Cd (ribo) $(H_2O)_2$] and [Cd (ribo) $(H_2O)_4$] complexes respectively. The peak at 137(m/z) corresponds to $C_5H_{11}O_4$ (part) of riboflavin ligand and 241(m/z) is $C_{12}H_9N_4O_2$ (other part) of riboflavin ligand.

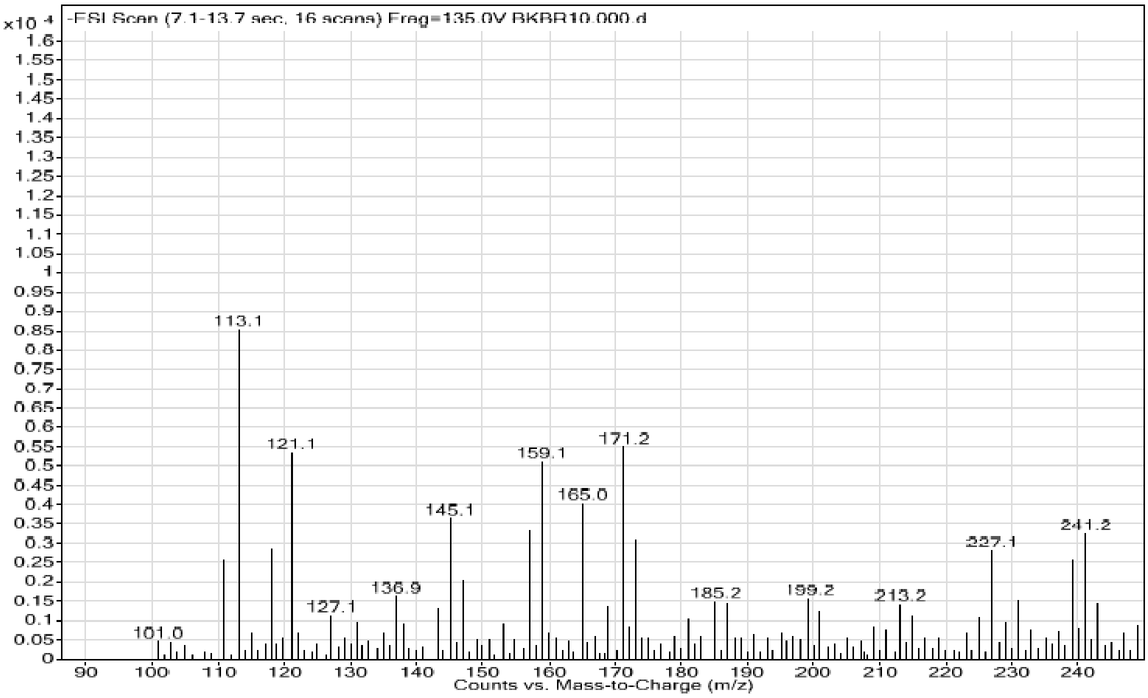


Fig 3 (a): LC-MS spectrum of Complex 1 (100-250 m/z)

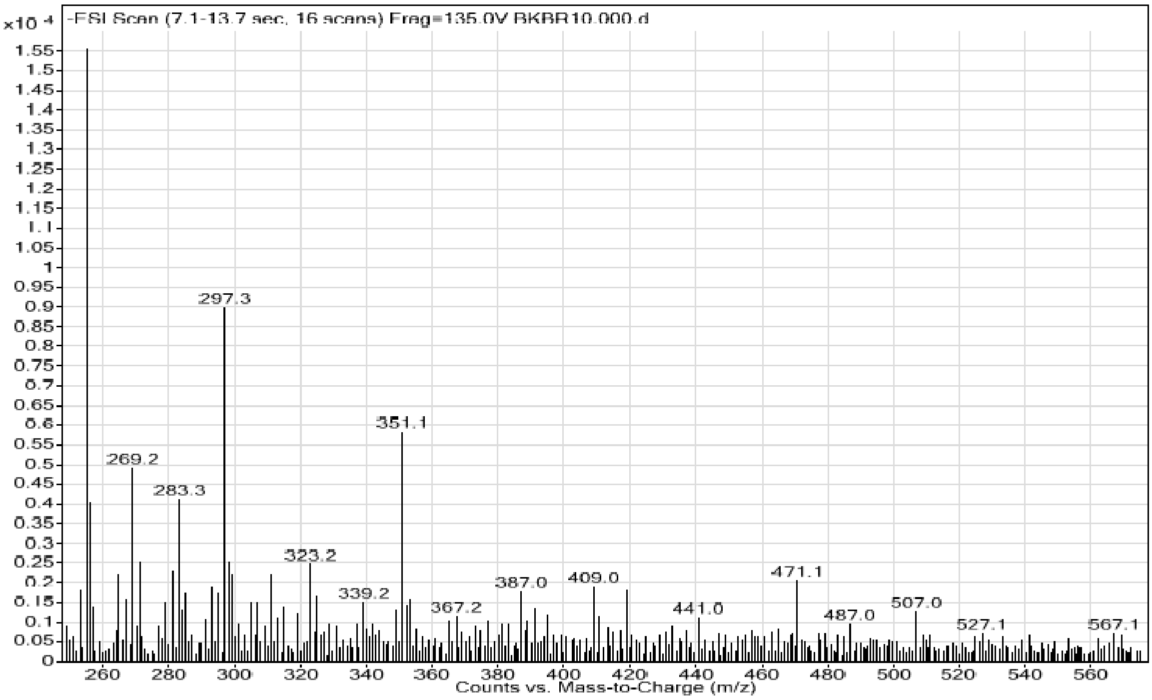


Fig 3 (b): LC-MS spectrum of Complex 1 (250-600 m/z)

LC-MS spectrum of Complex 2

The peak at 441(m/z) is complex bound to one riboflavin molecule, [Zn (L)] and the peak at 477(m/z) is complex bound to one riboflavin and two water molecule present in 1:2 ratio, [Zn (L) $(H_2O)_2$]. The peak at 137(m/z) corresponds to $C_5H_{11}O_4$ (part) of riboflavin ligand and 239(m/z) is $C_{12}H_9N_4O_2$ (other part) of riboflavin ligand.

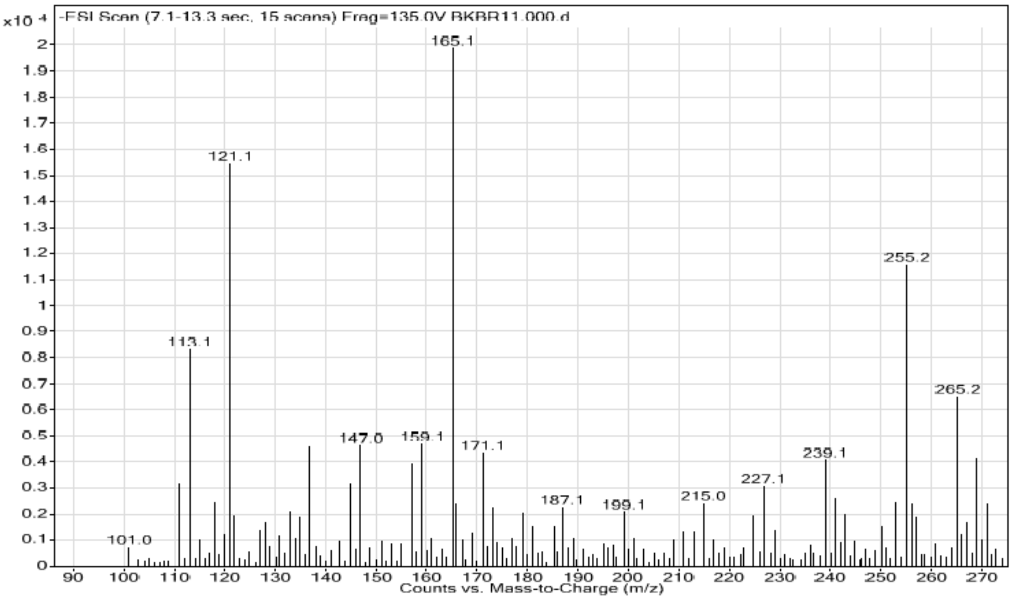


Fig 4 (a): LC-MS spectrum of Complex 1 (100-280 m/z)

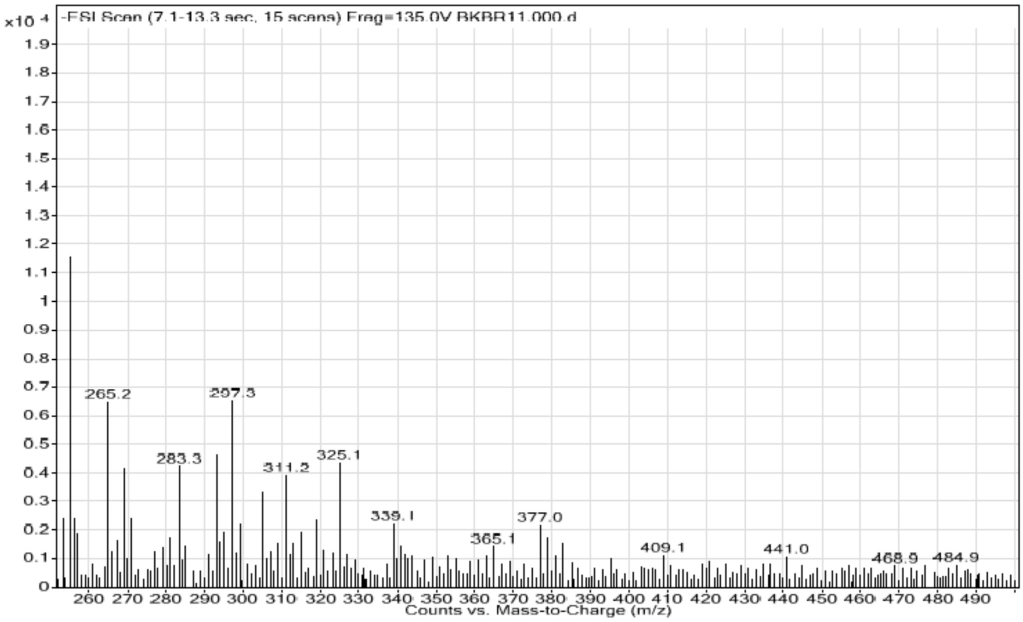


Fig 4 (b): LC-MS spectrum of Complex 2 (250-500 m/z)

LC-MS spectrum of Complex 3

The peak at 437(m/z) is complex bound to one riboflavin molecule, [Zn (ribo)] and the peak at 505(m/z) is complex bound to one riboflavin and one imidazole molecule present in 1:1 ratio, [Zn (ribo) (imd)]. The peaks around 525(m/z) and 561(m/z) refer to the [Zn (ribo) (imd) (H_2O)] and [Zn (ribo) (imd) $(H_2O)_3$] complexes respectively. The peak at 137(m/z) corresponds to $C_5H_{11}O_4$ (part) of riboflavin ligand and 241(m/z) is $C_{12}H_9N_4O_2$ (other part) of riboflavin ligand.

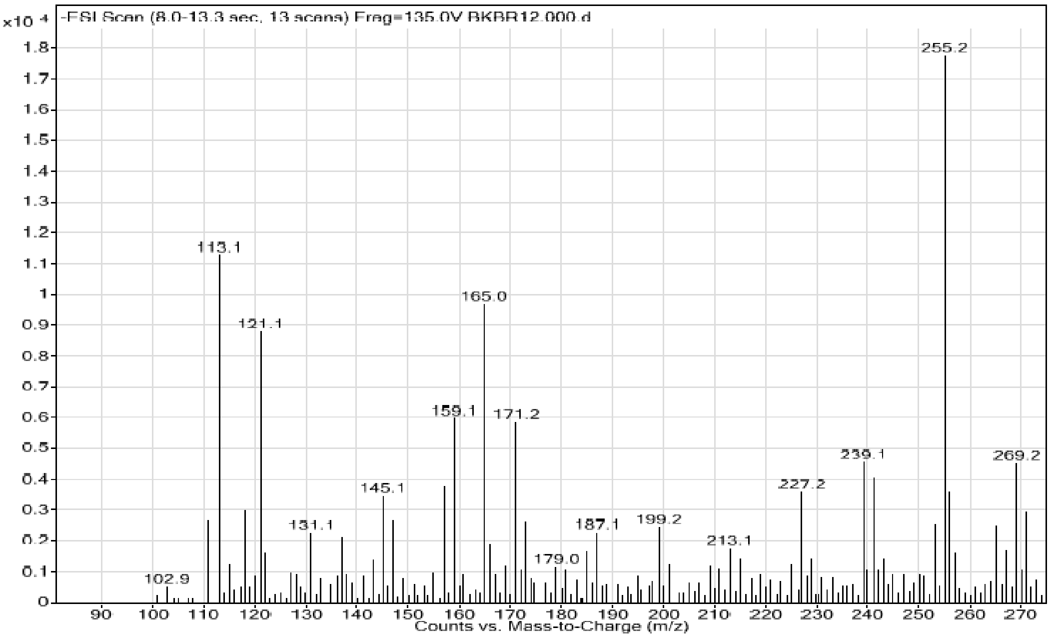


Fig 5 (a): LC-MS spectrum of Complex 3 (100-270 m/z)

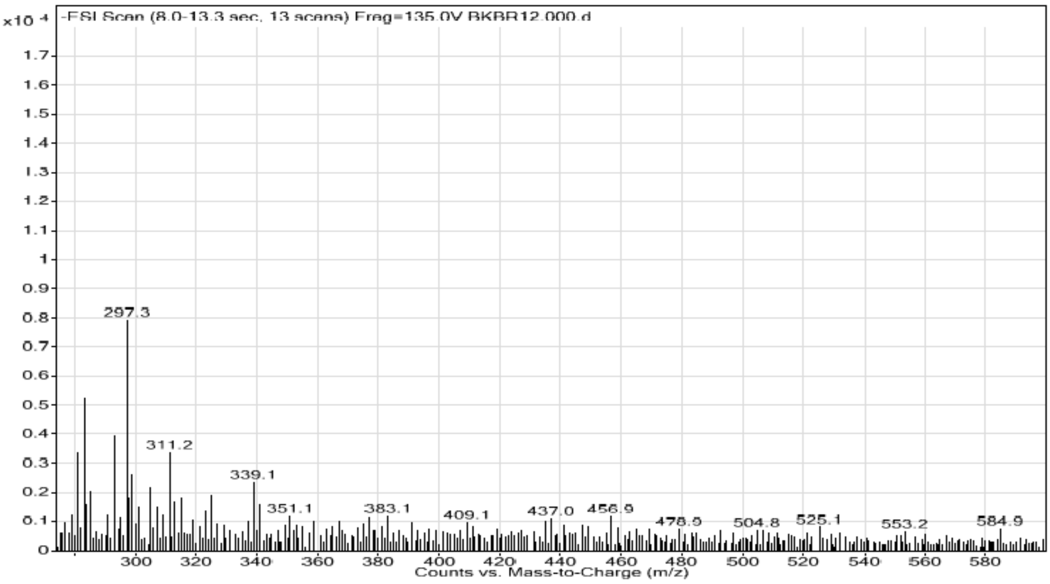


Fig 5 (b): LC-MS spectrum of Complex 3 (280-600 m/z)

Antimicrobial activity

Materials and method:

1. Muller Hinton agar (SRL)
2. Muller Hinton Broth (SRL)
3. Potato Dextrose Agar (Hi-Media)
4. Petri plates
5. Autoclave
6. Conical flasks 250ml
7. Laminar air flow
8. Required Bacterial strains (Clinically isolated Pathogens)

The required numbers of Muller Hinton agar plates and for fungal, potato dextrose agar plates were prepared.

Antibacterial activity:

The antibacterial activity was determined using the hole-in-plate bio assay procedure (Hugo *etal.*, 1983; Vlientick *et al.*, 1995). The pure cultures of the microorganisms were retrieved in Muller Hinton broth in proper dilution such that 10^8 cells/ml. ***Staphylococcus aureus, and Escherichia coli*** were used as the test microorganisms. 0.1ml of the organisms in the broth is evenly spreaded in the labelled petri plates. Using a sterile cork-borer of 6mm diameter, four holes were made into the Petri dishes seeded with bacterial culture. The sample compound Concentrations of 0.25, 0.5, 1, 2 mg/ml is made in 10% DMSO and transferred into the wells. The plates were incubated at temperature of 37°C for 24 hours. All bacterial cultures were maintained on nutrient agar slants at temperature of 4ºC and sub cultured onto nutrient agar broth for 24 hours prior to testing. The plates were kept for 30 min at room temperature to allow diffusion of the samples, and then were incubated at temperature of 37º C for 18 hours. After the incubation period, the zones of inhibition will be measured using a calliper. Studies were performed and the mean value was calculated.

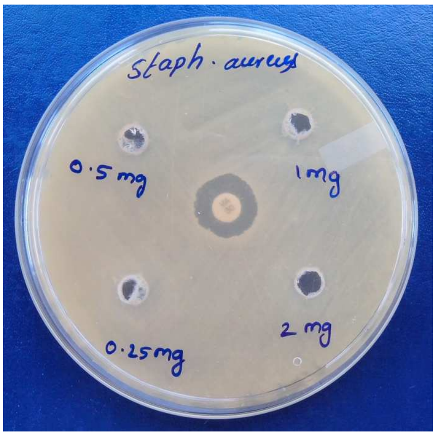
Antifungal Activity:

Holes were made into the Petri dishes containing inoculated medium as described by (Vlientick et al.,1995). Concentrations of 0.25, 0.5, 1, 2 mg/ml Samples were transferred into the wells and examined against ***Candida albicans.*** The diameter of the clear zone around the wells (inhibition diameter) was measured at the end of the incubation period. And four wells per plate against a single microorganism were used.

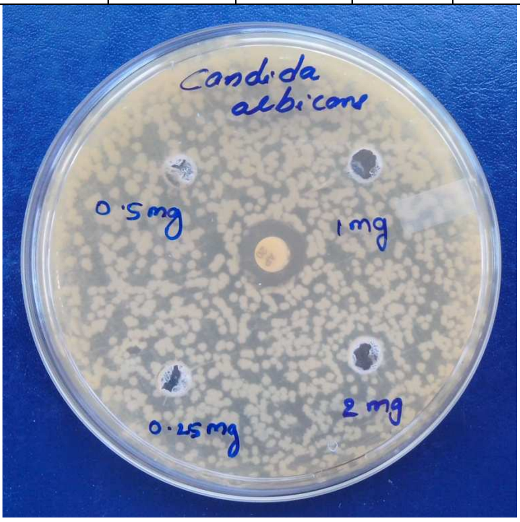
ANTIMICROBIAL ACTIVITY: WELL DIFFUSION METHOD

S.No.	**Organism**	**Zones of Inhibition in mm**				
		Zn-RF Complex				
		0.25 mg/ml	**0.5** mg/ml	**2** mg/ml	**1** mg/ml	**Std. Drug**
01.	*Staphylococcus aureus*	Nil	Nil	Nil	Nil	15 mm
02.	*Escherichia coli*	Nil	Nil	Nil	Nil	22 mm

RESULTS



S.No.	**Organism**	**Zones of Inhibition in mm**				
		Zn-RF Complex				
		0.25 mg/ml	**0.5** mg/ml	**2** mg/ml	**1** mg/ml	**Std. Drug**
01.	*Candida Albicans*	-	-	-	-	16 mm



CONCLUSION

We presented the results of the synthesis and characterization studies of Zinc and Cadmium complexes involving Riboflavin ligand. Among Three complexes, $Zn(RF)(H_2O)_2$ was tested for antimicrobial properties. The IR spectra reveals the existence of functional groups and coordinated RF and aqua ligands. The structures are predicted and proposed by using LC-MS Spectra.

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