Analytical, Physical, Spectral and Microbial Characteristics o f metal complexes of 4-aminopyridine

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Abstract

Cardanol, a renewable natural agro- based resource and a product of cashew nut shell liquid (CSNL). It's converted into a compound possessing two aldehydic groups that is di-α-formylmethoxybis(3-pentadecenylphenyl) methane (DFMPM) in three stages. The DFMPM so obtained is the starting material for the present study. The investigation involves synthesis and characterisation of bioactive schiff base complexes from cardnol and metal ions especially with transition metals.

Key Words: *di-α-formylmethoxybis(3-pentadecenylphenyl) methane, Cardanol*

1. INTRODUCTION

Schiff base is a nitrogen analogue of an aldehyde or ketone in which the carbonyl group has been replaced by an imine or azo-methine group . Schiff base ligands are easily synthesized and form complexes with almost all metal ions. In organic synthesis, Schiff base reactions are useful in making carbon-nitrogen bonds. Schiff bases appear to be important intermediates in a number of enzymatic reactions. Schiff bases, derived mostly from variety of heterocyclic rings, were reported to possess a broad spectrum of pharmacological activities. It has been widely used for industrial purposes and also exhibits a broad range of biological activities including antifungal, anti-bacterial, larvacidal, insecticidal, antimalarial, antiproliferative, anti-inflammatory, antiviral, antipyretic and DNA clevage properties. The ligand and complexes were characterised by UV-visible, FTIR, ^{1}HNMR, SEM, elemental analysis, melting point, conductivity, metal ion intake, anti bacterial, anti fungal, anti cancer, DNA cleavage, larvacidal and anti inflammatory activity[1-3].

1.1 Synthesis of ligand and complex

Ethanolic solution of DFMPM and 4-aminopyridine were mixed and taken in RB flask in 1:2 molar ratio and refluxed for an hour. The reaction mixture was poured in ice, an yellow compound of Schiff base ligands was obtained. All the metal complexes were prepared by mixing ethanolic solution of Schiff base ligand with the corresponding aqueous metal salt solution of Zr(IV) and Th(IV) in 1:2 molar ratio and refluxed for about twelve hours at 70-80^{0}C. Estimation of metal ion intake is done by, the filtrates obtained in the above method were collected and it is used for the estimation of Zr(IV) and Th(IV) intake for complexation using standard methods[4-6].

2. RESULT AND DISCUSSION

The analytical data of the complexes, together with their physical properties are mentioned in Table 1. The data suggested that the complexes are in ML_2 composition, they are coloured solids, stable towards air and have high melting points above (250^{o}C). The complexes are insoluble in water and common organic solvents but are soluble in DMF, CDCl3, and DMSO. Analytical data suggest that the metal to ligand ratio in all the complexes to be 1:2. Conductivities value were in the range 17-19 ohm^{-1}cm^{2} mol^{-1} and complexes are non electrolytes because their conductivity .From elemental analysis data, the complexes are mononuclear with the ligand coordinated to the central metal atom and the metal to ligand ratio was 1:2, and their empirical formulae also have been computed.

Table 1 Physical and Analytical data of ligand and Metal complexes

Complex / Ligand	Yield	Colour	Molecular formula	Mol. weight	Melting point	Elemental Analysis		
						C	H	N
Ligand L	61	grey	$C_{69}H_{104}N_4O_6$	1084	232	74.41 (76.38)	7.74 (9.59)	3.66 (5.16)
$[ZrL_2(NO_3)_2]\ 2H_2O$	58	Brown	$C_{138}H_{212}N_{10}O_{16}$	2355	>250	68.38 (70.31)	7.57 (9.00)	3.78 (5.64)
$[ThL_2(NO_3)_2]\ 2H_2O$	63	Brown	$C_{138}H_{212}N_{10}O_{16}$	2496	>250	63.44 (66.34)	6.71 (8.49)	2.86 (5.60)

2.1 FT- IR Spectrum analysis

FT-IR spectral data's are in table 2. The FT-IR spectrum of the free ligand fig 1 shows characteristic bands at 2767 cm^{-1},2990 cm^{-1}, 1651 cm^{-1},1499 cm^{-1} and 1439 cm^{-1} assignable to ν_{O-C} , ν_{C-H} , $\nu_{C=N}$, $\nu_{C=O}$ and free –COOH ; free (-O-H) stretching phenolic moiety, carbonyl (-C=O) and azomethine (-C=N), stretching modes. The spectra of metal of fig2,3 Zr(IV) and Th(IV) complexes showed new bands at 3408- 3303cm^{-1} which is assigned to ν_{O-H} , 2846 cm^{-1} for ν_{O-C} , 2923 - 2922 cm^{-1} for ν_{C-H} , 1650 - 1640 identify presence of $\nu_{C=N}$ bond, 1499 cm^{-1} point out bond at $\nu_{C=O}$ and bands at regions 689- 501cm^{-1} and 458- 399cm^{-1} can be assigned to ν_{M-N} and ν_{M-O} vibrations [7-8].

Table.2 FT IR frequencies and UV-spectrum of the ligand and its complexes

Ligand/ Complexes	ν_{O-H} cm^{-1}	ν_{O-C} cm^{-1}	ν_{C-H} cm^{-1}	$\nu_{C=N}$ cm^{-1}	$\nu_{C=O}$ cm^{-1}	free -COOH cm^{-1}	ν_{M-N} cm^{-1}	ν_{M-O} cm^{-1}	UV		aromatic ring proton ppm	H – C =N PPM	CO-NH PPM	olefinic proton PPM	$-CH_2$ PPM
Ligand L	-	2767	2990	1651	1499	1439	-	-			7.186-7.167	8.188-8.174	5.43-5.32	6.804-6.500	2.82-2.00
$[ZrL_5(NO_3)_2]$ $2H_2O$	3408	2846	2922	1640	1499	1400	501	399	500	650	7.798	-	5.34-5.33	6.99-6.65	2.58-2.00
$[ThL_5(NO_3)_2]$ $2H_2O$	3403	2846	2923	1650	1499	1400	689	458	650	750	-	-	-	-	-

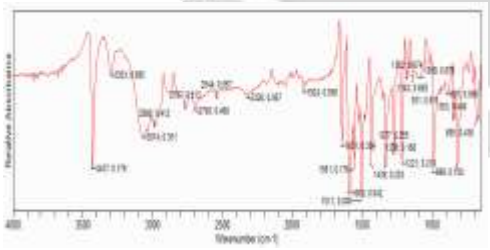


Fig. 1 FTIR Spectrum of ligand (L)

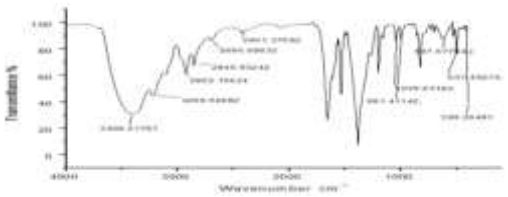


Fig.3 FTIR Spectrum of Zr(IV) complex

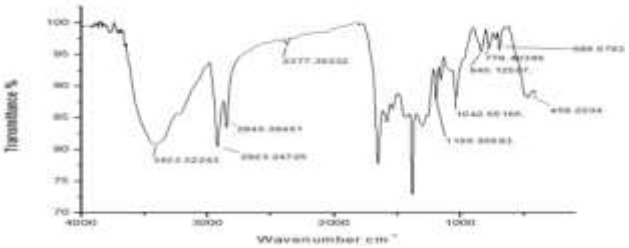


Fig. 4 FTIR Spectrum of Th(IV)complex

2.2 UV-Visible spectrum analysis

Electronic absorption spectral data of the ligand and Schiff base metal complexes are given in Table 2 . Zr(IV) and Th(IV) complexes show significant absorption in the region 500 cm^{-1}, 650 cm^{-1} and 650 cm^{-1}, 750 cm^{-1} support the geometry for the synthesised metal complexes[9].

2.3 ^{1}H NMR spectrum analysis

From table 2, Ligand shows a multiplet at δ= 7.186-7.167ppm aromatic ring proton and Zr(IV) shows a singlet at δ= 7.798 ppm. Ligand shows a multiplet at δ= 8.188-8.174 PPM due to H – C =N group. An olefinic proton ring is present for ligand and complex at δ= 6.804-6.500ppm and δ= 6.99-6.65ppm. mulplet were present for both ligand and complex at 2.82-2.00 PPM and 2.58-2.00ppm due to CH_2 group[10].

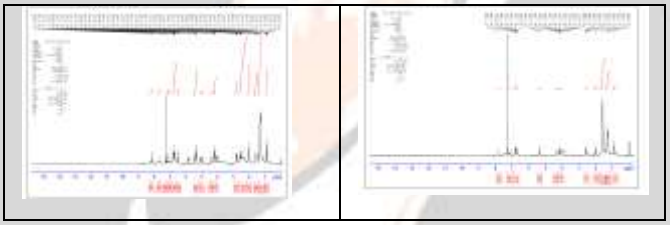


Fig.4 ^{1}HNMR spectrum of ligand and Zr(IV) complex

Based on the observations in elemental analysis, FT-IR, electronic and ^{1}H NMR spectral studies, the proposed structure of Ligand (L) and meal Schiff base complexes were given in Fig.5-6.

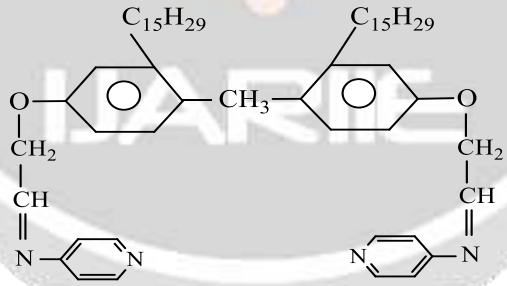


Fig .5 Structure of ligand

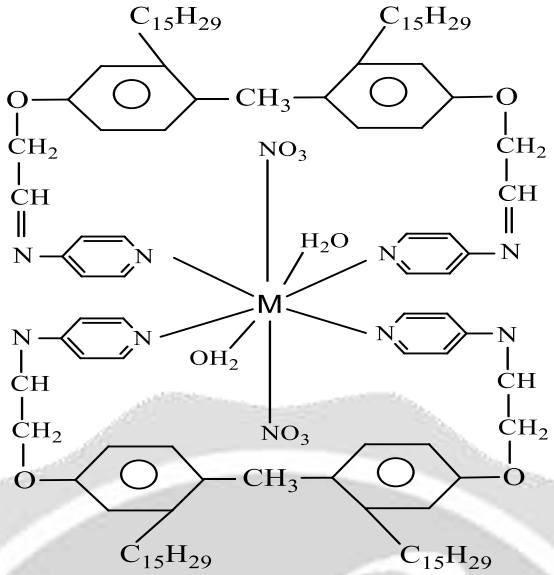


Fig.6 Structure of Schiff base complexes, M = Zr(IV) and Th(IV)

2.4 SEM Analysis

The surface morphology of the complexes has been examined using scanning electron microscope. Zr(IV) occur as flakes . Fig.7 showed that the complex is micro crystalline in nature. Careful examination of the single crystal clearly indicated the nano scale size of the single crystal of the complex [11].

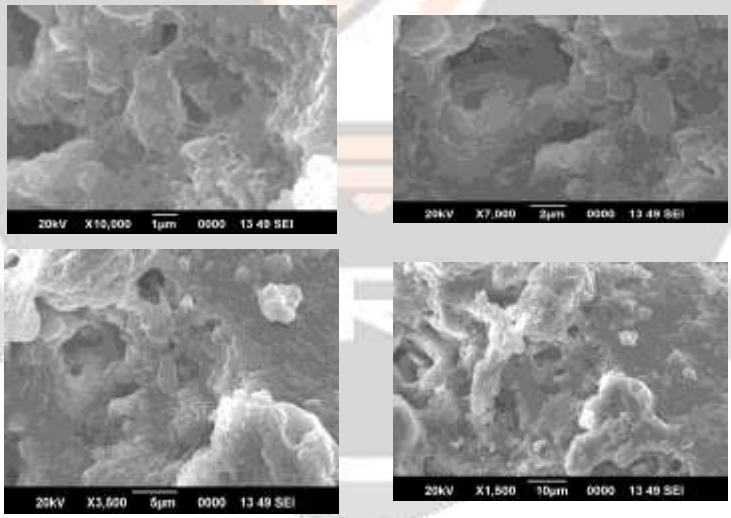


Fig.7 SEM images of Th(IV) complexes at 1µm, 2µm, 5µm, 10µm

2.5 Anti-bacterial activity

Metal complexes of Schiff base were screened against E. coli, Pseudomonas aeroginosa, Proteus vulgaris and Klebsiella pnemoniae. Table 3 shows Zr(IV) and Th(IV) were screened by disc diffusion method. Both complexes showed a higher effect on Proteus vulgaris. Metal chelates bear polar and nonpolar properties together; this makes them suitable for permeation to the cells and tissues. In addition, chelation may enhance or suppress the biochemical potential of bioactive organic species[12].

Table.3 Anti-bacterial activity data of complexes

Bacteria	Zr	Th
Pseudomonas aeroginosa	NZ	7mm
Proteus vulgaris	9mm	9mm
E.coli	8mm	7mm
Klebsiella pnemoniae	NZ	NZ

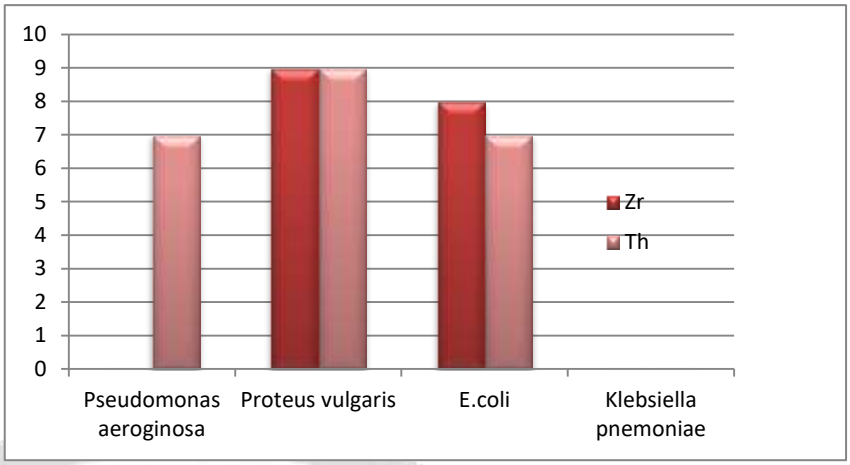


Fig.8 graphical representation of Anti-bacterial activity data of complexes

2.6 Anti-fungal activity

Metal complexes Zr(IV) and Th(IV) were treated aganist microorganisms Aspergillus niger and Aspergillus flavus. The metal complexes gave better results against the growth of fungi. It is found that the activity increases upon coordination. The increased activity of the metal chelates can be explained on the basis of chelation theory.a slight activity is shown by Zr(IV) towards Aspergillus flavus [12].

Table.4 Anti-fungal data of Schiff base complexes

FUNGAI	$[ZrL_2(NO_3)_2]2H_2O$	$[ThL_2(NO_3)_2]2H_2O$
Aspergillus niger	NZ	NZ
Aspergillus flavus	7mm	NZ

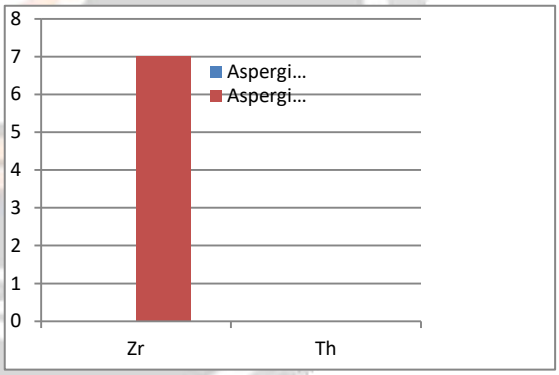


Fig.9 graphical pic of Anti-fungal data of Schiff base complexes

2.7 Anti-inflammatory activity

Schiff base complexes were screened for their anti-inflammatory activities. Bovine serum albumin and dichlorofenac sodium solution was used as the standard and distilled water as control. The percentage inhibition increases with increase in concentration. From data Th(IV) and Zr(IV) show statically significant activity in Invitro and Invivo percentage inhibition [13].

Table.5 Anti inflammatory activity data of complexes

Sample	Conc: (µg/ml)	Proteinase inhibitory %of inhibition	Proteinase denaturation %Inhibition
Zr(IV)	25	51.17	18.02
	50	56.77	30.22
	100	61.03	42.73
Th(IV)	25	49.46	15.9970
	50	52.19	20.653
	100	58.44	26.02

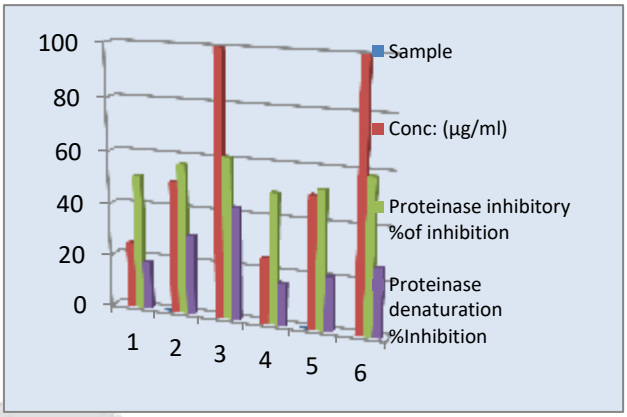
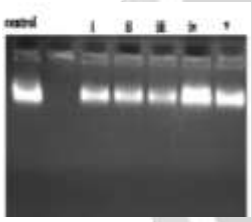


Fig.10 graphcal data of Anti inflammatory activity data of complexes

2.8 Nuclease activity

In the present study, metal complexes were incubated for 30 min at 37°C and the DNA was analyzed on 1.5% agarose gel in the presence of H_2O_2 as an oxidant. It was found that at very low concentration, the complexes exhibit nuclease activity in the presence of H_2O_2.from fig 11 ,it is clear that, Th(IV)complex cleaves DNA to a larger extent as compared with control DNA. This is due to the formation of redox couple of the metal ions. In oxidative mechanism, metal ions in the complexes react with H_2O_2 to generate the OH which attacks at the C_3 positions of the sugar moiety and finally cleaves DNA [14].



Lane 1-Control DNA
Lane 2-DNA treated with H_2O_2
Lane6-DNA+Zr (IV) complex + H_2O_2
Lane7-DNA+Th (IV) complex + H_2O_2

Fig.12 Gel electrophores diagram

2.8 Anticancer activity

Cytotoxic activity were conducted by MTT assay method ,using HeLa (cervical cancer) cell line. Observation from data it is cleared that when concentration increases the viability decreases that means the cytotoxicity of the cells decreases there by the cells are not cytotoxic . When increase in concentration number of dead cells increases there by indicate the anti cancerous property of complexes [15]. The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{Mean OD Samples x } 100}{\text{Mean OD of control group}}$$

LD 50 VALUE (2A4) – 177.416µg/ml & LD 50 VALUE (2A5) – 271.44µg/ml

Table 6 Anti cancer activity data of complexes

Sample Concentration (µg/ml)	Average OD at 540nm	Percentage Viability
Control	0.9852	100
Zr(IV)	0	0
25	0.838	85.05887
50	0.7399	75.1015
100	0.70405	71.46265
Th(IV)	0	0
25	0.8151	82.73447
50	0.7894	80.12586
100	0.7464	75.76127

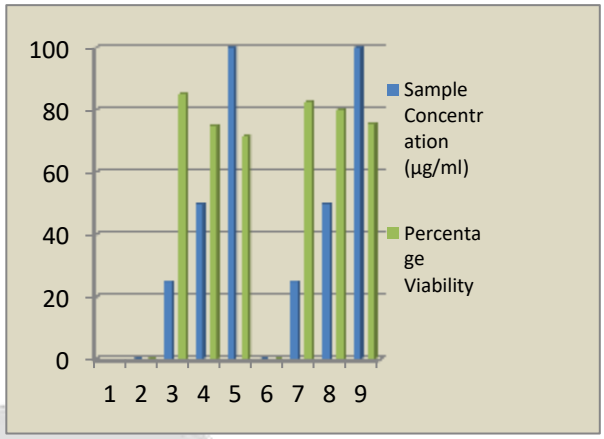


Fig.13 graphcal dataof Anti cancer activity data of complexes

2.9 Larvacidal activity

Brine shrimp cytotoxic assay of Ni(II) complex was performed to evaluate its cytotoxic activity. Sample exhibited 100% cytotoxic activity at 600 µg , 60% at 50 µg, 50% at 200 µg and 80% at 400 µg respectively. Chelation increases the liphophlic nature of central metal atom, which in turn favours the molecule in crossing the cell membrane of the microorganism and enhancing larvacidal activity of complexes [16].

Table.7 Brine Shrimp lethality assay of $[NiL_2(NO_3)_2]$

Concentration (µg/ml)	50 µg	200 µg	400 µg	600 µg	Control	-ve control
Number of brine shrimp per test sample	10	10	10	10	10	10
Average number of survivors	4	5	2	0	3	5
Average number of deaths	6	5	8	10	7	5
Percentage mortality	60	50	80	100	70	50

3. CONCLUSION

In this work the synthesis and characterization of a Schiff base ligand derived from 4-aminopyridine and forms stable complexes with metals zirconium (IV) and thorium (IV).The ligand and its complexes were characterized using spectral and analytical data. These analytical and spectral data suggests complex show tetrahedral geometry . SEM studies indicated the nano crystalline nature of the complexes. Th(IV)complex exhibit statically significant activity in Invitro and Invivo percentage inhibition in anti-inflammatory activity. Th(IV)complex cleaves DNA to a larger extent as compared with control DNA. inhibition ratio of the complexes in MTT analysis explains when increase in concentration number of dead cells increases there by indicate the anti cancerous property of complexes.

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