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# Synthesis Characterisation and Application of Manganese Complex with Dafone and Methanol

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**Abstract:** Manganese complex with dafone and methanol was prepared by solvent based synthesis method. Ligand 4,5 diazafluoren-9-one and the complex were characterised by elemental analysis and various spectroscopic methods. Application of the complex as antimicrobial agent, and its cytotoxic & catalytic activities were studied. The complex show octahedral geometry and its activity as antimicrobial agent is average. It is moderately active against HeLa and fibroblast cell.

**Key word:** 4,5diazfluoren-9-one (dafone); minimum inhibition concentration (MIC), dimethyl formamide (DMF); dimethyl sulfoxide (DMSO); Lethal Concentration, 50% (LC50); dye compound 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide used for measuring the functionality of animal and human cells (MTT assay)

## I. INTRODUCTION

4, 5diazfluoren-9-one (dafone) is a bidentate ligand similar to 1,10 phenanthroline (phen) and bipyridine (bpy). It is a derivative of 1, 10 phenanthroline, having an exocyclic keto group<sup>[1],[12]</sup>.

DNA interaction properties<sup>[2]</sup> of dafone attracted attention of many researchers. Unlike phen/bpy, co-ordination chemistry of bidentate neutral ligand dafone is restricted to a few metals<sup>[3]-[9]</sup>. The presence of reactive exocyclic keto functional group in dafone make it suitable for further derivatization, to yield multinuclear metal complexes having interesting catalytical and biological properties<sup>[10]</sup>.

The two coordinating nitrogens in dafone have a larger bite distance of 2.99Å<sup>0</sup> compared to phen (2.65Å<sup>0</sup>), resulting in unusual coordination modes and differences in electrochemical and biological properties<sup>[11]</sup>. The difference in the binding properties of phen and dafone may be because of the reduced overlap of nitrogen orbitals due to the larger bite distance in dafone. In this work, we have prepared and characterise a complex of manganese with dafone and methanol and its application in various areas were studied.

## II. EXPERIMENTAL

### A. Synthesis of Dafone

4.71g/.024mol 1,10 Phenanthroline monohydrate and 2.444g/.044mol KOH were added to 50ml of water and brought to reflux in an RB flask. 12.156g/.076mol KMnO<sub>4</sub> was dissolved in 100ml of water and was added drop by drop to the refluxing mixture. After the addition of KMnO<sub>4</sub>, the solution was refluxed for half an hour more and filtered to remove MnO<sub>2</sub>. On cooling the solution, dafone was precipitated as yellow needles<sup>[13]</sup>.

### B. Synthesis of [Mn(dafone)<sub>2</sub> (CH<sub>3</sub>OH)<sub>2</sub>] 2(ClO<sub>4</sub>) 6H<sub>2</sub>O

0.3619gm/1mmol Manganese perchlorate and 0.364gm/2mmol dafone were dissolved separately in minimum amount of methanol. Dafone in methanol solution was added slowly to Manganese perchlorate solution which is kept undisturbed. Yellow needles were formed slowly.

## III. RESULTS AND DISCUSSION

### A. General Properties

The colour and other physical properties are listed in table.1. The elemental analysis in Table.2 indicates the stoichiometry of the complex. The complex was highly soluble in DMSO and DMF and slightly soluble in methanol. Structure of the complex was concluded from elemental analysis and molar conductance value (table 3).

Table.1. Physical and analytical data of the ligand and complexes

Ligand/ Complex	Color	Mol.Wt	M.P(°C)	Yield (%)
Ligand - Dafone	Yellow-orange needles	182	140 <sup>0</sup> C	25
[Mn(dafone) <sub>2</sub> (CH <sub>3</sub> OH) <sub>2</sub> ] 2(ClO <sub>4</sub> ) 6H <sub>2</sub> O	yellow	788.94	320 <sup>0</sup> C	89.85

Table. 2. Elemental analysis data of complexes of dafone

Ligand/ Complex	C	H	N	Metal
Ligand - Dafone	65.76 (66.0)	2.74 (4.0)	13.77 (14.0)	-
[Mn(dafone) <sub>2</sub> (CH <sub>3</sub> OH) <sub>2</sub> ] 2(ClO <sub>4</sub> ) 6H <sub>2</sub> O	36.46 (36.5)	3.09 (4.05)	7.68 (7.00)	6.0 (6.96)

CHN analysis was done in SAIF STIC Cochin and metal percentage was found out gravimetrically.

*B. Molar conductance*

Table. 3. Molar Conductance value of the complex

Complex	Molar Conductance
[Mn(dafone) <sub>2</sub> (CH <sub>3</sub> OH) <sub>2</sub> ] 2(ClO <sub>4</sub> ) 6H <sub>2</sub> O	143.7

Molar conductance value was measured by preparing 10<sup>-3</sup>M solution of the complex in DMF. High molar conductance value indicate that the complex is an electrolyte contain ions in 1:2 ratio<sup>[14]</sup>.

*C. Characterisation of the ligand*

Ligand 4,5 diazafluren-9-one (dafone) was characterised through elemental analysis and various spectral studies.

- <sup>1</sup>H NMR spectroscopy:* <sup>1</sup>H NMR spectra of the synthesized ligand was recorded in CDCl<sub>3</sub> solvent, at the National Institute for Interdisciplinary Science and Technology, Thiruvananthapuram. The multiplets within the range δ = 8.73, 7.94 and 7.26ppm correspond to hydrogen of dafone.

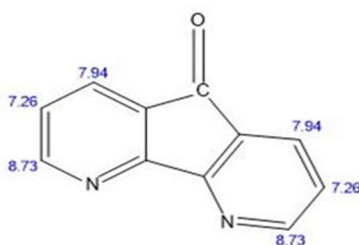


Fig.1. Structure of dafone

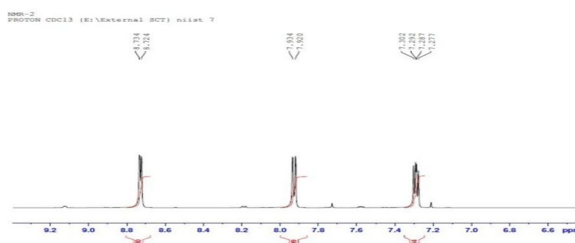


Fig.2. <sup>1</sup>H NMR spectra of dafone

2) *Infrared Spectroscopy*: IR Spectrum of dafone shows three characteristic bands, whose wavelengths corresponding to the stretching vibrations of its three types of bonds:  $3304\text{ cm}^{-1}$  (C-H str.aromatic),  $1714\text{ cm}^{-1}$  (C=O) and  $1461\text{ cm}^{-1}$  (C=N).

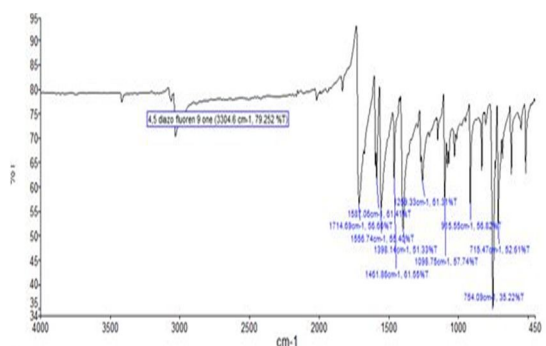


Fig..3. IR Spectrum of Dafone

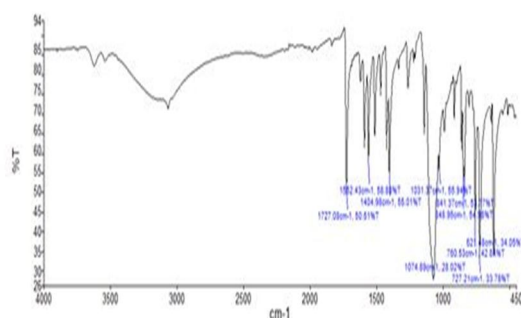


Fig..4. IR Spectrum of  $[\text{Mn}(\text{dafone})_2 (\text{CH}_3\text{OH})_2] 2(\text{ClO}_4) 6\text{H}_2\text{O}$

D. *Characterisation of the Complexes*

1) *Infrared spectroscopy of the complex*

Table. 4. Infrared spectral bands ( $\text{cm}^{-1}$ ) of dafone and its complexes

Ligand/ Complex	$\nu$ C=O	$\nu$ C=N
Ligand - Dafone	1714	1556
$[\text{Mn}(\text{dafone})_2 (\text{CH}_3\text{OH})_2] 2(\text{ClO}_4) 6\text{H}_2\text{O}$	1727	1552

The infrared spectral assignments in  $\text{cm}^{-1}$  of the ligand and complex are tabulated in table.4 and spectra was given in fig.2. The broad band at  $3000\text{cm}^{-1}$  in the case of the complex  $[\text{Mn}(\text{dafone})_2 (\text{CH}_3\text{OH})_2] 2(\text{ClO}_4) 6\text{H}_2\text{O}$  may be due to the presence of lattice water. Peak at  $1714\text{-}1727\text{cm}^{-1}$  and  $1552\text{-}1592\text{ cm}^{-1}$  indicate the stretching vibrations of C=O and C=N group respectively.

2) *Electronic Spectroscopy*

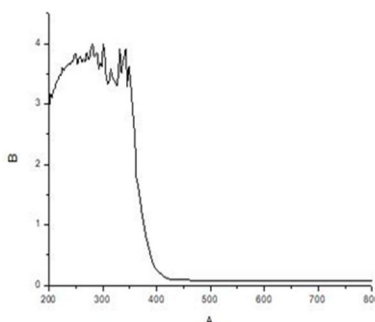


Fig.5. UV Spectrum of dafone

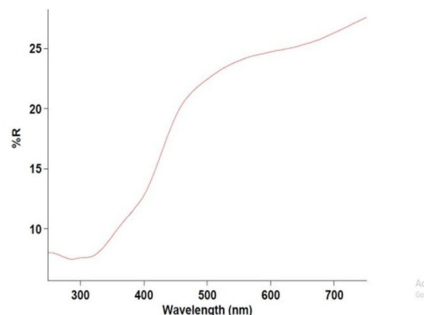


Fig.6. Solid state UV Spectrum of the complex

Table. 5. Electronic spectral bands ( $\text{cm}^{-1}$ ) of dafone and its complexes

Ligand/ Complex	Bands (nm)	Assigned Transitions	Geometry
Ligand - Dafone	300 360	$n \rightarrow \pi^*$ $\pi \rightarrow \pi^*$	
$[\text{Mn}(\text{dafone})_2 (\text{CH}_3\text{OH})_2] 2(\text{ClO}_4) 6\text{H}_2\text{O}$	480 700	${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{2g}$ ${}^4\text{T}_{1g} \rightarrow {}^4\text{A}_{2g}$	Octahedral

Absorption band at 480nm and 700nm in the case of  $[\text{Mn}(\text{dafone})_2 (\text{CH}_3\text{OH})_2] 2(\text{ClO}_4) 6\text{H}_2\text{O}$  may be due to the excitation of electron from  ${}^6\text{A}_{1g}$  to  ${}^4\text{T}_{2g}$  and  ${}^4\text{T}_{1g}$  to  ${}^4\text{A}_{2g}$  level indicate the octahedral structure of the complex.

#### E. Computational Analysis

Programme Gaussian 09 and base set B3lyp/6-311G(d,p) was used for the computational analysis.

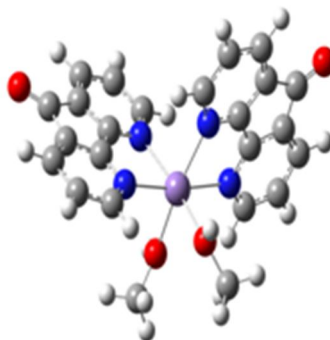


Fig.7. Optimised structure of  $[\text{Mn}(\text{dafone})_2 (\text{CH}_3\text{OH})_2] 2(\text{ClO}_4) 6\text{H}_2\text{O}$  from computational analysis

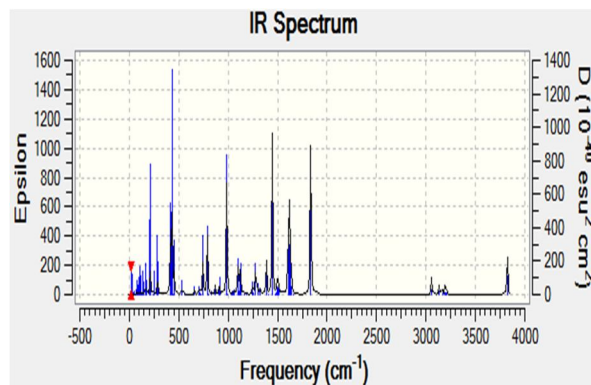


Fig.8. IR Spectrum of  $[\text{Mn}(\text{dafone})_2 (\text{CH}_3\text{OH})_2] 2(\text{ClO}_4) 6\text{H}_2\text{O}$  from computational analysis

#### IV. APPLICATION

##### A. Antimicrobial Activity

Antimicrobial activity MIC analysis and cytotoxicity studies were conducted from Biogenix Research centre, Thiruvananthapuram. It was done by agar-well diffusion method.

Procedure: The antimicrobials present in the samples are allowed to diffuse out into the medium (the medium was prepared by dissolving 33.8 g of the commercially available Muller Hinton Agar Medium (MHI Agar Media) in 1000ml of distilled water and it was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten and interact in a plate freshly seeded with the test organisms the gram positive bacteria, *Staphylococcus aureus* (ATCC 25923) and the gram negative bacteria *Pseudomonas aeruginosa* (ATCC 27853). Streptomycin was used as a positive control. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimetres<sup>[15]</sup>. Clotrimazole (concentration: 10mg / ml) was used as a standard antifungal agent<sup>[16]</sup>.

##### 1) Antibacterial Activity

Table.6. Antibacterial activity, Zone of inhibition (mm) of ligands

Complex	Concentration (µg/mL)	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>
[Mn(dafone) <sub>2</sub> (CH <sub>3</sub> OH) <sub>2</sub> (ClO <sub>4</sub> ) <sub>2</sub> 6H <sub>2</sub> O	Streptomycin (100µg)	19	23
	250	NIL	11
	500	NIL	12
	1000	NIL	13

From the results it was clear that [Mn(dafone)<sub>2</sub> (CH<sub>3</sub>OH)<sub>2</sub>] 2(ClO<sub>4</sub>) 6H<sub>2</sub>O was active against the gram positive bacteria *Staphylococcus aureus* but not against gram negative bacteria *Pseudomonas aeruginosa*



Fig.9. Activity of [Mn(dafone)<sub>2</sub> (CH<sub>3</sub>OH)<sub>2</sub>] 2(ClO<sub>4</sub>) 6H<sub>2</sub>O against gram negative bacteria *Pseudomonas aeruginosa*



Fig.10. Activity of [Mn(dafone)<sub>2</sub> (CH<sub>3</sub>OH)<sub>2</sub>] 2(ClO<sub>4</sub>) 6H<sub>2</sub>O against gram positive bacteria *Staphylococcus aureus*

2) Antifungal Activity

Table.7. Antifungal activity, Zone of inhibition (mm) of ligands

Complex	Concentration (µg/mL)	Candida albicans
[Mn(dafone) <sub>2</sub> (CH <sub>3</sub> OH) <sub>2</sub> ] 2(ClO <sub>4</sub> ) 6H <sub>2</sub> O	Clotrimazole	28
	250	Nil
	500	Nil
	1000	Nil

From the results it was clear that [Mn(dafone)<sub>2</sub>(CH<sub>3</sub>OH)<sub>2</sub>] 2(ClO<sub>4</sub>) 6H<sub>2</sub>O were not active against the fungus Candida albicans.



Fig.11. Activity of [Mn(dafone)<sub>2</sub>(CH<sub>3</sub>OH)<sub>2</sub>] 2(ClO<sub>4</sub>) 6H<sub>2</sub>O against the fungus Candida albicans

3) *Minimum inhibitory concentration (MIC) analysis*<sup>[17]</sup>: Minimum inhibitory concentration (MIC) is the lowest concentration of an anti microbial (like an antifungal antibiotic or bacteriostatic) drug that will inhibit the visible growth of a microorganism after overnight incubation.

Procedure: Minimal inhibitory concentration (MIC) was determined by using two fold serial dilution method. The growth of stock inoculum of test organisms (*Pseudomonas aeruginosa* and *Staphylococcus aureus*) was adjusted to 1% McFarland Standard. The broth dilution assay was done in 96 well microtiterplate. Each wells in the plate were added with 100µl of the diluted (two times) conidial inoculum suspensions (final volume in each well, 200 µl). Sample was dissolved in DMSO to a final concentration of 10mg/mL and was added in increasing concentration such as 62.5µg, 125µg, 250µg, 500µg, 1000µg to the wells respectively and incubated overnight at room temperature. A control well was kept with organism alone. Growth was observed by visual inspection and by measuring the optical density (OD) at 630 nm using an ELISA plate reader. The OD was measured immediately after the visual reading. The growth inhibition for the test wells at each extract dilution was determined by the formula:

$$\text{Percentage of inhibition} = (\text{OD of control} - \text{OD of test}) / (\text{OD of control}) \times 100$$

The results are given in table.8

Table.8. % Inhibition value of [Mn(dafone)<sub>2</sub>(CH<sub>3</sub>OH)<sub>2</sub>] 2(ClO<sub>4</sub>) 6H<sub>2</sub>O against *Staphylococcus aureus*

Organism	OD at 630 nm	% Inhibition
Control, <i>Staphylococcus aureus</i>	0.54905	0
Sample conc.in µg	OD at 630 nm	% Inhibition
62.5µg	0.1144	79.164%
125 µg	0.0462	91.585%
250 µg	0.0334	93.916%
500 µg	0.0178	96.758%
1000 µg	0.0109	98.0147%

MIC: 58.8076µg

**B. Cytotoxic Studies**

Cytotoxicity is the quality of being toxic to cells. Invitro toxicity is the scientific analysis of the effects of toxic chemical substances on cultured bacteria or mammalian cells.  $[Mn(dafone)_2 (CH_3OH)_2] 2(ClO_4) 6H_2O$  was tested for anticancer activity by standatd MTT Assay in Cervical carcinoma cells (HeLa) and Fibroblast cells (L929). Metal coordination complexes had been widely studied for their anticancer activities<sup>[18]-[31]</sup>. In this paper we conduct cytotoxicity studies of the complex  $[Mn(dafone)_2 (CH_3OH)_2] 2(ClO_4) 6H_2O$  against Cervical carcinoma cells (HeLa) and Fibroblast cells (L929).

Procedure: Cells are procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained in Dulbecco’s modified Eagles medium, DMEM (Sigma aldrich, USA). The cell line was cultured in 25 cm<sup>2</sup> tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate (Merck, Germany) and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphoteracin B (2.5µg/ml). Cultured cell lines were kept at 37°C in a humidified 5% CO<sub>2</sub> incubator (NBS Eppendorf, Germany). The viability of cells were evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method. Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension (5x10<sup>3</sup> cells/well) was seeded in 96 well tissue culture plate and incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator. 1mg of sample was weighed and dissolved in 1mL DMEM using a cyclomixer. The sample solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility. After 24 hours the growth medium was removed, freshly prepared each compounds in DMEM were five times serially diluted by two fold dilution (100µg, 50µg, 25µg, 12.5µg, 6.25µg in 500µl of DMEM) and each concentration of 100µl were added in triplicates to the respective wells and incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator. Non treated control cells were also maintained. Entire plate was observed after 24 hours of treatment in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity. 15ml of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization. After 24 hours of incubation period, the sample content in wells were removed and 30µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT Solubilization Solution (Dimethyl sulphoxide,DMSO, Sigma Aldrich, USA) was added and the wells were mixed gently by pipetting up and down in order to solubilize the crystals. The absorbance values were measured by using microplate reader at a wavelength of 540 nm (Laura B. Talarico et al., 2004).

The percentage of growth inhibition was calculated using the formula:

$$\text{Percentage of viability} = \frac{\text{Mean OD Samples} \times 100}{\text{OD of Control group}}$$

1) *Action of  $[Mn(dafone)_2 (CH_3OH)_2] (ClO_4)_2 6H_2O$  against HeLa cells:* Action of  $[Mn(dafone)_2 (CH_3OH)_2] 2(ClO_4) 6H_2O$  against HeLa cells was given in table.10. Concentration of the complex that can kill 50% of the HeLa cells (LC50 value) was found to be 233.714µg/mL

Table .9. % viability of  $[Mn(dafone)_2 (CH_3OH)_2] 2(ClO_4) 6H_2O$  against HeLa cells

Sample conce. in µg/mL	OD value I	OD value II	OD value III	Average OD	Percentage Viability
Control	0.8509	0.8566	0.8432	0.8502	100.00
6.25	0.7869	0.7977	0.7844	0.7897	92.88
12.5	0.7533	0.7418	0.7409	0.7453	87.67
25	0.7122	0.7039	0.7182	0.7114	83.68
50	0.6834	0.6766	0.6695	0.6765	79.57
100	0.6322	0.6411	0.6301	0.6345	74.63

LC50 Value : 233.714 µg/mL (Calculated using ED50 PLUS V1.0 Software)

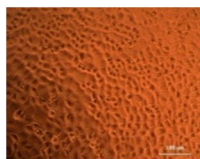


Fig.12. Microscopic image of Cervical carcinoma cells (HeLa) without  $[\text{Mn}(\text{dafone})_2 (\text{CH}_3\text{OH})_2] 2(\text{ClO}_4) 6\text{H}_2\text{O}$  (control)

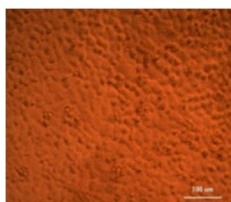


Fig.13. Microscopic image of Cervical carcinoma cells (HeLa) when treated with 6.25 μg/mL  $[\text{Mn}(\text{dafone})_2 (\text{CH}_3\text{OH})_2] 2(\text{ClO}_4) 6\text{H}_2\text{O}$

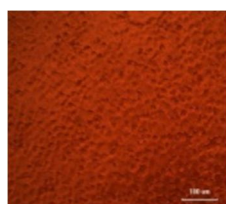


Fig.14. Microscopic image of Cervical carcinoma cells (HeLa) when treated with 12.5 μg/mL  $[\text{Mn}(\text{dafone})_2 (\text{CH}_3\text{OH})_2] 2(\text{ClO}_4) 6\text{H}_2\text{O}$

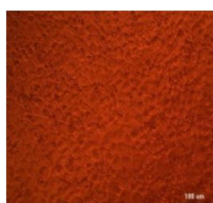


Fig.15. Microscopic image of Cervical carcinoma cells (HeLa) when treated with 25 μg/mL  $[\text{Mn}(\text{dafone})_2 (\text{CH}_3\text{OH})_2] 2(\text{ClO}_4) 6\text{H}_2\text{O}$

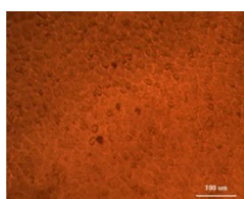


Fig.16. Microscopic image of Cervical carcinoma cells (HeLa) when treated with 50 μg/mL  $[\text{Mn}(\text{dafone})_2 (\text{CH}_3\text{OH})_2] 2(\text{ClO}_4) 6\text{H}_2\text{O}$

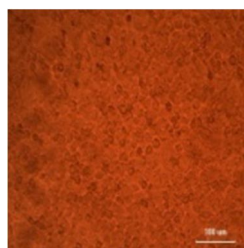


Fig.17. Microscopic image of Cervical carcinoma cells (HeLa) when treated with 100 μg/mL  $[\text{Mn}(\text{dafone})_2 (\text{CH}_3\text{OH})_2] 2(\text{ClO}_4) 6\text{H}_2\text{O}$

2) Action of  $[Mn(dafone)_2 (CH_3OH)_2] 2(ClO_4) 6H_2O$  against Fibroblast cells: Action of  $[Mn(dafone)_2 (CH_3OH)_2] (ClO_4)_2 6H_2O$  against Fibroblast cells was given in Table.11. Concentration of the complex that can kill 50% of the Fibroblast cells was found to be 143.252  $\mu g/mL$

Table .10. % viability of  $[Mn(dafone)_2 (CH_3OH)_2] 2(ClO_4) 6H_2O$  against Fibroblast cells

Sample Concentration ( $\mu g/mL$ )	OD value I	OD val	OD value III	Average OD	Percentage Viability
Control	0.8454	0.8493	0.8497	0.8481	100.00
6.25	0.8231	0.8257	0.8293	0.8260	97.40
12.5	0.7301	0.7332	0.7326	0.7320	86.31
25	0.6287	0.6251	0.6287	0.6275	73.99
50	0.5855	0.5889	0.5864	0.5869	69.21
100	0.5679	0.5613	0.5633	0.5642	66.52

LC50 Value : 143.252  $\mu g/mL$  (Calculated using ED50 PLUS V1.0 Software)

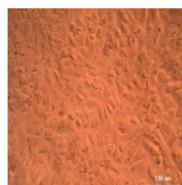


Fig.18. Microscopic image of Fibroblast (L929) without  $[Mn(dafone)_2 (CH_3OH)_2] 2(ClO_4) 6H_2O$  (control)

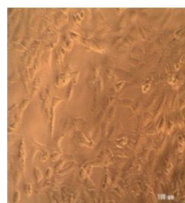


Fig.19. Microscopic image of Fibroblast (L929) when treated with 6.25  $\mu g/mL$   $[Mn(dafone)_2 (CH_3OH)_2] 2(ClO_4) 6H_2O$

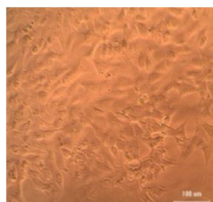


Fig.20. Microscopic image of Fibroblast (L929) when treated with 12.5  $\mu g/mL$   $[Mn(dafone)_2 (CH_3OH)_2] 2(ClO_4) 6H_2O$

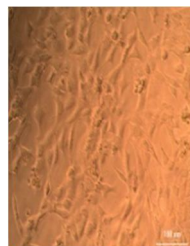


Fig.21. Microscopic image of Fibroblast (L929) when treated with 25  $\mu g/mL$   $[Mn(dafone)_2 (CH_3OH)_2] 2(ClO_4) 6H_2O$

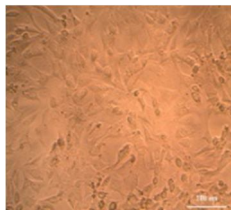


Fig.22. Microscopic image of Fibroblast (L929) when treated with 50 µg/ mL [Mn(dafone)<sub>2</sub> (CH<sub>3</sub>OH)<sub>2</sub>] 2(ClO<sub>4</sub>) 6H<sub>2</sub>O

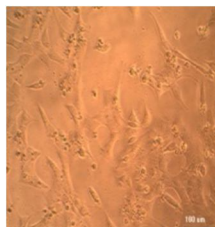


Fig.23. Microscopic image of Fibroblast (L929) when treated with 100 µg/ mL [Mn(dafone)<sub>2</sub> (CH<sub>3</sub>OH)<sub>2</sub>] 2(ClO<sub>4</sub>) 6H<sub>2</sub>O

Table .11. LC50 Value of the complex [Mn(dafone)<sub>2</sub> (CH<sub>3</sub>OH)<sub>2</sub>] (ClO<sub>4</sub>)<sub>2</sub> 6H<sub>2</sub>O

[Mn(dafone) <sub>2</sub> (CH <sub>3</sub> OH) <sub>2</sub> ] 2(ClO <sub>4</sub> ) 6H <sub>2</sub> O	Average LC50 Value in µg/mL
Cervical carcinoma cells	233.714
Fibroblast cells	143.252

### C. Catalytic Activity

One of the most important applications of coordination complexes is in its activity as catalysts. They can be selectively used as catalyst in various reactions such as oxidation, hydroxylation, aldol condensation and epoxidation<sup>[32]-[35]</sup>.

1) *Catalytic activities of dafone complexes on the basis of decomposition of Hydrogen Peroxide:* The decomposition of hydrogen peroxide has been used as a model reaction for the investigation of the catalytic activity of various metal complexes<sup>[36],[37]</sup>

Procedure: The metal complex (~ 2X10<sup>-4</sup> mmol) was mixed with 50ml of 10% H<sub>2</sub>O<sub>2</sub> in a flask at room temperature under constant stirring. Then the extent of hydrogen peroxide decomposed at different intervals of time was estimated through permanganometry. 1ml aliquot of reaction mixture was withdrawn in each 30 minutes and titrated against 0.02M KMnO<sub>4</sub> in presence of 1:5 H<sub>2</sub>SO<sub>4</sub> solution. The difference in titre values of permanganate solution before and after the catalysed decomposition was recorded<sup>[36,37]</sup>  
 % decomposition of H<sub>2</sub>O<sub>2</sub> = (C<sub>0</sub>-C<sub>t</sub>/C<sub>0</sub>) X 100

Table.12. Catalytic action of [Mn(dafone)<sub>2</sub> (CH<sub>3</sub>OH)<sub>2</sub>] 2(ClO<sub>4</sub>) 6H<sub>2</sub>O in the decomposition reaction of hydrogen peroxide

Time in minutes	Vol. Of KMnO <sub>4</sub> in ml	% decomposition
0	73.4	-
30	72.6	1.1
60	70.4	4.08
90	69.3	5.59
120	68.1	7.22
150	67.0	8.72
180	65.5	10.76

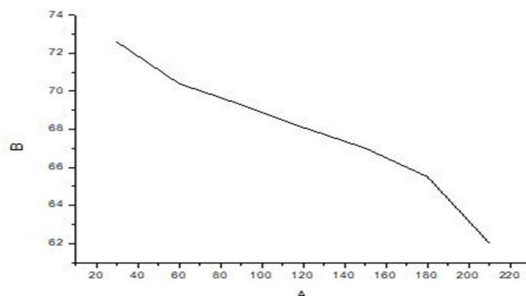


Fig.24. A-time in minutes; B-vol. of KMnO<sub>4</sub> in ml

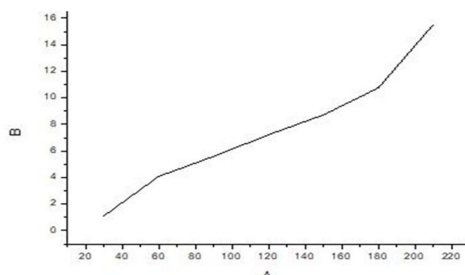


Fig.25. A-time in minutes; B-% decomp. of H<sub>2</sub>O<sub>2</sub>

2) *Photocatalytic activity of [Mn(dafone)<sub>2</sub>(CH<sub>3</sub>OH)<sub>2</sub>] 2(ClO<sub>4</sub>) 6H<sub>2</sub>O*: The degradation reaction of methylene blue dye has been used as a model reaction for the investigation of the photocatalytic activity of various metal complexes.

**Procedure:** The metal complexes (~0.01g) and aqueous solution of methylene blue (70ml) were mixed in a beaker under constant stirring at room temperature. The mixture was equilibrated by stirring in dark for thirty minutes to allow the adsorption of methylene blue dye, if any, by the complex. The solution is stirred under UV light. The sample was allowed to absorb UV light and 5ml aliquots were taken and filtered at a definite time interval of 30 minutes. Filtration was done to avoid errors due to scattering of UV radiation. The filtrate was analysed using UV-Visible spectrophotometer. The intensity of the absorption peak of methylene blue at 663nm gets diminished gradually with extension of the exposure time indicating the degradation of methylene blue dye.

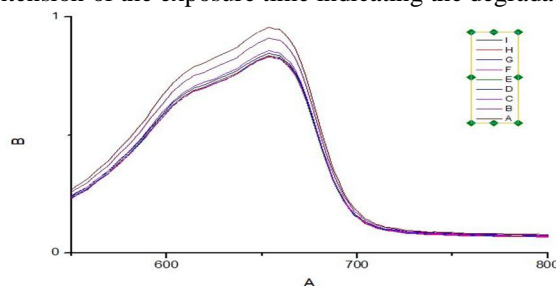


Fig.26. Photodegradation plot of methylene blue dye degradation under UV light using [Mn(dafone)<sub>2</sub>(CH<sub>3</sub>OH)<sub>2</sub>] 2(ClO<sub>4</sub>) 6H<sub>2</sub>O; A-dark; B-0min; C-30min; D-60min; E-90min; F-120min; G-150min; H-180min; I-210min;

## V. CONCLUSION

Complex [Mn(dafone)<sub>2</sub>(CH<sub>3</sub>OH)<sub>2</sub>] 2(ClO<sub>4</sub>) 6H<sub>2</sub>O was prepared by solvent based synthesis using acetonitrile/methanol as solvent. Characterisation of ligand and complexes were done by elemental and various spectral analysis. The complex shows octahedral geometry. Its application as antimicrobial agent was studied with the test organisms the gram positive bacteria, *Staphylococcus aureus* (ATCC 25923); the gram negative bacteria *Pseudomonas aeruginosa* (ATCC 27853) and the fungus *Candida albicans*. The complex was active against the gram positive bacteria *Staphylococcus aureus* but not against gram negative bacteria *Pseudomonas aeruginosa* and the fungus *Candida albicans*. Cytotoxicity studies are conducted on the complex and was found to be active against both HeLa and fibroblast cell. Its catalytic activity was studied by considering decomposition reaction of hydrogen peroxide and photocatalytic degradation of methylene blue dye. These complexes show low catalytic activity

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