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### RESEARCH ARTICLE

## Synthesis and Spectroscopic Investigation of Imminent Bioactivity in the Cardanol Based Tetradentate CU (II), CO (II), ZR (IV), TH (IV) Complexes

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### ABSTRACT

Cardanol Schiff bases derived synthetic compounds are an important class of ligands that were coordinate to metal ions and have been studied extensively. Considering the importance of metal complexes in the development of medical science four different Schiff base metal complexes such as Cu(II), Co(II), Zr(IV), Th(IV) were synthesized with the DFMPMNA ligand comprises of di- formylmethoxy-bis (3-pentadecenyl phenyl) methane i.e. DFMPM and 2-nitroaniline. The current experimental compounds were characterized by molar conductance, UV-Vis, IR, <sup>1</sup>HNMR, <sup>13</sup>C NMR, XRD. Furthermore, the synthesized experimental complexes have been screened for invitro antibacterial as well as antifungal activity on microorganisms such as Proteus mirabillus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa and fungi like Aspergillusflavus, Aspergillusniger, Rhizopus species, by well diffusion technique using DMF as the solvent. The invitro anticancer activities were tested for HeLa cancer cell lines using MTT assay. Hence, the present result was concluded that the Cu(II) complex contains a superior bacterial and fungicidal activity against the experimental pathogens and Zr(IV) complex possessed remarkable anticancer activity against the HeLa cell lines.

**Keywords:** DFMPM, 2-nitro aniline, DFMPMNA, Schiff base, microbial activity, in-vitro anticancer activities

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## 1. Introduction

DFMPM is derived from the natural source of *Anacardium occidentale* (L) plant nut shell oil. It is otherwise known as cardanol is a unique by product of the cashew nut small and large scale industry manufactured or prepared from the cashew nut shell liquid obtained by vacuum distillation process. Previously described about the CNSL derived cardanol based Schiff complexes could be accommodate the different metal orientation involving multiple coordination methods, thereby allowing successfully synthesized along with homo and hetero metallic complexes depends upon its stereochemistry. Synthesis and characterization of Schiff base metal complexes are much familiar throughout the field of coordination Chemistry (Tarafder *et al.*, 2001). While Schiff base ligand plays a vital role as tetra dentate Schiff base ligand have maximum potential of coordination with various metal ions for forming stable compounds (Yu *et al.*, 2007). With the preparative accessibility and potential biological properties it is the Schiff bases with O and N donor that attracted considerable attention towards the coordination Chemistry (Kannappan *et al.*, 2003). Later, Borisova *et al.* (2007) had been studied the Schiff bases of cardanol based metal complexes of nitrogen – Oxygen chelating agents are derived from the natural source of CNSL and its diverse spectrum activity towards the biological studies. Since the discovery and development of antibiotics are considered as the most powerful and successful achievements of modern medical science especially as a prominent application for controlling fungal as well as bacterial infections, the potential for further development of Schiff base complexes as antimicrobial agent for the treatment for enormous epidemic diseases. Tracing out the significance of Schiff base complexes as a part of our continuing research, the synthesis, characterization and biological studies of Schiff base tetra dentate ligand (DFMPMNA) derived from 2-nitroaniline with di-formylmethoxybis (3-pentadecenylphenyl) methane i.e. (DFMPM) is done in order to analyze the anticancer and its ability for DNA cleavage. Still, there is a little amount of work has been analyzed about this ligand complex. Hence the present study was designed the following objectives. Initially synthesized and characterization of Cardanol based DFMPMNA ligand and metal complexes Cu(II), Co(II), Zr(IV), Th(IV) by spectroscopic investigation of Electronic, <sup>1</sup>HNMR, <sup>13</sup>CNMR, IR and XRD techniques. Analyzed the metal complex product possessed the biological activities like antibacterial antifungal and DNA cleavage on He, La cell lines.

## 2. Materials and Methods

### Procedure and conduction of experiment:

The whole study was conducted with reagent and solvents of analytical grade while the complexes were purified and dried by standard methods. It is from the merck(Ltd) 2nitroaniline, formaldehyde, epichlorohydrine, sodiumperiodide, Cu(II), Co(II), Zr(IV), Th(IV) Nitrates where obtained for the experiment. In addition to elemental analysis using

Perkin- Elmer elemental analysis IR spectra was also recorded in 4000 – 400 cm<sup>-1</sup> region using pellet with K Br. The spectrum of proton NMR(<sup>1</sup>HNMR) and carbon NMR(<sup>13</sup>CNMR) was recorded using CDCl<sub>3</sub> along with a solvent from sophisticated analytical instrument facility (Cochin) and the XRD analysis is also conducted in the same while the measurement of molar conductance of the complexes in DMSO solution was recorded with an ELICO model conductivity meter.

### Synthesis of Schiff base ligand:

Ethanol solution of DFMPM and 2- nitroaniline at the ratio 1:2 was mixed by stirring and was refluxed at 70°C for two hours. The product of the reaction was then poured in to beaker with ice cubes, which gave a yellowish solution with the deposition of yellowish precipitate. Then the product (DFMPMNA) ligand was purified by adding anhydrous ether which gave a yield of 63%.

### Synthesis of complexes:

Aqueous solution of Cu (II)nitrate, Co (II) nitrate, Zr(IV) nitrate, Th(IV) were slowly added to a stirred ethanolic solution of ligand in 1:2 molar ratios and refluxed for 12 hr at 80°C. The solution was filtered under hot conditions and the filtrate was kept at room temperature. The brown precipitate get as the result was washed with ethanol and diethyl ether to remove the impurities and was finally dried in a dedicator to get the product yield of 55% and are found to be stable at room temperature which is insoluble in water and soluble in DMSO, DNF and CDCl<sub>3</sub>.

### Antimicrobial Assay

#### Target Bacterial and Fungal Organisms:

The present study was done with the following experimental microbial pathogens such as bacteria and fungi viz., *E. coli*, *B. subtilis*, *P. aeruginosa*, *p. mirabilis* and *A. flavus*, *A. niger* and *Rhizopus*.

#### Qualitative Antimicrobial Assay:

The method that has been used for the antibacterial assay was the disc diffusion assay reported by Kelmanson *et al.* (2000) with some modifications, while the antifungal assay was also performed in a way similar to the disc diffusion assay with slight modifications. The positive controls used against bacteria were SBDTC, streptomycin, neomycin, and chloramphenicol, while amphotericin B and SBDTC were the positive controls used against the fungus. Dimethyl sulfoxide (DMSO) was used as a negative control against all the microbes investigated.

#### Cytotoxicity assay:

Cytotoxicity of sample on tumor cells was measured by microculture tetrazolium (MTT) assay (Mosmann, 1983). For the assays, 96-well microplates were seeded with 100 µl medium containing 10, 000 HeLa cells in suspension. After 24 h incubation and attachment, the cells were treated with 6 fourfold dilution of crude extracts. Exactly from the stock solution (40 mg/ml), each extract sample was applied in a series of 6 dilutions (final concentrations ranging from 15.6 to 500 µg/ml) with a final DMSO concentration of 0.1% and was tested in quadruplicate. After 48 h incubation, cell viability was determined by adding (Sigma) tetrazolium salt as cytotoxicity indicator and by reading absorbance at 590 nm with a scanning multi wall spectrophotometer. Tetrazolium salts are cleaved to

formazan dye by cellular enzymes (only in the viable cells). The level of absorbance directly correlates to the metabolically active cells. Mitomycin C (~ 95 % HPLC, sigma-Aldrich) was used as a positive control.

#### Statistical Analysis:

Antibacterial, anti-fungal susceptibility data and cytotoxic viability experiments were performed 3 times in triplicated and the experimental data were statistically analyzed with pair sample 'T' test using SPSS packages (Excel-2010 version)

### 3. Results and Discussions

#### Structural Determination of Experimental Complexes and Ligand by UV, IR, <sup>1</sup>H-NMR, <sup>13</sup>C NMR and XRD :

The general characteristic properties of the ligand and its metal complexes are shown in Table 1. The result of elemental analysis suggests that all the complexes are mononuclear with the ligand coordinated to the central metal atom and the metal to ligand ratio in all complexes were 1:2. The molar conductivity  $\Lambda^m$  lies between 2 to 4  $\text{cm}^2 \text{mol}^{-1}$  which shows that the complexes were non-electrolyte in nature. The structure of synthesized ligand (DFMPMNA) was established by UV-Vis spectra, IR spectra, <sup>1</sup>H-NMR spectra, <sup>13</sup>C NMR spectra and XRD.

#### Electronic absorption spectra:

The electronic absorption spectra of the cardanol based Schiff base and i.e. DFMPMNA and its Cu(II), Co(II), Zr (IV), Th (IV) complexes were recorded using DMSO solvent. The ligand showed a broad band at 280nm due to  $\pi \rightarrow \pi^*$  transition of conjugated benzene ring and another band at 350nm due to  $n \rightarrow \pi^*$  transition of hetero atom  $\text{C}=\text{N}$  chromophores. In the metal complex this band is shifted to a longer wavelength which shows the donation of lone pair of electrons of nitrogen of Schiff base to metal ion.

#### Infrared spectra:

A wide range of information regarding the nature of functional group attached to the metal atom was obtained from the IR spectra and the characteristic IR bands of the complexes are vividly mentioned in the Table 2. The detection of IR absorption band in the range of 3350  $\text{cm}^{-1}$  – 3400  $\text{cm}^{-1}$  is due to the presence of coordinated lattice water molecule present in the ligand and complexes by observing atmospheric moisture. Then azomethine group appears at 1624  $\text{cm}^{-1}$  region for the free ligand and in the complexes, the  $\text{C}=\text{N}$  band was found shifted 20-40  $\text{cm}^{-1}$  towards the negative side, invariably indicating the involvement of azomethine nitrogen atom in coordination with metal ion, because of the donation of electrons from nitrogen to the empty d-orbitals of the metal atom. The appearance of bands in the region of 2924  $\text{cm}^{-1}$  indicates the presence of alkane group and the band at 1446  $\text{cm}^{-1}$  – 1432  $\text{cm}^{-1}$  indicates that alkane methyl group. The intense band at 1344  $\text{cm}^{-1}$  shows the presence of nitro group and the aromatic ether group is confirmed by the band at 1255  $\text{cm}^{-1}$ . In the complexes a new band appears in the region of 451  $\text{cm}^{-1}$  – 412  $\text{cm}^{-1}$  shows the M – N coordination in the complexes. (Ferrare, 1971).

**<sup>1</sup>H-NMR Spectra:** The NMR technique is highly useful to confirm the structure of the compound. The <sup>1</sup>H-NMR

spectrum (400MHz, CDCl<sub>3</sub>) shows the following signals: 0.863-0.907(m, 6H, free methyl group), 1.253-1.307(d, 29H, CH<sub>2</sub> protons), 2.549-2.588(t, 4H, Ar-CH<sub>2</sub> protons), 3.962-3.989(t, bridging CH<sub>2</sub> protons), 4.31-4.185(m, OCH<sub>2</sub> protons), 5.331-5.42(m, ArH), 7.359(s, N=CH), 7.183-7.189(d, ArH-NO-2) and 8.110-8.135(q, Ar-H5-NO-2). A slight down field shift is observed in metal complexes 6.5111-6.635(m, ArH) indicates the coordination of the ligand, with the central metal ion.

#### <sup>13</sup>C NMR:

The <sup>13</sup>C NMR (100MHz, CDCl<sub>3</sub>) shows the following signals. The DFMPMNA ligand showed a singlet at 22.66 ppm which was due to aliphatic CH<sub>3</sub>, while quartet at 29.33 ppm was due to alkane group. A singlet at 68.68 ppm was due to O – CH<sub>2</sub>, and a singlet at 129 ppm was due to aldehyde benzene to bridging carbon.

#### XRD pattern:

Since getting good single crystal of the complex is not practical, the powder X-ray diffraction pattern of DFMPMNA Zr (IV) complex is considered for the characterization of the complex, as it has specific d values. With the help of Scherrer's formula  $d_{XRD} = k\lambda/\beta \cos\theta$  where  $\lambda$  is the wavelength,  $\beta$  is the full width at half maxima and  $\theta$  is the diffraction angle the crystalline size was determined from the XRD pattern. Further the analysis shows that Zr (IV) complexes have an average crystalline size of 0.122 nm have sharp peak that reveals the crystalline nature of the Zirconium complex.

**Probable structure of Experimental ligand and the complexes:** The results exhibit that the synthesized ligand binds with metal ions in tetra-dentate through N atom of the azomethine group. The proposed structure of the ligand shown in fig 4 and 5.

#### Results about Biological Effect of these experimental pathogens

##### Effect of Anti-microbial activity on the special Bacteria and Fungi:

Commonly Schiff base complexes have been possessed a wide spectrum activity towards the biological response especially in pharmaceutical effects. Apart from this common concept the present research work was focused the cardanol based DFMPMNA metal complex. Initially, the present study denoted biological activity was antimicrobial assessment of these chemically synthesized complexes of Cu (II), Co (II), Zr (IV), Th (IV). The synthesized complexes were tested for their in-vitro antibacterial and antifungal activity also compared this effect along with the commercially available antibacterial and antifungal drugs as streptomycin and Fluconazole respectively. The result of antibacterial susceptibility test was expressed in terms of diameter zone of inhibition (in mm) size totally four various pathogenic bacterial species are subjected in this antibacterial effect. Among the four *Bacillus subtilis* was a gram (+<sup>ive</sup>) remaining three bacterial strains are belongs to gram negative type such as *P. aeruginosa*, *E. coli* and *P. mirabilis*. From the present results were clearly showed that copper complex possessed that maximum activity against the organism of *P. aeruginosa* (18±3.11mm) followed by similar zone of inhibition 11±2.54 mm size observed on both organisms named as *B. subtilis* and *P. mirabilis*.

Though, an optimum level zone of inhibition ( $12 \pm 3.08$  mm) also been noticed against the Gram (-<sup>ve</sup>) organism of *E. coli*. Moreover, the tested other three complexes, Zirconium complex showed the positive as well as similar zone of inhibition ( $10 \pm 1.95$ ) and ( $10 \pm 2.54$  mm) was noticed against *B. subtilis* and *E. coli* respectively. Even though, a minimum  $7 \pm 1.32$  mm zone of inhibition has been noticed on *P. mirabilis* organism. However, DFMPMNA-Cu complex denoted significantly maximum efficiency ( $11 \pm 3.53$  mm) against *P. mirabilis* followed by ( $11 \pm 2.54$ ) and ( $12 \pm 3.08$ ) mm size, zone of inhibition has been noticed on *B. subtilis* and *E. coli* respectively. Finally, focusing the experimental complex of the Thorium expressed the least level of activity against  $9 \pm 1.23$  mm zone the *P. aeruginosa* and  $8 \pm 1.65$  mm zone of inhibition in *P. mirabilis*. According to the present result clearly expressed among the four metal complexes copper complex denoted the significantly quite be positive effect more against the tested experimental pathogenic bacterial strains or organisms, when compared with other three complexes. Whereas other three complexes of Co, Zr and Th showed that nil effect against *P. aeruginosa*, *B. subtilis* and these three complexes are quite not worth. From, the overall results was definitely showed copper complex that possessed noteworthy positive antibacterial zone was observed on all the four pathogenic isolates rather than the other three DFMPMNA complexes. So the current research clearly expressed copper complex act as a bactericidal agent of those experimental pathogenic bacterial organism (Table - 3).

The antifungal efficiency also been represented the result in Table - 4. The current research expressed that the four experimental chemical complexes were that subjected with three different fungal organisms such as *A. flavus*, *A. niger* and *R. species*. Among the four experimental complexes copper complex effect was highly significantly maximum ( $21 \pm 2.05$ ) zone of the inhibition on *A. niger* fungi. It was interestingly highest antifungal when compared with the commercialized antifungal dose or antibiotics of *Flucanazole*. Subsequently, the other two fungal pathogens expressed the activity also significantly optimum range  $14 \pm 2.75$  mm observed in *R. species* and  $10 \pm 1.83$  mm zone in *A. flavus* represented the same copper complex. In addition, secondary level of maximum activity ( $13 \pm 1.26$ ) also been noticed in the Zirconium complex against *A. niger* fungi, it was also significantly highest findings followed by *R. species* denoted  $11 \pm 2.81$  mm size zone of inhibition and lowest zone  $7 \pm 1.45$  mm has been observed in *A. flavus* organism, it was stated that statistically insignificant level of observation. However, the Cobalt complex also given the antifungal efficiency maximum  $10 \pm 2.14$  mm and minimum  $8 \pm 0.59$  mm size of zone of inhibition noticed against *R. species* and *A. flavus* respectively finally the fourth complex possessed antifungal effect on *A. niger*  $12 \pm 2.26$  mm but minimum  $9 \pm 1.43$  mm size zone of inhibition has been noticed. From the overall antifungal result where clearly depicted that significantly that highest level of antifungal efficiency possessed the copper complex on the three experimental pathogenic fungal organism followed by

Zirconium, Thorium and Cobalt also denoted the antifungal property though Cobalt and Thorium like two complexes does not show any other antifungal influence against the *A. flavus* and *A. niger* organism. So it was replied that noteworthy effect of antifungal property of those two organism (Table 2).

#### Quantitative Anticancer study by Cytometric assay:

The present result was explained the cytotoxic effect of four Schiff based complexes on the He, La cancer cell lines by cytometry assay data we represented in Table-5. In the four tested complexes molecules correctly showed that the highest anticancer activity possessed in Zirconium complex at the concentration of  $100 \mu\text{g/ml}$ . It was significantly maximum dead cells are observed in this concentration from the uncontrolled cancer cell lines. For this anticancer or cytotoxic studies totally five different concentrations has been made for each complexes such as 6.25, 12.5, 25, 50 and  $100 \mu\text{g/ml}$ . among the five concentration six fold to 12 and 18 fold increasing cell death was observed on each concentrations. However, the current result was indicated whenever the concentration has been increased anticancer or cell death also been increased in the particular He, La cell lines. Similar complexes have been stands for lowest concentration to other three onwards given the somewhat worthy manner of the significant level anticancer activity against the treated He, La cell lines. Subsequently the other three complexes and its concentrations are indicated anticancer activity of second most maximum noticed in Thorium and followed by Cobalt and finally copper complex. Hence, these four complexes posed cytotoxic effect depicted the following order like  $\text{Zr} > \text{Th} > \text{Co} > \text{Cu}$  from the present result was clearly indicated most promising anticancer activity consisted Zirconium complex is act as a good anticancer agent against the He, La cell lines. It was statically significant level at  $p < 0.05$  % paired sample 'T' test. The overall result of the MTT Assay (or) Cytotoxic efficiency concluded the statically highest Leethal Dose responded in Zirconium complex 14.22. In addition, secondary, tertiary level of least values also been observed.  $\text{LD}_{50}$  values were expressed in Thorium (44.69), Copper (64.65), and Cobalt (74.04) respectively.

#### DNA Cleavage studies:

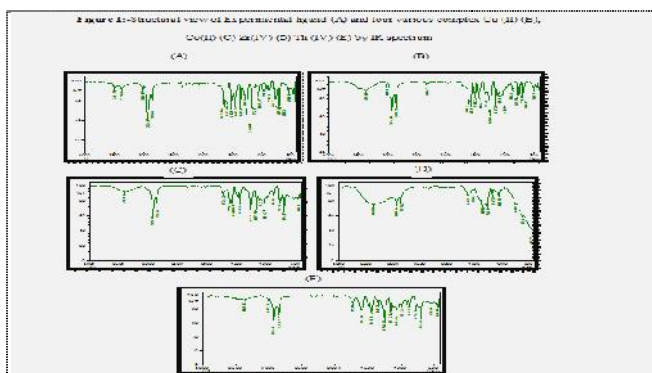
After treated the four synthesized metal complexes subjected on He, La cell lines and its resulted products separating and visualizing DNA fragments through Agarose gel electrophoresis (AGE) technique was adopted. After treatment the stained gel was visualized using a gel documentation system. Fainted nature of DNA fragment appeared on Zirconium (IV) complex followed by least intensity of DNA pattern also been observed compared with control fragment on Cu(II) and Th(IV) complexes. This due to free hydroxyl radical formed by metal ions reacts with  $\text{H}_2\text{O}_2$  which could be damaged DNA segment into pieces of fragments in the base.

#### Discussion

Commonly Schiff – base metal complexes play an essential role in various economically important fields such as Agriculture (Patel *et al.*, 2000; Shalinet *et al.*, 2009; Gobalakrishnan *et al.*, 2011). Pharmaceutical industry for drug manipulation (Pillai and Mathew 1993, Mukherjee *et*



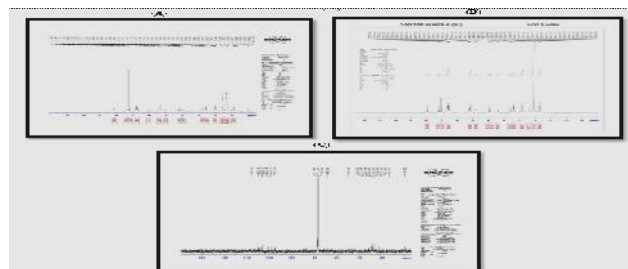
al.,1995); Biological especially antimicrobial efficiency (Issac *et al.*, 2011; Issac *et al.*, 2014;Harinath *et al.*, 2011; Shivhareand Mangala, 2011) Industrial Chemistry (Shalin *et al.*, 2009, Lekshmi.*et al.*, 2011). The present research paper focusing ligand molecule of this Schiff base complexes was cardanol, this is a lay product of cashew nut shell oil (CNSO) source from *Anacardiumoccidentale* (L). plant seed coat. It is a typically unique natural vast spectrum of natural source for unsaturated long chain phenol compounds described previously Gopalakrishnan and Sujatha (2010);Kumar *et al* (2013);Anushaet *al.* (2015).Cardanol assisted with various complexes like Co, Cu,Th definitely such a ligand complex combined products reflected some essential as well as novel potential activity against the various microorganisms through, bacterial isolates are *Klepsiellasp.*, *S.aureus*, *E.coli*,*P.auroginosa* and *B.cereus* .Subsequently other fungal pathogenic organisms. This kind of similar findings already been identified by various researches (Danyet *al.*, 2004; Issac.*et al.*,2011; Harinath.*et al.*, 2011; Gopalakrishnanet *al.*,2011).Apart from the present result clearly depicted that was antibacterial activity was estimated depends upon the size especially the zone of inhibition against the aseptic identical conditions, hence the cardinal consisted Schiff – based complex had better to moderate level of antibacterial and anti-fungal activity against these experimental microorganisms. Due to this antibacterial activity substantiate the findings by previous researches described the following reliable valid reasons that biologically inactive components become active as soon as reduced level of biologically active compounds become an active it will be happened depends upon the coordination capability of cardanol based ligand with four different complex molecules (Praphakaran *et al.*,2001;Raman *et al.*, 2004).



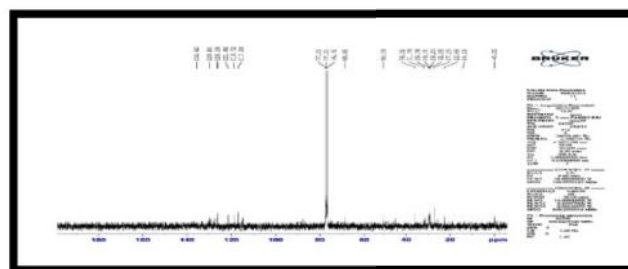
**Fig 1:** structural view of experimental ligand (A) and four various complex Cu(II), (B) Co (II), (C) Zr (II), (D) Th(IV),(E) by IR Spectrum

**Table 1:** Physico-chemical parameters of the experimental complexes

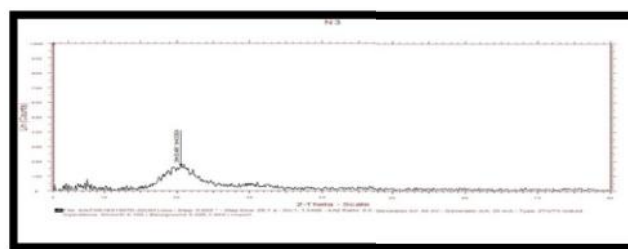
Compounds	Yield (%)	Colour	Molecular Formula	Molecular Weight	Melting Point °C	Molar conductance Ohm <sup>-1</sup> cm <sup>-1</sup> mol <sup>-1</sup>
DFMPMNA(Ligand)	63	Orange	C <sub>59</sub> H <sub>92</sub> N <sub>4</sub> O <sub>6</sub>	941	218	—
DFMPMNA- Cu	59	Black	C <sub>118</sub> H <sub>160</sub> CuN <sub>10</sub> O <sub>18</sub>	2070	>250	2.5
DFMPMNA- Co	54	Brown	C <sub>118</sub> H <sub>160</sub> CoN <sub>10</sub> O <sub>18</sub>	2065	>250	3.7
DFMPMNA- Zr	51	Brown	C <sub>118</sub> H <sub>160</sub> N <sub>12</sub> O <sub>24</sub> Zr	2221	>250	3.4
DFMPMNA- Th	56	Black	C <sub>118</sub> H <sub>160</sub> N <sub>12</sub> O <sub>24</sub> Th	2362	>250	3.1



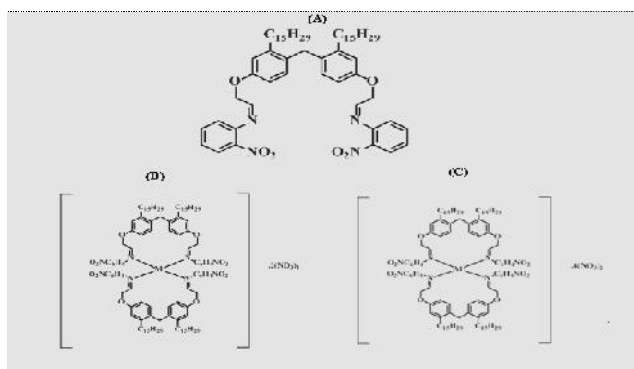
**Fig 2:** Structural view of Ligand (A) and Cu(II) complex by <sup>1</sup>H NMR (B) Ligand by <sup>13</sup>C NMR (C)



**Fig 3:** Structural view of Ligand by <sup>13</sup>C NMR



**Fig 4:** Structural view of Ligand by XRD



**Fig 5:** Structural view of Experimental Ligand (A) and complexes (M=Cu (II), Co (II) (B), (M=Zr (IV),Th (IV)) (C) elucidated by Chem Draw technique

**Table 2:** IR spectral data (cm<sup>-1</sup>) of the Experimental Ligand and their four metal complexes

Ligand/Complex	O-H(H <sub>2</sub> O)	O-C	C-H	C=N	M-N
DFMPMNA(Ligand)	3373	2924	2852	1624	-
DFMPMNA-Cu	3369	2924	2852	1608	445
DFMPMNA-Co	3410	2924	2852	1604	449
DFMPMNA-Zr	3352	2926	2854	1629	412
DFMPMNA-Th	3375	2924	2852	1583	451

**Table 3:** Antibacterial activity of Schiff base four complexes on selected pathogenic bacterial organisms

Name of the Sample	<i>B. subtilis</i> (mm)	<i>P.aeruginosa</i> (mm)	<i>E.coli</i> (mm)	<i>P.mirabilis</i> (mm)
Streptomycin	18 ± 0.61	23 ± 2.56	22 ± 4.67	13 ± 2.04
DFMPMNA-Cu	11 ± 2.54	18 ± 3.11**	12 ± 3.08*	11 ± 3.53**
DFMPMNA-Co	9 ± 2.17 <sup>Is</sup>	-	8 ± 1.86	10 ± 2.43*
DFMPMNA-Zr	10 ± 1.95*	-	10 ± 2.54*	7 ± 1.32
DFMPMNA-Th	-	9 ± 1.23	-	8 ± 1.65*

Is--- stands for Insignificant, \*---stands for significant at the level of p<0.05%,

\*\*----stands for highly significant at p<0.001%level

**Table 4:**Anti-fungal effect of four synthesized complexes on three pathogenic fungi

Sample	<i>Aspergillusflavus</i> (mm)	<i>Aspergillus Niger</i> (mm)	<i>Rhizopus species</i> (mm)
<i>Flucanazole</i>	21 ± 0.54	20 ± 2.51	21 ± 2.11
DFMPMNA-Cu	10 ± 1.83*	21 ± 2.05**	14 ± 2.75*
DFMPMNA-Co	8 ± 0.59 *	-	10 ± 2.64 *
DFMPMNA-Zr	7 ± 1.45 <sup>Is</sup>	13 ± 1.26 *	11 ± 2.81
DFMPMNA-Th	-	12 ± 2.16*	9 ± 1.43 <sup>Is</sup>

Is--- stands for Insignificant, \*---stands for significant at the level of p<0.05%,

\*\*----stands for highly significant at p<0.001%level

**Table 5:** Cytotoxic effect of four Schiff base complexes against the MTT Assay of HeLa cell lines cancer cells

Sample .Con(µg/ml)	OD value at 540nm	Percentage of viability dead cells
Control	0.5215	03.14
<b>DFMPMNA- Cu</b>		
6.25	0.43±0.021 *	18.31±3.14 <sup>Is</sup>
12.5	0.35±0.01	34.87±3.86 <sup>Is</sup>
25	0.033±0.002	40.66±2.93*
50	0.28±0.017	47.72±3.81*
100	0.22±0.015	60.30±5.64**
<b>DFMPMNA- Co</b>		
6.25	0.40±0.023*	23.22±3.45*
12.5	0.34±0.015	34.57±2.13
25	0.031±0.026	41.85±3.47
50	0.27±0.011*	47.41±2.39*
100	0.24±0.018	54.12±4.75**
<b>DFMPMNA- Zr</b>		
6.25	0.36±0.05	31.88±5.24*
12.5	0.27±0.07	42.12±3.46*
25	0.23±0.05	64.55±5.08*
50	0.17±0.003	81.67±4.53**
100	0.13±0.004	93.48±5.28**
<b>DFMPMNA- Th</b>		
6.25	0.32±0.007	39.81±5.24
12.5	0.31±0.006	43.43±6.17
25	0.27±0.004	47.38±4.08
50	0.26±0.003	50.72±3.21
100	0.24±0.005	53.73±7.13

#### 4. Conclusion

This study is the outcome of the need we have against selected pathogenic microbial organisms and cancer cell lines. Apart from the DNA cleavage study showed the whenever the concentration of experimental complex was increased cell death also been increased. While synthesis and structural determination of the ligand and complexes open a new phase in the study of bioactivity of the mentioned complexes are the boon for the future medical treatment of cancer as well as microbial infections.

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