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TEMPLATE DIRECTED SYNTHESIS AND CHARACTERIZATION OF MACROCYCLIC COMPLEXES: *In-silico* DOCKING AND ANTIMICROBIAL STUDIES

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ABSTRACT

A new series of macrocyclic complexes have been synthesized by template condensation between 3,4-diaminotoluene and acetylacetone in the presence of trivalent metal ions to form macrocyclic complexes having four coordination sites. Different spectroscopic and physicochemical techniques were used to establish the geometry and structural framework of the synthesized macrocyclic complexes. Further, these complexes were also investigated for their antibacterial, antifungal and antioxidant activities. Removal of methylene blue dye from the aqueous by iron complexes was also investigated.

Keywords: Antibacterial, Antifungal and Antioxidant Activities, Macrocyclic Complexes.
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INTRODUCTION

The pioneering work on template synthesis of macrocyclic complexes started by Curtis[1] and Busch[2] has become a major area of research during the past few decades. The macrocyclic moiety-containing complexes are of great interest due to their resemblance with naturally occurring macrocyclic compounds like cobalamines, porphyrins and chlorophyll.[3] The macrocyclic complexes of lanthanides have attracted much attention in catalytic cleavage of RNA, in radioimmunography as well as in positron emission tomography. Some macrocyclic complexes have been reported to exhibit potential antibacterial and antifungal activities.[4,5] Prompted by these applications, in the present paper, we wish to report the macrocyclic complexes synthesized from 3,4-diaminotoluene and acetylacetone and their antibacterial, antifungal and antioxidant activities.[6–8] Furthermore, the removal of methylene blue dye from aqueous solution by using iron complexes was also studied.

EXPERIMENTAL

Materials and Methods

Analytical grade chemicals and solvents were procured and used without further purification. 3,4-diaminotoulene and acetylacetone were procured from Molychem, Mumbai, India while Cr(III) and Fe(III) salts were purchased from Sigma-Aldrich.

Template Synthesis of Macrocyclic Complexes

Briefly, to a hot and well stirred methanolic solution of 3,4-diaminotoulene (10 mmol) was added methanolic solution of Cr(III) or Fe (III) salts (5 mmol) respectively, giving a colored precipitate. This reaction mixture was refluxed for 0.5 h followed by the addition of acetylacetone (10 mmol). This reaction mixture was allowed to reflux again for ~6-8 hours [
Scheme-1]. Macrocyclic complexes of different colors were formed. These complexes were isolated and washed with organic solvents. Then these complexes were dried *in vacuo.* Yield, solubility and melting points of well-dried complexes were measured.

Pharmacology: (*In vitro* Antimicrobial Activity)

Four microbial strains were selected, including one Gram-positive bacterium (*Paenibacillus polymyxa)*, one Gram-negative bacterium (*Pseudomonas aeruginosa*) and two fungi strains i.e. *Macrophomina phaseolina* and *Alternaria brassicae* for evaluation of the antimicrobial activity of these complexes.

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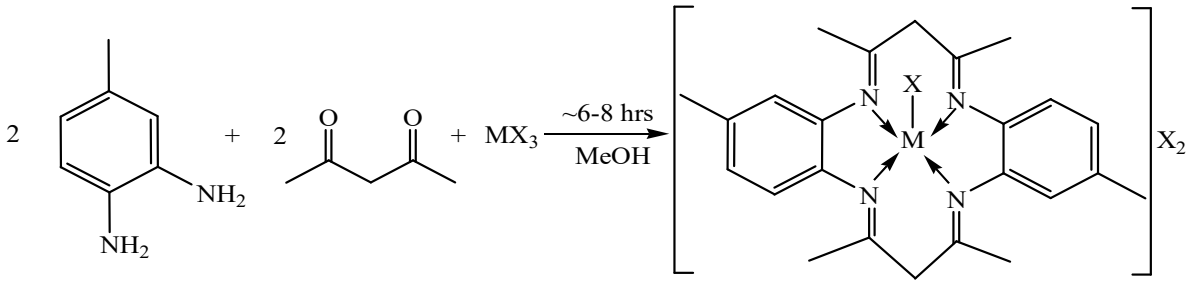

Where M = Fe (III) and Cr (III)
X = Cl^-, NO_3^-, OAc^-

Scheme-1: Synthesis of Macrocyclic Complexes

Primary Screening

The antifungal and antibacterial activities of all macrocyclic complexes were investigated with the help of agar well diffusion method.[9] In this procedure, Luria Bertani agar plates were inoculated with a particular bacterial or fungal strain. Further, 10 µl of complexes were incubated overnight (37°C). Commercially available drugs i.e. Rifampicin and Amphotericin-B were used for comparative studies. CLSI (Clinical and Laboratory Standards Institute, USA) guidelines were adopted for the determination of zone of inhibition [
Table-1]. Negative control was also observed.

No.	Complexes/ Standard Drugs	Diameter of Zone of Inhibition (mm)[a]			
		Paenibacillus polymyxa	*Pseudomonas aeruginosa*	Fungi	
				Macrophomina phaseolina	*Alternaria brassicae*
C-1	[Cr(C24H28N4)NO3](NO3)$_2$	12	13	-	20
C-2	[Cr(C24H28N4)Cl](Cl)$_2$	21	-	-	18
C-3	[Cr(C24H28N4)OAc](OAc)$_2$	16	-	-	19
C-4	[Fe(C24H28N4)NO3](NO 3)$_2$	23	15	-	21
C-5	[Fe(C24H28N4)Cl](Cl)$_2$	18	-	-	11
C-6	[Fe(C24H28N4)OAc](OAc)$_2$	15	8	-	17
SD-1	Amphotericin-B	-	-	15	25
SD-2	Rifampicin	22	10	-	-

Table-1: Zone of Inhibition Shown by Synthesized Complexes and Standard Drugs

No.	Complexes/ Standard Drugs	Diameter of Zone of Inhibition (mm)[a]			
		Paenibacillus polymyxa	*Pseudomonas aeruginosa*	Fungi	
				Macrophomina phaseolina	*Alternaria brassicae*
C-1	[Cr($C_{24}H_{28}N_4$)NO_3](NO_3)$_2$	12	13	-	20
C-2	[Cr($C_{24}H_{28}N_4$)Cl](Cl)$_2$	21	-	-	18
C-3	[Cr($C_{24}H_{28}N_4$)OAc](OAc)$_2$	16	-	-	19
C-4	[Fe($C_{24}H_{28}N_4$)NO_3](NO_3)$_2$	23	15	-	21
C-5	[Fe($C_{24}H_{28}N_4$)Cl](Cl)$_2$	18	-	-	11
C-6	[Fe($C_{24}H_{28}N_4$)OAc](OAc)$_2$	15	8	-	17
SD-1	Amphotericin-B	-	-	15	25
SD-2	Rifampicin	22	10	-	-

[a]Values = means of three replicates (including diameter of well); (-) = no activity

Determination of Minimum Inhibitory Concentration

Broth dilution method was adopted for determining MIC (minimum inhibitory concentration) of the synthesized macrocyclic complexes. In brief, overnight grown colonies of bacteria and fungi were suspended to a turbidity equivalent to that of a 0.5 McFarland standard and then diluted further in BHI (Brain-Heart Infusion Broth) to get bacterial suspension (1.1×10^6cfu/mL). For the determination of MIC, all complexes were dissolved in DMSO (200µg/ml) and different concentrations were obtained from this stock solution after dilution. 500µl of bacterial cells (1.1×10^6cfu/mL) were added with a complex solution of different concentrations ranging from 20 to 100µg/L and incubated for 18-22 h at 37 °C. Further, the absorbance of 100 µl of each suspension was recorded spectrophotometrically at 620nm. The lowest concentration of these complexes that inhibited bacterial growth was considered as MIC [Table-2]. All assays were conducted in triplicate.

Antioxidant Activity

Macrocyclic complexes were also screened for their antioxidant activities by adopting DPPH radical scavenging method.[10] In brief, a freshly prepared, 3.0 ml methanolic solution of DPPH (0.1 mM) was added to the solution of macrocyclic complexes.

Table-2: MIC Value of the Complexes and Standard Drugs

Comp. No.	Complexes/ Standard Drugs	Minimum Inhibitory Concentration (µg/ml)			
		Paenibacillus polymyxa	*Pseudomonas aeruginosa*	Fungi	
				Macrophominap haseolina	*Alternaria brassicae*
C-1	$[Cr(C_{24}H_{28}N_4)NO_3](NO_3)_2$	40	80	-	60
C-2	$[Cr(C_{24}H_{28}N_4)Cl](Cl)_2$	60	-	-	>100
C-3	$[Cr(C_{24}H_{28}N_4)OAc](OAc)_2$	60	-	-	40
C-4	$[Fe(C_{24}H_{28}N_4)NO_3](NO_3)_2$	20	40	-	40
C-5	$[Fe(C_{24}H_{28}N_4)Cl](Cl)_2$	40	-	-	60
C-6	$[Fe(C_{24}H_{28}N_4)OAc](OAc)_2$	80	40	-	60
SD-1	Amphotericin-B	-	-	10	10
SD-2	Rifampicin	10	10	-	-

Absorbance was recorded after 0.5h at 517 nm. Negative control was observed using a methanolic solution of DPPH. For positive control ascorbic acid was used. IC50 value and scavenging activity in percentage was calculated using the formula given below;

$$\%\ \text{of Radical Scavenging} = \frac{A_o - A_c}{A_o} \times 100$$

Where; A_c = Absorbance shown by the sample of "c" concentration; A_o = Absorbance shown by the control solution.

Removal of Methylene Blue Dye From Aqueous Solution

An aqueous solution of methylene blue dye (100 ml, 50 mg/L) was prepared. The initial absorbance (A_o) of this solution was measured. The pH of the solution was adjusted by using the $Na_2CO_3/NaHCO_3$ buffer. Fe(III) complexes were added in different amounts to the different samples of the above prepared aqueous solution. The temperature was kept constant throughout the experiment. To the above solution, 2.0 ml of H_2O_2 was added. After 0.5 h, the absorbance of the aqueous solution (A_t) was measured. Effect of amount of iron complex (in mg) on adsorption of methylene blue dye from aqueous solution was observed. The dye removal efficiency of complexes (in %) was calculated using the following formula:

$$\text{Dye Removal Efficiency}(\%)\text{of Complexes} = \frac{A_o - A_t}{A_o} \times 100$$

Molecular Docking

Autodock 4.2 software[11] was used for *in-silico* interaction studies of complexes with hydrolase from *Paenibacillus polymyxa*(PDB id: 2O9P). The synthesized macrocyclic complexes were treated as a ligand.[12] Docking studies have been carried out by starting with the initiation of protein formation by Autodock 4.2 with Kollaman charge as -7.0 for PDB: 2O9P. After the protein preparation, complexes were prepared by setting a number of torsions. Aromaticity criteria of complexes were set to 7.5. The size of the grid box was taken as 126, 126, and 126 for PDB structure. The maximum number of generations was 27000.

Analytical and Spectral Studies

The IR spectra of the synthesized macrocyclic complexes, as well as pure diamino and dicarbonyl compounds from which these complexes are derived, were recorded on the FTIR spectrometer (MB-3000). The mass spectral studies were carried out on XEVO G2-XS QTof mass spectrometer. Elemental analysis was determined by using EuroEA elemental analyzer. By using conductivity meter CM-183, the conductance of synthesized macrocyclic complexes was measured. Magnetic susceptibilities of complexes were measured using "PAR 155 magnetometer". Thermogravimetric studies of the complexes were carried out using Perkin Elmer thermal analyzer. The electronic spectra of complexes were obtained from Perkin Elmer Lambda U.V. spectrophotometer.

RESULTS AND DISCUSSION

The molar conductance of reported complexes in DMSO was found in the range of 140-170 ohm^{-1} cm^2 mol^{-1} suggesting these complexes to be 1:2 electrolytes.[13] The solubility of all complexes was tested in various organic solvents. However, all complexes were found completely soluble in DMSO only and partially soluble in other organic solvents. The melting points of all complexes were found between 240 to 260ºC.

Analytical Data of the Synthesized Complexes

Yield, colour, melting point, molar conductance and elemental analyses data (cacld. and found) of the Cr(III) and Fe(III) complexes are summarized below:

For $[Cr(C_{24}H_{28}N_4)NO_3](NO_3)_2$: Yield = 45%, Black, 247ºC, 156.71 ohm^{-1} cm^2 mol^{-1}.
anal. Calcd.% : C =47.21, H =4.62, N =16.06, Cr=8.52
Found (%) : C =47.12, H =4.23, N =15.85, Cr=8.32

For $[Cr(C_{24}H_{28}N_4)Cl](Cl)_2$: Yield = 51%, Black, 259ºC, 164.22 ohm^{-1} cm^2 mol^{-1}.
anal. Calcd.% : C =54.30, H =5.31, N =10.55, Cr=9.79
Found (%) : C =53.96, H =5.11, N =10.13, Cr=9.25

For $[Cr(C_{24}H_{28}N_4)OAc](OAc)_2$: Yield = 50%, Black, 252ºC, 146.21 ohm^{-1} cm^2 mol^{-1}.
anal. Calcd.% : C =59.89, H =6.20, N =9.31, Cr=8.64
Found (%) : C =59.28, H =6.06, N =9.19, Cr=8.45

For $[Fe(C_{24}H_{28}N_4)NO_3](NO_3)_2$: Yield = 54%, Dark Brown, 242ºC, 151.16 ohm^{-1} cm^2 mol^{-1}.
anal. Calcd.% : C =46.92, H =4.59, N =15.96, Fe=9.09
Found (%) : C =46.45, H =4.21, N =15.23, Fe=8.82

For $[Fe(C_{24}H_{28}N_4)Cl](Cl)_2$: Yield = 55%, Reddish Brown, 255ºC, 159.69 ohm^{-1} cm^2 mol^{-1}.
anal. Calcd.% : C =53.90, H=5.27, N =10.48, Fe=10.44
Found (%) : C =53.06, H =5.01, N =10.03, Fe=9.91

For $[Fe(C_{24}H_{28}N_4)OAc](OAc)_2$: Yield= 49%, Brown, 250ºC, 141.15 ohm^{-1} cm^2 mol^{-1}.
anal. Calcd.% : C =59.51, H =6.15, N =9.25, Fe=9.22
Found (%) : C =59.07, H =5.94, N =9.05, Fe =9.04

Infrared Spectral Study of Chromium and Iron Complexes

IR spectra of 3,4-diaminotoluene, acetylacetone and all macrocyclic metal complexes were recorded for comparative studies. All macrocyclic complexes exhibited IR peak of medium intensity in the region 1600-1625 cm^{-1} corresponding to C=N group which was not observed in IR spectra of 3,4-diaminotoluene and acetylacetone itself. The appearance of this new peak in the IR spectra of all complexes confirmed the complete condensation between 3,4-diaminotoluene and acetylacetone. Generally, the IR peak corresponding to C=N group is observed in the range ~1645-1690 cm^{-1}, however, in the IR spectra of all complexes this peak appeared at a lower wavenumber. The decrease in wavenumber of the peak corresponding to (C=N) stretch in all complexes may be justified based on coordination between the nitrogen of C=N group and metal ion[14] resulting in weakening of C=N bond. The IR peaks in the region 410-460 cm^{-1} may be assigned to(M-N) stretching while the peaks observed in the range 310-330 cm^{-1} corresponds to (M-Cl) vibration. The peaks corresponding to (M-O) stretching vibration were observed within the range 200-240 cm^{-1}.

Magnetic Studies and Electronic Spectral Studies

For $[Cr(C_{24}H_{28}N_4)Cl](Cl)_2$, $[Cr(C_{24}H_{28}N_4)NO_3](NO_3)_2$, $[Cr(C_{24}H_{28}N_4)OAc](OAc)_2$

Magnetic moment (at R.T.) of chromium complexes was observed between 4.20-4.55 B.M. This indicates that metal ions in the above-mentioned complexes contain three unpaired electrons. Electronic spectra of the all chromium complex showed bands in the range; 1070-1088, 695-750, 520-560 and 341-340 nm. The various bands observed in electronic spectra of the chromium complexes may be attributed to the following electronic transitions respectively, $^4B_1 \rightarrow {}^4E^a$, $^4B_1 \rightarrow {}^4B_2$, $^4B_1 \rightarrow {}^4A_2$, $^4B_1 \rightarrow {}^4E^b$. Further, five coordinated complexes chromium complexes having square pyramidal geometry reported in the literature have exhibited the electronic transition in the same region[15] and thus these complexes are supposed to have square pyramidal geometry.

For $[Fe(C_{24}H_{28}N_4)Cl](Cl)_2$, $[Fe(C_{24}H_{28}N_4)NO_3](NO_3)_2$, $[Fe(C_{24}H_{28}N_4)OAc](OAc)_2$

All of these complexes showed μeff between 4.20-4.55 B.M., suggesting five unpaired electrons in the metal ion. The electronic transitions for these complexes were recorded in the range; 990-1008 nm (corresponds to $d_{xy} \rightarrow d_{xz}/d_{yz}$transition), 655-665 nm (corresponds to $d_{xy} \rightarrow d_{x^2-y^2}$transition) and 361-370 nm (corresponds to $d_{xy} \rightarrow d_{z^2}$ transition). All of these transitions are in good agreement with transitions shown by already reported five coordinated complexes having square pyramidal geometry.[16]

Mass Spectra

m/z value of molecular ion i.e. $[M]^+$ of these complexes gave idea about the molecular weight of these complexes [Fig.-1]. The results so obtained are in close agreement with the calculated M.W.

For $[Cr(C_{24}H_{28}N_4)NO_3](NO_3)_2$, m/z =611.29; Cacld. M.W.=610.51

$[Cr(C_{24}H_{28}N_4)Cl](Cl)_2$, m/z =529.14; Cacld. M.W.=530.86

$Cr(C_{24}H_{28}N_4)OAc](OAc)_2$, m/z =619.14; (include weight of moisture also) Cacld. M.W.=601.63

$[Fe(C_{24}H_{28}N_4)NO_3](NO_3)_2$ m/z =616.29;Cacld. M.W.=614.37

$[Fe(C_{24}H_{28}N_4)Cl](Cl)_2$, m/z=534.14;Cacld. M.W.=534.71

$[Fe(C_{24}H_{28}N_4)OAc](OAc)_2$, m/z =605.09;Cacld. M.W.=605.48

PXRD Studies

The PXRD pattern of complexes $[Cr(C_{24}H_{28}N_4)NO_3](NO_3)_2$ [Fig.-2] and $[Fe(C_{24}H_{28}N_4)OAc](OAc)_2$ [Fig.-3], respectively, showed sharp peaks indicating the crystalline nature of the complexes. To calculate various lattice dimensions, a computer program (Full proof suite software via dicvol04) was used.[17] By comparison of observed and calculated 2q values, the indexing of parameters was confirmed. The calculated values of miller indices (h,k,l) and interplanar-spacing for $[Cr(C_{24}H_{28}N_4)NO_3](NO_3)_2$ and $[Fe(C_{24}H_{28}N_4)OAc](OAc)_2$ complexes are shown in [Table-3] and [

Table-4], respectively. In case of complex $[Cr(C_{24}H_{28}N_4)NO_3](NO_3)_2$ the various dimensions of unit cell may be given as; a =4.6068, b =4.1441, c =4.5754; α =76.277 β =109.419, γ =110.387 and volume =76.49 ;while in case of $[Fe(C_{24}H_{28}N_4)OAc](OAc)_2$ the parameters of unit cell may be given as a =7.8330, b =4.8910, c =3.428; α =76.277 β =94.986, γ =110.387 and volume =76.49. Both of these complexes satisfy conditions $\alpha \neq \beta \neq \gamma$ and $a \neq b \neq c$ which indicate that the complexes under study may belong to triclinic crystal system.

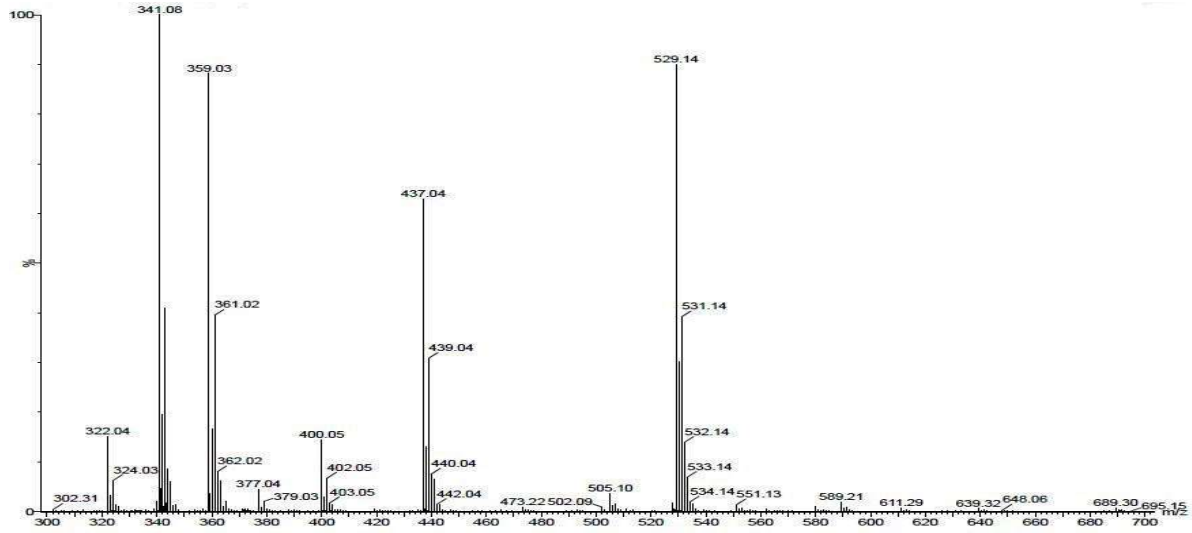

Fig.-1: Mass Spectrum of $[Cr(C_{24}H_{28}N_4)Cl](Cl)_2$

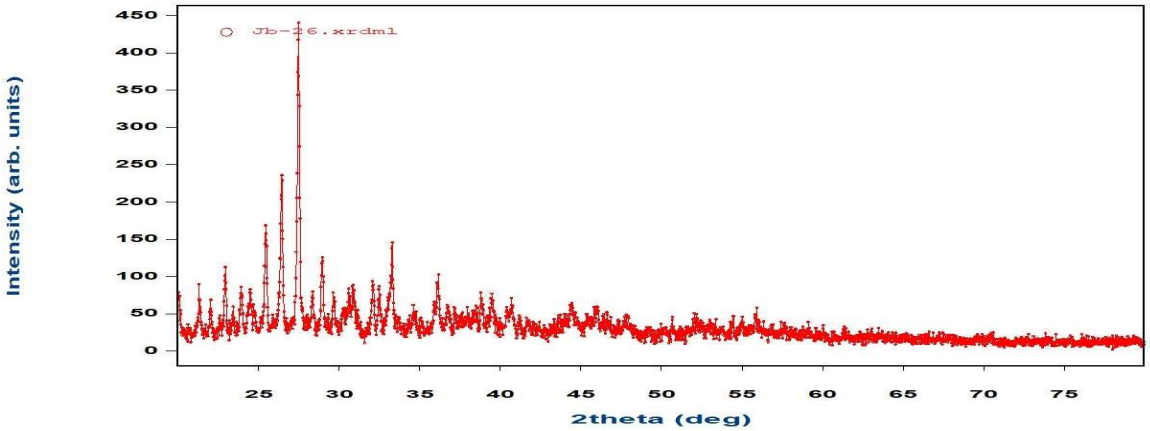

Fig.-2: Powder XRD Diffractogram of $[Cr(C_{24}H_{28}N_4)NO_3](NO_3)_2$

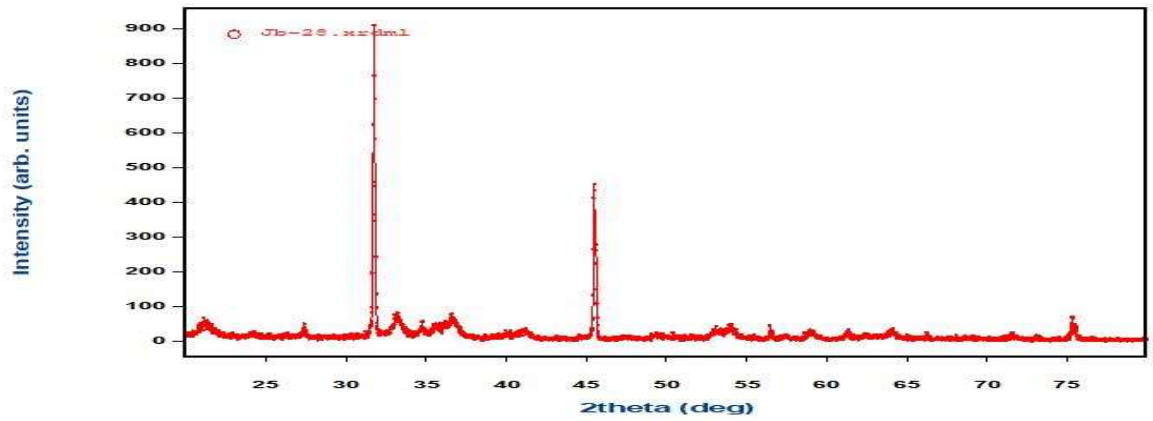

Fig.-3: Powder XRD Diffractogram of $[Fe(C_{24}H_{28}N_4)OAc](OAc)_2$

Table-3: XRD Data of $[Cr(C_{24}H_{28}N_4)NO_3](NO_3)_2$

H	K	L	DOB	DCAL	2TH.	OBS	2TH. CAL	DIFF.2TH
1	0	0	4.15863	4.15918	-0.00056	21.349	21.346	0.003
0	1	0	3.85334	3.85337	-0.00003	23.063	23.063	0.000
1	0	-1	3.50122	3.50078	0.00044	25.419	25.422	-0.003
1	-1	0	3.36203	3.36160	0.00043	26.490	26.494	-0.003
1	-1	-1	3.23376	3.23416	-0.00040	27.561	27.558	0.003
0	1	1	3.08134	3.08115	0.00019	28.954	28.956	-0.002
0	1	-1	2.68489	2.68505	-0.00016	33.345	33.343	0.002
1	1	0	2.48407	2.48406	0.00001	36.130	36.130	0.000

Table-4: XRD Data of $[Fe(C_{24}H_{28}N_4)OAc](OAc)_2$

H	K	L	DOBS.	DCAL.	DOBS-DCAL	2TH.OBS	2TH.CAL.	DIFF.2TH
1	1	0	4.15863	4.15714	0.00148	21.349	21.357	-0.008
1	0	-1	3.23376	3.23863	-0.00487	27.561	27.519	0.042
0	1	1	2.80781	2.80403	0.00377	31.486	31.890	-0.044
2	0	-1	2.69329	2.69198	0.00132	33.238	33.255	-0.017
1	1	1	2.58039	2.58117	-0.00078	34.738	34.727	0.011
0	2	0	2.44901	2.44983	-0.00082	36.666	36.653	0.013
0	2	1	1.98963	1.99043	-0.00080	45.556	45.536	0.019

Thermogravimetric Studies

The thermogravimetric analyses of all synthesized complexes were carried out at a temperature ranging from 20°C to 800°C (under N_2 atmosphere; rate of heating=10°C/min). The initial decomposition of the complexes, at temperatures 60-120°C may be attributed to the loss of the moisture from the complexes. No significant decomposition pattern was observed from 120° to 260°C, suggesting the absence of water inside or outside the coordination sphere.[18] This also indicated about the complexes were thermally stable up to 260°C. Further weight loss of the complexes between 260-420°C was observed due to the gradual breakdown of the organic macrocyclic ligand moiety.

Removal of Methylene Blue Dye From Aqueous Solution by Fe(III) Complexes

The Fe(III) complexes were added gradually, to the aqueous solution of methylene blue dye. After 30 minutes, a decrease in the intensity of blue color in the aqueous solution of dye was observed. The effect of the amount of Fe(III) complexes (05 mg – 25 mg) on the removal of dye from aqueous solution was evaluated at constant temperature (25°C) while maintaining the pH between 7-8. It was found that the dye removal efficiency of Fe(III) complexes enhanced with an increase in their amount, respectively[Fig.-4].

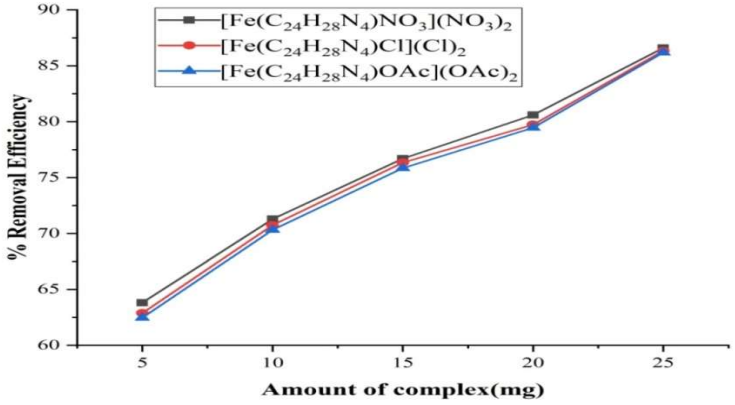

Fig.-4: Effect of Amount of Complexes on the Removal of Methylene Dye From Aqueous Solution

Biological Results and Discussion

Minimum Inhibitory Concentration

The results of biological studies revealed that all complexes exhibited remarkable antibacterial and antifungal activity against the different test pathogens under study except one fungal strain, i.e.

Macrophomina phaseolina. The complexes showed MIC in the range of 20 to >100 µg/ml against different microbial strains [Table-2]. However, each of the standard drugs showed 10 µg/ml MIC value against each microbial strain under test. Among all complexes, $[Fe(C_{24}H_{28}N_4)NO_3](NO_3)_2$ showed the best antibacterial activity against *Paenibacillus polymyxa* (20 µg/ml) [Fig.-5].

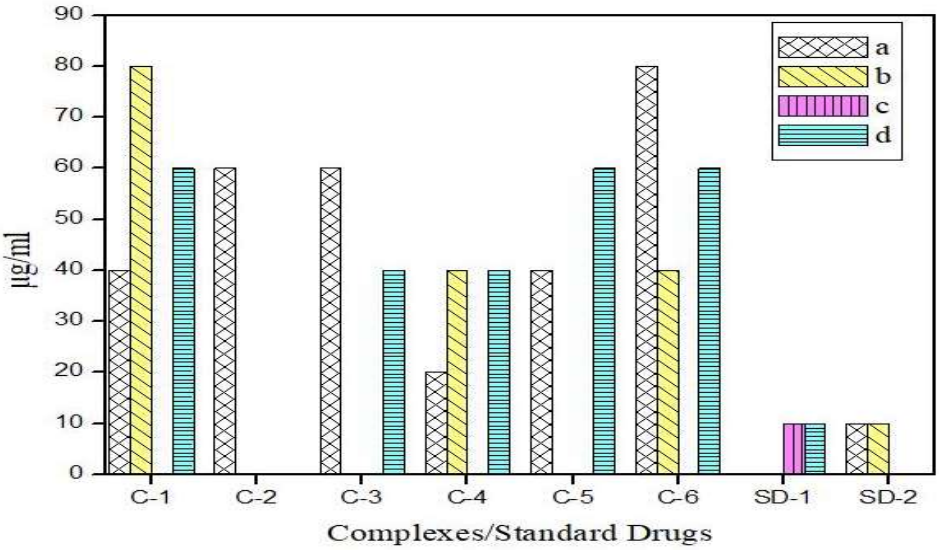

Fig.-5: Minimum Inhibitory Concentration of the Complexes and Standard Drugs

Where, a=*Paenibacillus polymyxa*; b=*Pseudomonas aeruginosa*; c=*Macrophomina phaseolina*; d=*Alternaria brassicae*; and SD-1= Amphotericin-B (Antifungal Drug); SD-2= Rifampicin Standard drug.

Docking

The results of docking of a hydrolase from *Paenibacillus polymyxa*(PDB id: **2O9P**) with complex $[Fe(C_{24}H_{28}N_4)NO_3](NO_3)_2$ using Autodock 4.2 software are given in [Table-5]. The docking results showed that out of 10 best runs, the third run exhibited the lowest binding energy -8.18 kcal/mol and RMSD 71.43.

Table-5: Binding Energy of Hydrolase from *Paenibacillus polymyxa* with $[Fe(C_{24}H_{28}N_4)NO_3](NO_3)_2$.

Run	Binding Energy	RMSD
1st	-6.74	92.18
2nd	-6.27	88.90
3rd	-8.18	71.43
4th	-7.27	64.07
5th	-7.30	72.04
6th	-6.15	90.96
7th	-7.28	63.95
8th	-5.90	79.51
9th	-5.74	79.86
10th	-7.22	96.43

In the third run, various types of interactions of the complex $[Fe(C_{24}H_{28}N_4)NO_3](NO_3)_2$ with hydrolase from *Paenibacillus polymyxa*(PDB id: **2O9P**) were scrutinized using "Discovery Studio 2020" software. It was observed that GLU A: 225, LEU A: 174, ARG A: 243, GLU A: 167, CYS A: 170, TYR A: 298, TRP A: 123, ILE A: 247, TRP A: 410, TRP A: 328. of hydrolase bind significantly with the complex *via* different interactions at various distances [Fig.-6].

Antioxidant Activity

A significant effect on the antioxidant activity of the complexes was observed with variation in the concentration of the complexes**.** All macrocyclic complexes were found to possess moderate to excellent antioxidant activity. Among all reported complexes; complexes 2 and 3 [Table-6] showed the best antioxidant activity having IC50 value lesser than 10ug/ml. Further, complexes 1, 4 and 6 have also

shown remarkable antioxidant activity with an IC50 value in the range of 10-20 µg /ml. In contrast, complex 5 registered the least antioxidant activity. Higher antioxidant activity observed in Cr(III) complexes (d^3) may be attributed to their virtue of more electron affinity as compared to half-filled Fe(III) (d^5) complexes.[19]

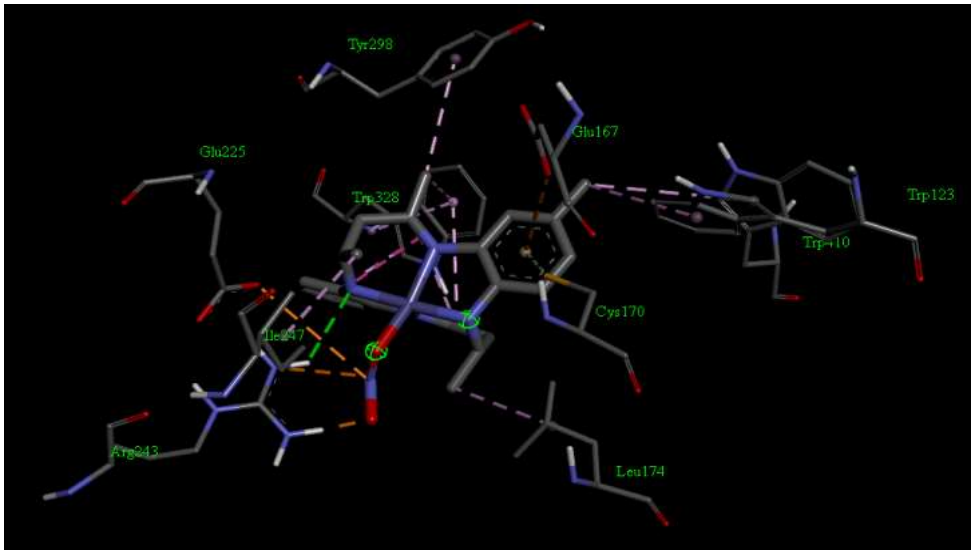

Fig.-6: Best Docking Score (Run 3rd: -8.18 kcal/mol) of the interaction of $[Fe(C_{24}H_{28}N_4)NO_3](NO_3)_2$ with hydrolase from *Paenibacillus polymyxa.*

Table-6: Percentage Antioxidant Activity of the Complexes

Complex No.	Complexes	10µg	20 µg	30 µg	40 µg
		% Antioxidant Activity			
	Ascorbic Acid	46.06	50.9	53.78	58.93
1.	$[Cr(C_{24}H_{28}N_4)NO_3](NO_3)_2$	52.72	59.54	67.57	71.51
2.	$[Cr(C_{24}H_{28}N_4)Cl](Cl)_2$	41.36	46.06	52.57	59.24
3.	$[Cr(C_{24}H_{28}N_4)OAc](OAc)_2$	51.21	58.63	64.39	70.15
4.	$[Fe(C_{24}H_{28}N_4)NO_3](NO_3)_2$	36.36	42.12	44.54	54.39
5.	$[Fe(C_{24}H_{28}N_4)Cl](Cl)_2$	43.33	48.33	54.69	60.3
6.	$[Fe(C_{24}H_{28}N_4)OAc](OAc)_2$	48.18	56.36	61.21	67.27

CONCLUSION

Macrocyclic complexes of Cr(III) and Fe(III) metal ions were synthesized and characterized with the help of various spectroscopic and physicochemical techniques. All these studies suggested that complexes have five coordinated square pyramidal geometry. Besides this, *in vitro* biological and antioxidant activities of all complexes were carried out and it was observed that complex $[Fe(C_{24}H_{28}N_4)NO_3](NO_3)_2$ exhibited remarkable antibacterial activity while $[Cr(C_{24}H_{28}N_4)NO_3](NO_3)_2$ showed best antioxidant activity. In molecular docking studies, it was found that complex $[Fe(C_{24}H_{28}N_4)NO_3](NO_3)_2$ exhibited excellent binding affinity with hydrolase from *Paenibacillus polymyxa* (PDB id: 2O9P). Further, Fe(III) complexes were found to have excellent capability to remove the methylene blue dye from the aqueous solution.

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