Part-A Anti-Microbial Studies

6.1 Introduction

The biological and medicinal potency of coordination compounds has been established by their antitumor[1,2], antiviral[3] and anti-malarial activities. This characteristic property has been related to the ability of the metal ion to form complexes[4] with ligand containing sulphur, nitrogen and oxygen donor atoms. The biological activity of any compound or complex is the combination of steric, electronic and pharmacokinetic factors. A possible explanation for the toxicity of the complexes has been postulated in the light of chelation theory[5].

It was suggested that the chelation reduces considerably the charge of the metal ion mainly because of partial sharing of its positive charge with the donor groups and possible n-electron delocalization over the whole chelate ring. This increase the lipophillic character of metal chelate which favors its permeation through lipid layers of fungus membranes. Furthermore the mode of action of the compounds may involve the formation of a hydrogen bond through the -N=C- group of the chelate or the ligand with the active centers of the fungal cell constituents resulting in the interferences with the normal cell process.

In addition to this Schiff bases containing azomethine group ortho to the hydroxy group are known to yield chelates which posse's fungicidal properties[5]. A Comparative study of the ligands and their complexes indicate that most of the metal chelates exhibit higher antimicrobial activity than that of the free ligand. The increased antifungal activity of metal chelates with increase in concentration is due to the effect of metal ion on the normal cell process. Such increased activity of the metal chelates can be explained on the basis of Overtone's concept[6] and chelation theory.[7] According to overtone's concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid soluble materials due to which liposolubility is an important factor that decides antimicrobial activity. On chelation, the polarity of the metal ion will be reduced to a greater extent due to the overlap of positive charge of metal ion with donor groups.

Further, it increases the delocalization of the pi-electrons over the whole chelate ring and enhances the lipophilicity of thee complexes. This increased lipophilicity enhances the penetration of the complexes into lipid membrane and

blocking the metal binding sites on the enzymes of microorganism. These complexes also disturb the respiration process of the cell and thus block the synthesis proteins which restrict further growth of the organism. The transition metal complexes having oxygen and nitrogen donor Schiff bases possess unusual configuration, structural liability and are sensitive to molecular environment[8].

The environment around the metal center 'as coordination geometry', number of coordinated ligands and their donor group is the key for metalloproteinase to carry out a specific physiological function[9]. About 20 Zinc enzymes are known in which zinc is generally tetrahedrally four coordinate and bonded to hard donor atoms such as nitrogen or oxygen[10]. Manganese plays an important role in several biological redox-active system[11], a number of cupper proteins including enzymes have been reported[12] and proteins containing iron participate in oxygen transport[13]. The preparation of model complexes having similar spectroscopic features is perhaps the most important step to understand the structure and behavior of these biological systems. Schiff base metal complexes attract considerable interest and occupy an important role in the development of the chemistry of chelate systems[14] due to the fact that especially these with N_2O_2 tetradentate ligands, such system dosely resemble metallo-proteins.

Survey of the literature reveals an excellent work devoted to synthesis and characterization of many metal complexes of Schiff base. Schiff base derivatives followed by studying their complexation with di-and trivalent transition metal ions which have the subject of several studies due to the fact that these ligands and their metal complexes exhibit different biological activity and very industrial applications[12]. The benzofuran nucleus is associated with various biological activities. The natural product containing benzofuran nucleuses are well known for their useful medicinal properties. There have been several examples of plant extracts containing benzofuran compounds, being used for the treatment of various diseases. This led several workers to search for such molecules in the natural sources initially. These facts stimulated interest in the exploration of synthetic analogous.

In the substituted furan, the benzofuran ring system has received little attention. The investigation of benzofuran derivatives as antimicrobial agents was initiated only in recent years, within this short period of 2-3 decades, several benzofuran[15] derivatives have been fund to possess such an activity[16-21] furylbenzofuran[22], benzofuran analogs of chalcones[23-26] are also reported to possess antibacterial properties.

The testing of a series of substituted benzofuran, benzofuran[3,2-d]pyrimidin, 4H-benzofuro[3,2-d]-m-oxygen-4-one derivatives prepared in this laboratory are found to be significantly active against *S. aureus and E. coli*[27]

M. M. Atalla et. al.,[28] prepared a series of tetrahydropyridazine substituted benzofuran derivatives and screened them for antimibacterial and antifungal activities. Among these some compounds were found to be most active against fungi yeast and bacteria. Albert[29] reported antibacterial and antifungal agents exert their toxic effect on micro-organisms through chelation.

6.1.1 Experimental

Reagents

Dimethylformamide (A.R. Grade) was distilled before use. Peptone, pancreatic digest of casein yeast extract, beef extract, dextrose and agar were used as purchased.

6.1.2 Antibacterial activity

The antibacterial activity of the test compounds was assessed against *Staphylococcus-aureus* (gram-positive) and *Escherichia-coli* (gram-negative) organisms. In view of their potent biological activity of all the ligands, metal salts and their metal complexes under present investigation were tested. These bacterial strains were chosen as there are the potential pathogen's of human beings. The biological screening was done by cup plate method.

6.1.3 Materials and methods

The following materials were used.

i. Nutrient broth and nutrient agar.

ii. Sterilized Petri-dishes.

iii. Bacterial cultures.

iv. Sterilized cork borer of 8 mm diameter

v. Sterilized micro tips (1-200μ).

vi. Micropipette (1-200μl).

6.1.4 Test organism

The test organisms were selected from both gram positive and gram-negative organisms to test the antibacterial activity. These organisms were cultured on agar slants and incubated for 24 hrs. at 37° C. From these slants a suspension was made

using sterile saline solution (saline solution was prepared by dissolving 0.9 gm of sodium chloride in 100 ml distilled water and then sterilized).

6.1.5 Preparation of media

The Nutrient agar was prepared by dissolving bacteriological peptone (10g/L), Beef extract (5g/L), Sodium chloride (10g/L) in 1000ml distilled water and the pH of the solution was adjusted to 7.40 by sodium hydroxide (1M) or hydrochloric acid (1M). This solution was filtered and agar (20g/L) was added. Then it was sterilized for 15 min at 15 lb per kg pressure. This was used as the media for antibacterial activity testing.

6.1.6 Preparation of sub-culture

The organisms used in the present study were obtained from the laboratory stock on the day of testing, the organisms were sub-cultured in the sterilized nutrient broth. After incubating the same for 24 hrs, the growth was used as inoculums for the test.

6.1.7 Sterilization of media and glass were

The media used for nutrient agar and nutrient broth were sterilized in a conical flask of suitable capacity by autoclaving the same at 15 lb/kg pressure for 15 min. The cork borer and glass wares, i.e., Petri-dishes, test tubes and micro tips etc, were sterilized by employing autoclave at 15 lb/kg pressure for 15 minutes.

6.1.8 Preparation of solution for test compound

The 10 mg of test compound was dissolved in 10ml of dimethylformamide in serially labeled sterilize test tubes, from the stock 0.10 ml (100µl) of solution was used for antimicrobial assay.

6.1.9 Method of testing

About 15-20 ml of molten nutrient agar was poured into each of the sterilized Petri-dishes of 3.50 inches diameter, with the help of sterile cork borer two cups of each with 8 mm diameter were pouched and scooped out the set agar (two cups were numbered for the particular test compounds). The agar plates so prepared are divided into two sets and each set of the plates were inoculated with the suspension of particular organisms by spread plate techniques.

The cups of inoculated plates were then filled with 0.1ml of the test solution the plates were allowed to stay for 2 hrs in refrigerator further the plates were

incubated at 37° C for 24 hrs. The zone of inhibition developed if any, was then measured for the particular compound with particular organism.

Gentamycine (10mg/10ml) was used as a standard and DMF control was also put to know the activity of the solvent.

6.2 Antifungal activity

The antifungal activities of the ligands and their metal complexes were tested against *Aspergillus niger* and *C. albicans* by cup-plate method.

6.2.1 Materials and methods

The following materials were used

i. Nutrient agar and nutrient broth,
ii. Sterilized Petri-dishes,
iii. Antifungal cultures,
iv. Sterilized cork borer of 8 mm diameter,
v. Sterilized micro tips (1-200ml),
vi. Micropipette (1-200ml),

6.2.2 Preparation of sub-cultures

The organisms used in the present study were obtained from the laboratory stock on the day of testing the organisms were sub-cultured in the sterile nutrient broth, after incubating the same for 3hrs. The growth thus obtained was used as inoculums for the test.

6.2.3 Preparation of media

The media used for antifungal activity was the potato-dextrose agar. It was prepared as follows, potato pieces (250gm) were dissolved in 20mL distilled water by steaming for 30 min. The solution was filtered while hot and the volume was made up to 400 mL. To this solution of dextrose (20gm) and agar (8gm) were added and dissolved by steaming for 30 min. The so formed potato-dextrose agar (PDA) media was poured into two separated conical flasks and were separately inoculated with above fungus using sterile metal wire loop.

6.2.4 Sterilization of media and glass were

Potato-dextrose agar and nutrient broth were sterilized in a conical flask of suitable capacity by autoclaving the same at 15 lb/kg pressure for 15 min. The cork

borer, glass wares, Petri-dishes, test tubes and pipettes were sterilized by employing autoclave at 15 lb/kg pressure for 15 min.

6.2.5 Preparation of test compound

The 10 mg of test compound was dissolved in 10mL of dimethylformamide in serially labeled sterile test tubes from the stock solution 0.1mL (1000mL) of solution was used for antimicrobial assay.

6.2.6 Method of testing

About 15-20 mL of molten potato-dextrose agar was poured into each of the sterilized Petri-dishes of 3.50 inches diameter with the help of sterile cork borer, two cups of each with 8 mm diameter were punched and scoped out from the set PDA medium (two cups were numbered for the particular test compounds). The plates so prepared are divided into two sets and separate set of plates were inoculated with the suspension of particular organism by spread plate techniques.

The cups of inoculated plates were then filled with 0.1 mL of the test solution the plates were allowed to stay there, as they are in their upright position for 2 hrs, further the plates were incubated at 37°C for 72 hrs. The zone of inhibition developed, if any was then measured for the particular compound with particular organism.

Amphotericin (10mg/10mL) was used as a standard and DMF control was also put to known the activity of the solvent.

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1.	Concentration of the test compound	1 mg/mL in DMF
2.	Quantity of the test compound in each cup	0.10 mL
3.	Diameter of the cup	8mm
4.	Standard for antibacterial activity	Gentamycine
5.	Standard for antifungal activity	Amphotericin
6.	Control	DMF

]Key for antibacterial interpretation

	Zone of inhibition in mm	Activity
1.	Less than 10 mm	Inactive
2.	10-12	Weakly active
3.	13-15	Moderately active
4.	16 and above	High active

Key for antifungal interpretation

	Zone of inhibition in mm	Activity
1.	Less than 12 mm	Inactive
2.	12-14 mm	Weakly active
3.	15-17 mm	Moderately active
4.	18 and above	High active

Results and discussion

All the synthesized ligands such as BCAP, BCAT, BCACP, BCMeOACP, BCClACP, BCMeTPC, BCMeTB and BCEtHB and their Cu(II), Co(II), Ni(II), Zn(II), Cd(II) and Hg(II) complexes and metal salts were tested for their antibacterial activity against *E. coli* and *S. aureus*, and antifungal activity against *A. niger* and *C. albicans*. The results of the antibacterial and antifungal activity studies have been presented in Table 6 (1-3).

The antibacterial and antifungal activity for the ligand BCAP and its complexes along with standards are shown in Table 6 (1). From the results it is clear that the ligand and its Cd(II) and Hg(II) complxes showed good activity against both bacteria *E. coli* and *S. aureus* and both fungi *A. niger* and *C. albicans*. The Cu(II) complex show good activity against bacteria *S. aureus* and fungi *C. albicans.* All other complexes were found to be weak to moderate active when compared with the standard drug Gentamycine which shows 24 mm and 26mm growth of inhibition

against *E. Coli* and *S. aureus* and Amphotericin which shows 25 mm and 26mm growth of inhibition against *A. niger* and *C. albicans* respectively.

The antibacterial and antifungal activity for the ligand BCAT and its complexes along with standards are shown in Table 6 (1). From the results it is clear that Hg(II) complex show good activity against both bacteria *E. coli* and *S. aureus* and both fungi *A. niger* and *C. albicans.* The Ni(II) complex show good activity against fungi *C. albicans* and all other remaining complexes were found to be weak to moderate active when compared with the standard drug.

The antibacterial and antifungal activity for the ligand BCACP and its complexes along with standards are shown in Table 6 (1). From the results it is clear that Cu(II), Zn(II) and Hg(II) complxes showed good activity against both bacteria *E. coli* and *S. aureus* and both fungi *A. niger* and *C. albicans*. All other complexes were found to be weak to moderate active when compared with the standard drug.

The antibacterial and antifungal activity for the ligand BCMeOACP and its complexes along with standards are shown in Table 6 (1). From the results it is clear that the ligand show good activity against bacteria *S. aureus* and fingi *C. albicans.* Cu(II), Cd(II) and Hg(II) complex show good activity against both bacteria *E. coli* and *S. aureus* and both fungi *A. niger* and *C. albicans.* All other complexes were found to be weak to moderate active when compared with the standard drug.

The antibacterial and antifungal activity for the ligand BCClACP and its complexes along with standards are shown in Table 6 (2). From the results it is clear that the ligand and its Cu(II), Zn(II) and Cd(II) show good activity against bacteria *S. aureus.* Co(II) and Ni(II) complexes shows good activity against fungi *C. albicans.* Hg(II) complex show good activity against both bacteria *E. coli* and *S. aureus* and both fungi *A. niger* and *C. albicans*. All other complexes were found to be weak to moderate active when compared with the standard drug.

The antibacterial and antifungal activity for the ligand BCMeTPC and its complexes along with standards are shown in Table 6 (2). From the results it is clear that Cu(II) complex show good activity against bacteria *E. coli* and fungi *A. niger* and Ni(II) complex show good activity against fungi *A. niger*. The Hg(II) complex show good activity against both bacteria *E. coli* and *S. aureus* and both fungi *A. niger* and *C. albicans*. All other complexes were found to be weak to moderate active when compared with the standard drug.

The antibacterial and antifungal activity for the ligand BCMeTB and its complexes along with standards are shown in Table 6 (2). From the results it is clear that Cu(II) and Hg(II) complexes shows good activity against both bacteria *E. coli* and *S. aureus* and both fungi *A. niger* and *C. albicans.* The ligand and its Ni(II) and Cd(II) complexes shows good activity against fungi *A. niger* and all other remaining complexes were found to be weak to moderate active when compared with the standard drug.

The antibacterial and antifungal activity for the ligand BCEtHB and its complexes along with standards are shown in Table 6 (3). From the results it is clear that Cu(II) and Hg(II) complexes shows good activity against both bacteria *E. coli* and *S. aureus* and both fungi *A. niger* and *C. albicans.* The Co(II) and Zn(II) complexes shows good activity against fungi *A. niger* and all other remaining complexes were found to be weak to moderate active when compared with the standard drug.

Table 6(1)
Antibacterial and antifungal activity data of the ligands and their metal complexes

Ligands / Complexes	Zone of inhibition in mm			
	Antibacterial activity		Antifungal activity	
	E. coli	*S. aureus*	*A. niger*	*C. albicans*
BCAP [$C_{16}H_{13}N_3O_2$]	**19**	**21**	**19**	**19**
$[Cu(C_{16}H_{13}N_3O_2)Cl_2]_n$	13	**16**	08	**25**
$[Co(C_{16}H_{12}N_3O_2)_2Cl_2]$	09	08	07	13
$[Ni(C_{16}H_{12}N_3O_2)_2Cl_2]$	10	11	07	07
$[Zn\ (C_{16}H_{12}\ N_3O_2)_2]$	14	10	11	07
$[Cd\ (C_{16}H_{12}\ N_3O_2)_2]$	**18**	**22**	**23**	**23**
$[Hg\ (C_{16}H_{12}\ N_3O_2)_2]$	**19**	**23**	**23**	**19**
BCAT $[C_{15}H_{12}N_2O_2S]$	07	07	08	07
$[Cu(C_{15}H_{12}N_2O_2S)Cl_2]_n$	10	12	07	07
$[Co(C_{15}H_{11}N_2O_2S)_{2.}2H_2O]$	11	10	07	07
$[Ni(C_{15}H_{11}N_2O_2S)_{2.}2H_2O]$	09	11	09	**18**
$[Zn\ (C_{15}H_{11}N_2O_2S)_2]$	11	14	07	09
$[Cd\ (C_{15}H_{11}N_2O_2S)_2]$	11	11	07	08
$[Hg\ (C_{15}H_{11}N_2O_2S)_2]$	**20**	**21**	**21**	**20**
BCACP $[C_{17}H_{14}N_2O_2\ ]$	12	10	07	07
$[Cu(C_{17}H_{14}N_2O_2)Cl_2]_n$	**17**	**18**	**18**	**19**
$[Co(C_{17}H_{14}N_2O_2)Cl_2]_n$	15	13	11	10
$[Ni(C_{17}H_{14}N_2O_2)Cl_2]_n$	11	10	07	07
$[Zn(C_{17}H_{14}N_2O_2)Cl_2]$	**16**	**17**	08	07
$[Cd(C_{17}H_{14}N_2O_2)Cl_2]$	09	08	09	09
$[Hg(C_{17}H_{14}N_2O_2)Cl_2]$	**18**	**19**	**20**	**22**
BCMeOACP$[C_{18}H_{16}N_2O_3\ ]$	11	**19**	13	**24**
$[Cu(C_{18}H_{16}N_2O_3)Cl_2]_n$	**16**	**17**	**18**	**25**
$[Co(C_{18}H_{16}N_2O_3)Cl_2]_n$	12	12	07	16
$[Ni(C_{18}H_{16}N_2O_3)Cl_2]_n$	07	07	07	08

$[Zn(C_{18}H_{16}N_2O_3)Cl_2]$	09	10	07	07
$[Cd(C_{18}H_{16}N_2O_3)Cl_2]$	**20**	**21**	**21**	**18**
$[Hg(C_{18}H_{16}N_2O_3)Cl_2]$	**16**	**20**	**21**	**22**
Standard				
Gentamycine	24	26	--	--
Amphotericin	--	--	25	26

Table 6(2)

Antibacterial and antifungal activity data of the ligands and their metal complexes

Ligands / Complexes	**Zone of inhibition in mm**			
	Antibacterial activity		**Antifungal activity**	
	E. coli	***S. aureus***	***A. niger***	***C. albicans***
BCClACP$[C_{17}H_{13}N_2O_2Cl]$	09	**16**	10	08
$[Cu(C_{17}H_{13}N_2O_2Cl)Cl_2]_n$	12	**17**	08	13
$[Co(C_{17}H_{13}N_2O_2Cl)Cl_2]_n$	11	12	09	**18**
$[Ni(C_{17}H_{13}N_2O_2Cl)Cl_2]_n$	07	15	09	**19**
$[Zn(C_{17}H_{13}N_2O_2Cl)Cl_2]$	09	**16**	07	07
$[Cd(C_{17}H_{13}N_2O_2Cl)Cl_2]$	09	**18**	07	09
$[Hg(C_{17}H_{13}N_2O_2Cl)Cl_2]$	**20**	**21**	**18**	**19**
BCMeTPC $[C_{15}H_{12}N_2O_2S]$	07	08	15	08
$[Cu(C_{15}H_{12}N_2O_2S)Cl_2]_n$	**16**	08	**19**	10
$[Co(C_{15}H_{12}N_2O_2S)Cl_2]_n$	07	07	07	08
$[Ni(C_{15}H_{12}N_2O_2S)Cl_2]_n$	07	08	18	08
$[Zn(C_{15}H_{12}N_2O_2S)Cl_2]$	10	08	08	08
$[Cd(C_{15}H_{12}N_2O_2S)Cl_2]$	08	08	07	08
$[Hg(C_{15}H_{12}N_2O_2S)Cl_2]$	**21**	**20**	**22**	**20**
BCMeTB $[C_{17}H_{14}N_2O_2S]$	07	08	**20**	08
$[Cu(C_{17}H_{14}N_2O_2S)Cl_2]_n$	**17**	**16**	**22**	**21**
$[Co(C_{17}H_{14}N_2O_2S)Cl_2]_n$	07	08	08	07

$[Ni(C_{17}H_{14}N_2O_2S)Cl_2]_n$	08	07	**21**	09
$[Zn(C_{17}H_{14}N_2O_2S)Cl_2]$	10	11	09	10
$[Cd(C_{17}H_{14}N_2O_2S)Cl_2]$	08	09	**23**	09
$[Hg(C_{17}H_{14}N_2O_2S)Cl_2]$	**21**	**16**	**22**	**23**
Standard				
Gentamycine	24	26	--	--
Amphotericin	--	--	25	26

Table 6(3)
Antibacterial and antifungal activity data of the ligand and its metal complexes and metal salts

Ligand/Salts/Complexes	**Zone of inhibition in mm**			
	Antibacterial activity		**Antifungal activity**	
	E. coli	***S. aureus***	***A. niger***	***C. albicans***
BCEtHB $[C_{18}H_{16}N_2O_4]$	07	08	13	08
$[Cu(C_{18}H_{16}N_2O_4)Cl_2]_n$	**18**	**16**	**23**	**22**
$[Co(C_{18}H_{16}N_2O_4)Cl_2]_n$	08	08	**20**	07
$[Ni(C_{18}H_{16}N_2O_4)Cl_2]_n$	08	07	12	10
$[Zn(C_{18}H_{16}N_2O_4)Cl_2]$	11	10	**19**	11
$[Cd(C_{18}H_{16}N_2O_4)Cl_2]$	09	10	11	10
$[Hg(C_{18}H_{16}N_2O_4)Cl_2]$	**22**	**16**	**23**	**23**
$CuCl_2.2H_2O$	11	12	14	13

$CoCl_2.6H_2O$	13	12	12	13
$NiCl_2.6H_2O$	11	10	12	13
$ZnCl_2$	13	11	10	12
$CdCl_2.2H_2O$	13	14	10	11
$HgCl_2$	14	15	12	13
Standard				
Gentamycine	24	26	--	--
Amphotericin	--	--	25	26

Part B: DNA-Cleavage Activity Studies

6.3 Introduction

In some of the bioactive ligands with low molecular weight which also recognize and interact with DNA, are of chemical and medicinal significance as potential artificial gene regulators or cancer chemotherapeutic agents[30,31]. Interaction of transition metal complexes with DNA has been extensively studied because of diverse applications in molecular biology, biotechnology and therapeutic agents[32]. Small agents bind unspecifically or with lower sequence specificity to dsDNA are often capable of influencing or inhibiting these processes and intrinsically exhibit magnetic properties. Consequently these molecules find application as Pharmaceuticals mainly in the treatment of Cancer others are employed as straining agents.[33]

Deoxyribonucleic acid (DNA) is the primary target molecules for most anticancer and antiviral therapies according to cell biologists. Investigations on the interaction of DNA with small molecules are important in the design of new types of pharmaceutical molecules. Since the chemical nuclease activity of transition metal complexes was discovered in 1980 studying the interaction model and the mechanism of transition metal complexes with DNA and exploring the application of metal complexes in anti-neoplatstic medication, molecular biology and bioengineering have become hotspots in recent years. Some kind of metal complexes interacted with DNA could induce the breakage of DNA strands by appropriate methods. In the case of cancer genes after DNA strands are cleaved, the DNA double strands break. The replication ability of cancer is destroyed[34] with DNA.

6.3.1 Experimental Preparation of culture media

DNA cleavage experiments were done according to the literature[35]. Nutrient broth [Peptone, 10, Yeast extract, 5 and NaCl, 10; in (g/L)] was used for culturing of *E.Coli*. The 50mL media was prepared, autoclaved for 15min at 121^0C under 15 lb pressures. The autoclaved media were inoculated with the seed culture and incubated at 37^0 C for 24h.

6.3.2 Isolation of DNA

The fresh bacterial culture (1.50mL) is centrifuged to obtain the pellet which is then dissolved in 0.5mL of lysis buffer (100mM tris pH 8.0, 50mM EDTA, 50mM lysozyme). To this 0.5mL of saturated phenol was added and incubated at 55^0C for 10min. Then centrifuged at 10,000rpm for 10min and to the supernatant, equal volume of chloroform: isoamyl alcohol (24:1) and $1/20^{th}$ volume of 3M sodium acetate (pH 4.8) was added. Then centrifuged at 10,000rpm for 10min and to the supernatant, 3 volumes of chilled absolute alcohol was added. The precipitated DNA was separated by centrifugation and the pellet was dried and dissolved in Tris buffer (10mM tris pH 8.0) and stored in cold condition.

6.3.3 Agarose gel electrophoresis

Cleavage products were analyzed by agarose gel electrophoresis method[35]. Test samples (10mg/mL) were prepared in DMSO. The samples (100µg) were added to the isolated DNA of *E.Coli*. The samples were incubated for 2h at 37^0C and then 20µL of DNA sample (mixed with bromophenol blue dye at 1:1 ratio) was loaded carefully into the electrophoresis chamber wells along with standard DNA marker containing TAE buffer (4.84g Tris base, pH 8.0, 0.5M EDTA/1L) and finally loaded

on agarose gel and passed the constant 50V of electricity for around 30min. Removed the gel and stained with 10μg/mL ethidium bromide for 10-15min and the bands observed under UV transilluminator and photographed to determine the extent of DNA cleavage. Then the results are compared with standard DNA marker.

Results and discussion

The DNA cleavage activities of Schiff base [BCAP] and their Cu(II), Ni(II), Zn(II), Cd(II) and Hg(II) complexes were studied by agarose gel electrophoresis method, and are given in Fig. 6(1). The gel after electrophoresis clearly revealed that the intensity of all the treated DNA samples has diminished, possibly because of the cleavage of the DNA. The difference was observed in the bands of the complexes compared to that of the control DNA of *E coli*. This shows that the control DNA alone does not show any apparent cleavage where as complexes show cleavage. The complete cleavage of DNA was observed by Cu(II), Ni(II) and Hg(II) complexes and partial cleavage of DNA was observed by Cd(II) and Zn(II) complexes.

The Schiff's base [BCACP] and their Cu(II), Co(II), Zn(II), Cd(II), and Hg(II) complexes were studied for their DNA cleavage activity by agarose gel electrophoresis method, and are given in Fig. 6(2). The gel after electrophoresis clearly revealed that the intensity of all the treated DNA samples has diminished, possibly because of the cleavage of the DNA. The difference was observed in the bands of the complexes (lanes L-Hg) compared to that of the control DNA of *E coli*. This shows that the control DNA alone does not show any apparent cleavage where as complexes show cleavage. The complete cleavage of DNA was observed by Co(II) complex and partial cleavage of DNA was observed by Cu(II) and Hg(II) complexes.

The Cu(II), Co(II), Ni(II), Zn(II) and Cd(II) complexes of the Schiff's base [BCMeTPC] were studied for their DNA cleavage activity by agarose gel electrophoresis method, and presented in Fig. 6(3). The gel after electrophoresis clearly revealed that the intensity of all the treated DNA samples has diminished, possibly because of the cleavage of the DNA. The difference was observed in the bands of the complexes (lanes L – Cd) compared to that of the control DNA of *E coli*. This shows that the control DNA alone does not show any apparent cleavage where as complexes show cleavage. The complete cleavage of DNA was observed by Cu(II), Co(II) and Cd (II) complexes and partial cleavage of DNA was observed by Ni(II) complex of the Schiff base [BCMeTPC].

The Schiff base [BCMeTB] and their Cu(II), Co(II), Ni(II), Cd(II) and Hg(II) complexes of the Schiff base [BCMeTB] were studied for their DNA cleavage activity by agarose gel electrophoresis method, and presented in Fig. 6(4). The gel after electrophoresis clearly revealed that the intensity of all the treated DNA samples has diminished, possibly because of the cleavage of the DNA. The difference was observed in the bands of the complexes (lanes Cu-Co) compared to that of the control DNA of *E coli*. This shows that the control DNA alone does not show any apparent cleavage where as complexes show cleavage. The complete cleavage of DNA was observed by Co(II) complex and partial cleavage of DNA was observed by Ni(II) complex of the Schiff base [BCMeTB].

The results indicated the important role of metal ions in these isolated DNA cleavage reactions. As the compound was observed to cleave the DNA, it can be concluded that the compound inhibits the growth of the pathogenic organism by cleaving the genome[36].

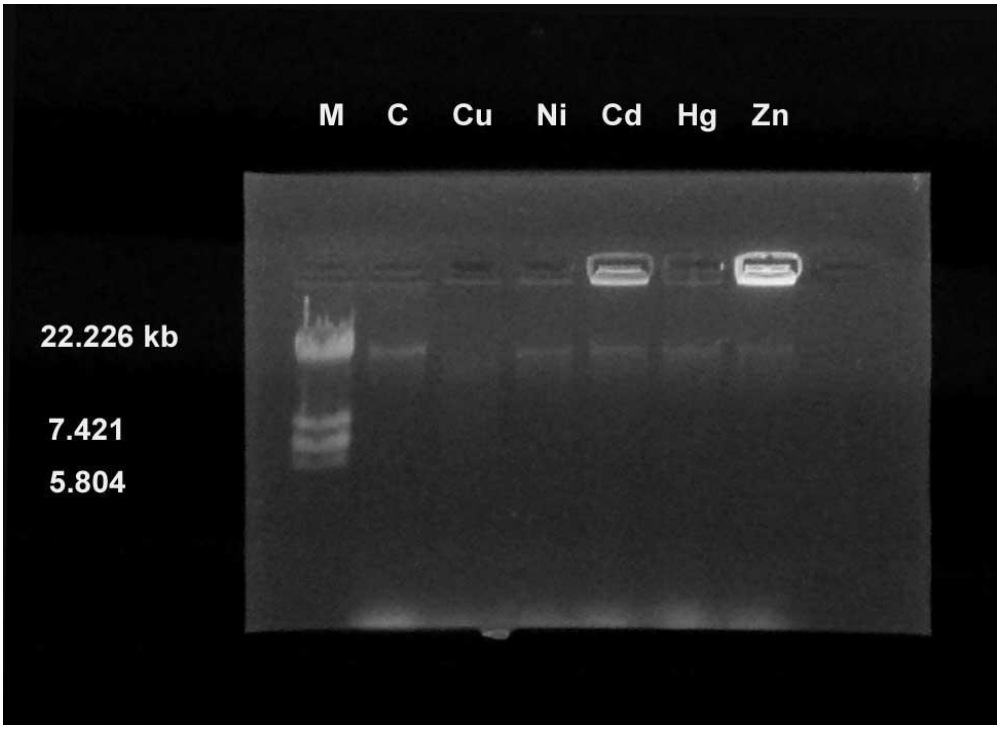

Fig. 6(1) DNA cleavage of Schiff base, [BCAP] and their Cu(II), Ni(II), Zn(II), Cd(II) and Hg(II) complexes

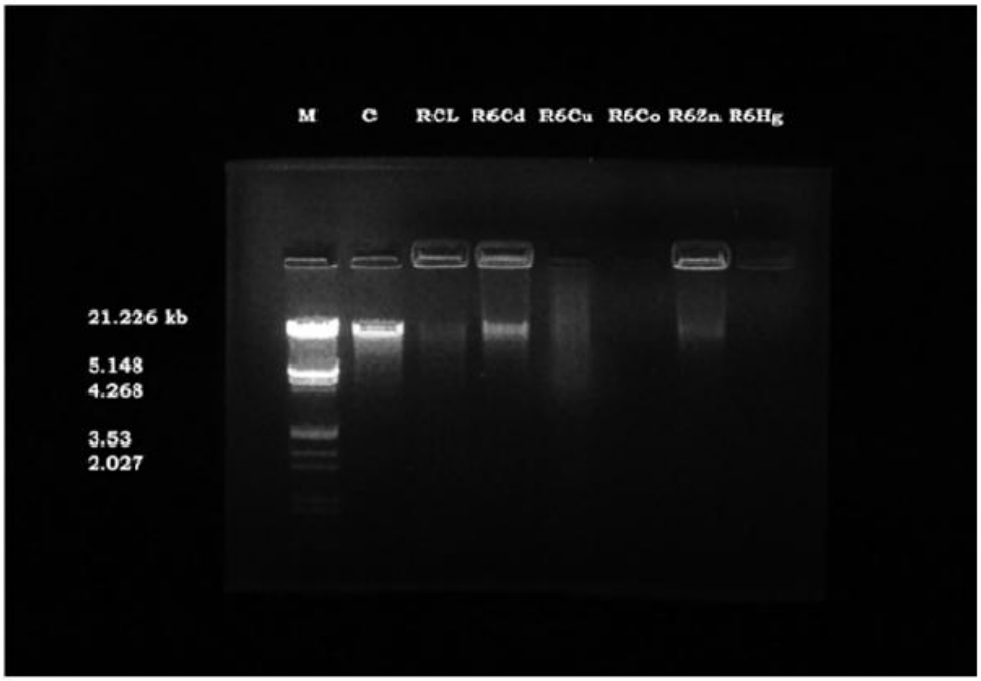

Fig. 6(2) DNA cleavage of Schiff base [BCACP] and their Cu(II), Co(II), Zn(II), Cd(II) and Hg(II) complexes

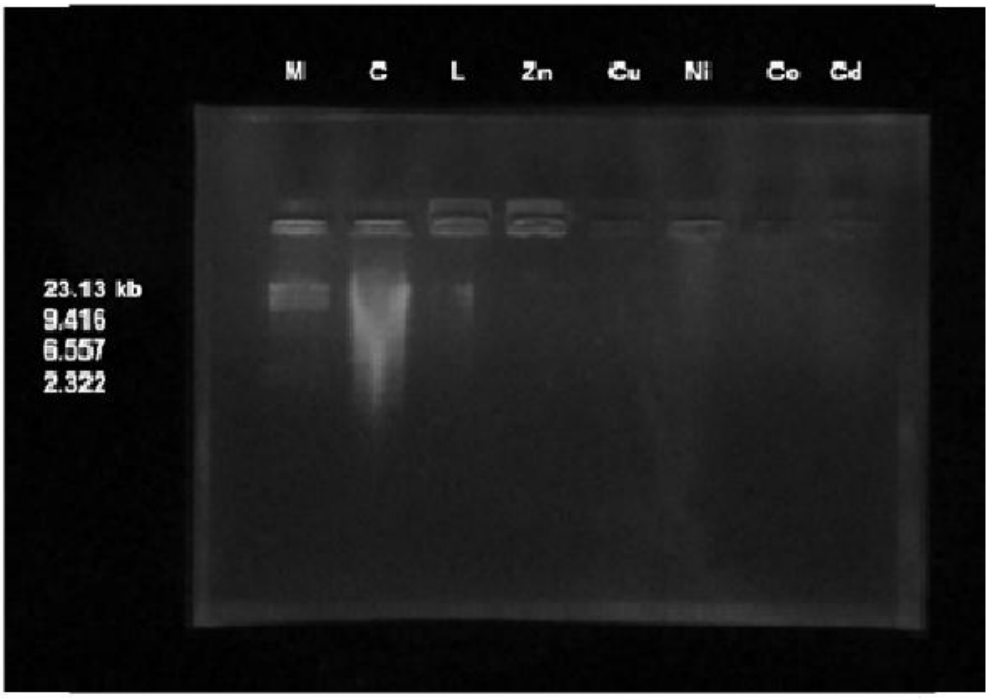

Fig. 6 (3) DNA cleavage of Schiff base [BCMeTPC] and their Cu(II), Co(II), Ni(II), Zn(II) and Cd(II) complexes

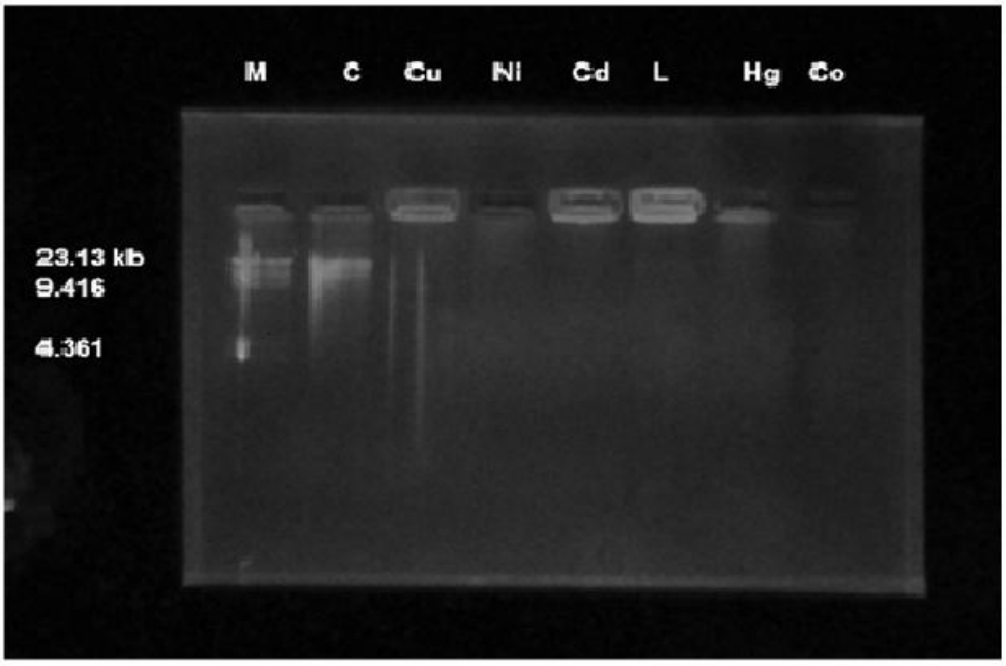

Fig. 6(4) DNA cleavage of Schiff base [BCMeTB] and their Cu(II), Co(II), Ni(II), Cd(II) and Hg(II) complexes

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