Design and Characterization of Benzofuran-Derived Mixed Ligand Metal Complexes: Antimicrobial Properties and DNA Cleavage Assessment

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Abstract. Metal complexes following the formula [M(L)(L')Cl_2] (**1–6**) were prepared, with M being Copper (II), Cadmium (II), Cobalt (II), Zinc (II), Nickel (II), Mercury (II). The ligand primary (L) was obtained by the condensation of benzofuran-2-carbohydrazide with piperonaldehyde, and the secondary ligand (L') is 1,10-phenanthroline (phen). The structures of the compounds were confirmed through various analytical techniques. The complexes exhibit non-electrolity which is confirmed from the molar conductivity measurements. The antimicrobial activities were evaluated for all synthesized compounds against selected fungal and bacterial strains using the agar diffusion method. Metal complexes exhibitited enhanced activity than the ligand. The DNA cleavage studies is done by gel electrophoresis technique. Redox characteristics were examined for Cu^{+2} complex.

Key words: Mixed ligand complexes, Characterization, Cyclic voltammetry, antimicrobial, gel electrophoresis

1 Introduction

Numerous Complexes are found to display a variety of biological activities [1] and helpful in the transport and storage of certain substances using membranes [2]. While, bioactive compounds contain benzofuran frameworks. Due to the widespread of structural variations in biologically active benzofurans, the benzo[b]furan framework has gained significant importance as a core structure in various pharmaceutical compounds. These derivatives are found to exhibit diverse biological activities [3]. It is been observed that benzofuran derivatives exhibit fluorescent properties and enjoy good antimicrobial [4], cytotoxic, anti-inflammatory, anti-analgesic etc activities.

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The significant role exhibited by benzofurans has motivated us to in the current study, aiming to synthesize new compounds with intensive biological activities. Till date, no investigations are been reported on the following complexes of $[M(L)(L')Cl_2]$ (1–6) synthesized by condensation product of benzofuran-2-carbohydrazide with piperonaldehyde, using 1,10-phenanthroline as a secondary ligand. This research was undertaken to address this gap. The new synthesized compounds were confirmed using various analytical techniques, and their molecular structures were proposed. The DNA cleavage and antimicrobial activity was carried out.

2. Experimental

The starting material which we used for the present work was Benzofuran-2-carbohydrazide obtained by reported method [5]. By using established literature methods, the metal and chloride concentrations were estimated [6].

2.1 Ligand synthesis, BMBFC

0.01 mole of Benzofuran-2-carbohydrazide is solvated in 30 mL ethanol added to a solution of 0.01 mole of piperonaldehyde in 20 mL of ethanol. The reaction mixture was refluxed on a water bath. The product was filtered and purified through recrystallization process (Scheme 1). Molecular Formula = $C_{17}H_{12}N_2O_4$, M.P. = 205 ºC, yield = 70%

2.2 Preparation of the Complexes:

A 20 mL alcoholic solution of $M(II)Cl_2$ (0.01 mole), where M = Copper (II), Cadmium (II), Cobalt (II), Zinc (II), Nickel (II) or Mercury (II) mixture containing 0.01 mole of Schiff base [BMBFC] in 30 mL alcohol and 0.01 mole of 1,10-phenanthroline (phen) in 20 mL ethanol was taken. The prepared reaction mixture was refluxed. The complexes precipitated were dried with fused calcium chloride. Yields: 51%

2.3 Measurements

The compositions of Carbon, Hydrogen and Nitrogen of the synthesized solid chelates was determined using a PerkinElmer 240C microanalyzer. FTIR spectrophotometer is used to record Infrared spectra. Far-infrared (FAR-IR) spectra were taken in the region 700–30 cm^{-1}. Copper (II) complex was recorded on a EPR spectrometer for ESR studies. The ^1H Nuclear Magnetic Resonance studies were done on a BRUKER 500 Mega Hertz spectrometer. Electronic absorption spectra of Copper (II), Nickel (II) and Cobalt (II) complexes are studied on a UV-Visible instrument. Electrochemical behavior of Cu^{+2} complex was studied using a electrochemical analyzer in DMF. A GC-Mate mass spectrometer was used for GC-MS study of the ligand. The conductivity measurements were carried out using an ELICO conductivity bridge. Magnetic susceptibility studies are done using the Gouy balance technique.

3. Biological Activity

3.1 Antimicrobic activity

The reported procedure [7] was followed to carry out the antibacterial and antifungal activities.

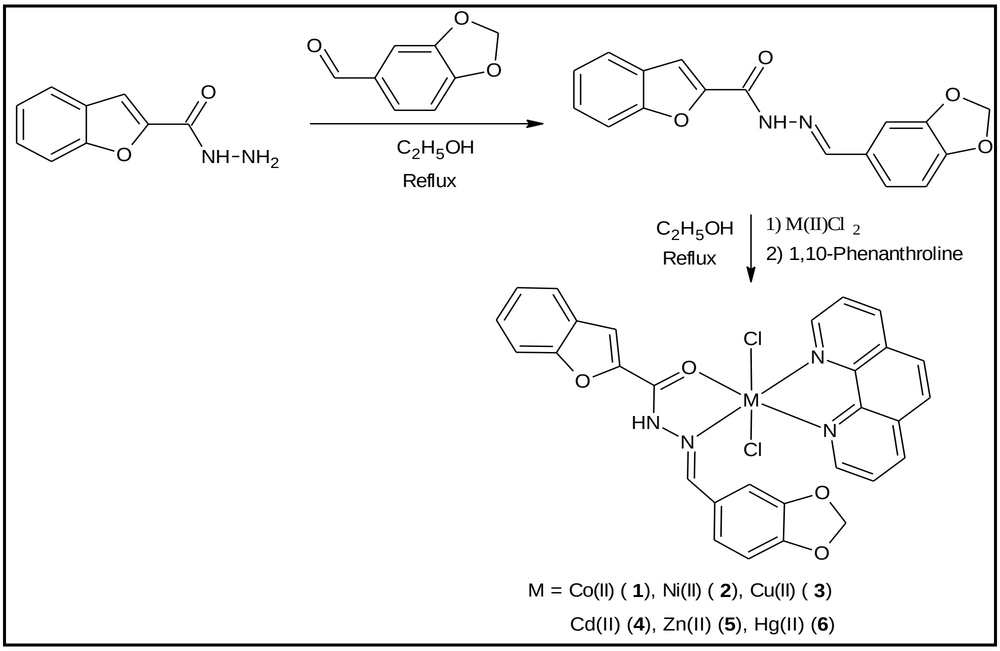
3.2 DNA cleavage

Standard protocols were followed to prepare the culture media, and the DNA cleavage was performed following literature methods [7].

4 Result and Discussion

4.1 Synthesis

All the complexes (**Scheme 1**) were colored and stable at RT. The complexes exhibited 1: 1: 1 (M: L: L) stoichiometry. The proposed molecular formulae supports the calculated values (Table 1). Capillary tubes were used to carried out the M. P of the compounds. The molar conductivity results are experimentally determined [8].



Scheme 1: Synthesis of Ligand and the proposed structure for the complexes.

4.2 Infrared absorption spectra studies

The medium broad bands were exhibited by the ligand [BMBFC] at 3301 cm^{-1} and 3236 cm^{-1}, corresponding to ν (NH) stretching vibrations which are assigned to the asymmetric (ν_{asym}) and symmetric (ν_{sym}) stretches, which shifts to wavenumbers by 6–16 cm^{-1} in the complexes, which indicates non-coordination and noninvolvement of the NH group nitrogen. Bands observed at 1663 cm^{-1} and 1624 cm^{-1} are due to the carbonyl and amide carbonyl, ν(Carbon-Nitrogen double bond) stretching vibrations. Approximately 33–50 cm^{-1} shift of the bands in the complexes is observed. Bonding with N atom of the hydrazine moiety is observed at 1036 cm^{-1}. This ν(N–N) stretch which shifts by 5–15 cm^{-1} in complexes, **1–6**. At 1174 cm^{-1} ν(C–O–C) stretching is observed. Change is not observed in the band of **1–6** complexes which indicates oxygen atom is not involved in bonding with the furan ring. At 1643 cm^{-1} 1,10-

phenanthroline exhibits ν(Carbon nitrogen double bond) stretching vibration. This band shifts in all complexes to lower wavenumbers between 1597–1601 cm⁻¹. Weak bands are observed between 341–356 cm⁻¹ for ν(M–Cl) stretching vibrations.

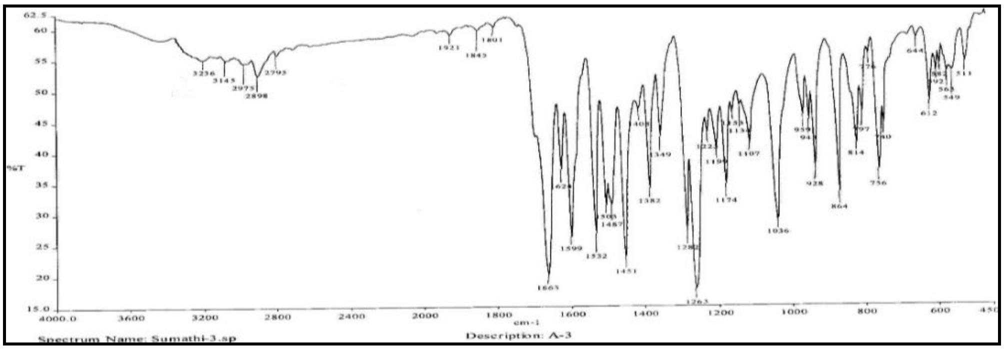


Fig. 1 IR spectrum of the ligand

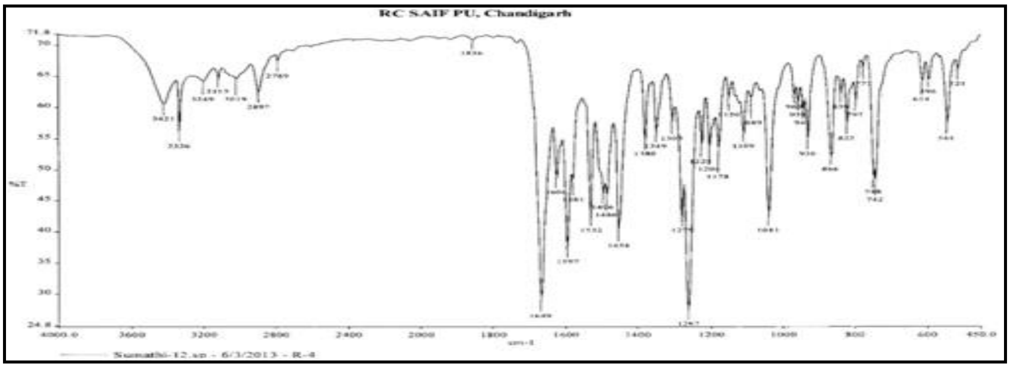


Fig. 2 IR spectrum of Ni(II) complex

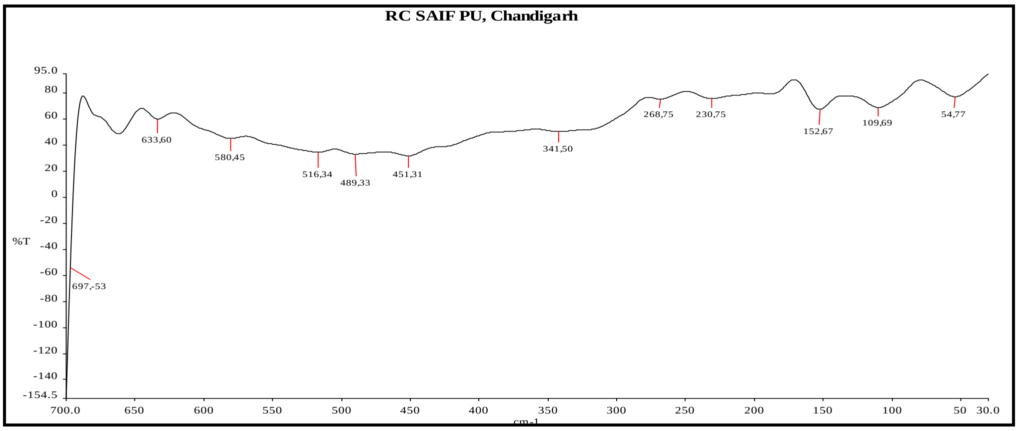


Fig. 3 Far-IR spectrum of Ni(II) complex

4.3 ^{1}H NMR spectral studies

The **Fig. 4** displays ^{1}H NMR spectrum of the ligand. Two distinct singlets at δ 12.04 and δ 8.42 corresponding –CONH– group and –N=CH– group, respectively. In ^{1}H NMR spectrum of Cd^{+2} complex (Fig. 5), signal of amide proton shifts downfield from δ 12.04 to δ 12.48, indicating coordination of the –CONH– oxygen. Aromatic protons appear in the region δ 7.01–7.86. The δ 6.10 signal indicates protons of –CH_2. Similarly, the azo methine proton shifts from δ 8.42 to δ 8.63, suggesting nitrogen of the –N=CH– moiety in coordination to the metal. Aromatic protons are increased to twelve in number, resonating a multiplet between δ 7.03 and 8.22 (m, 12H). The –CH_2 protons remain unchanged, as a singlet appearing at δ 6.10 (s, 2H).

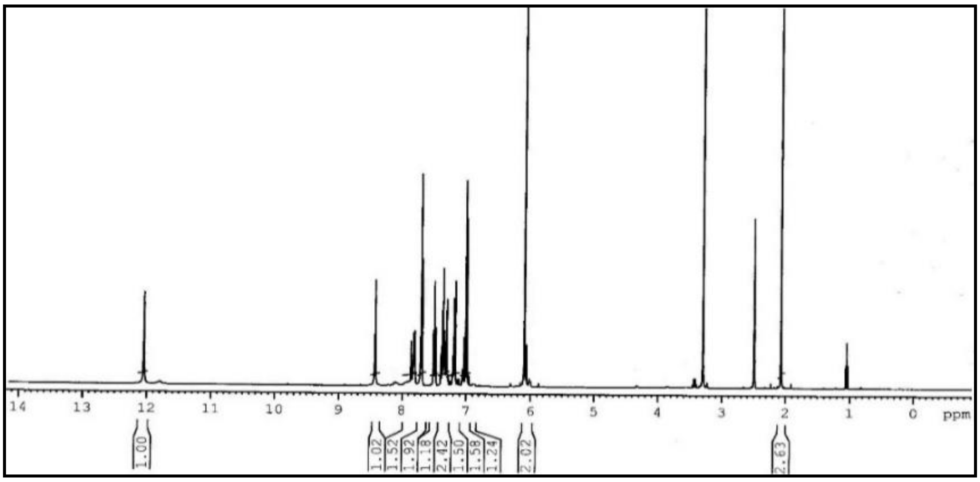
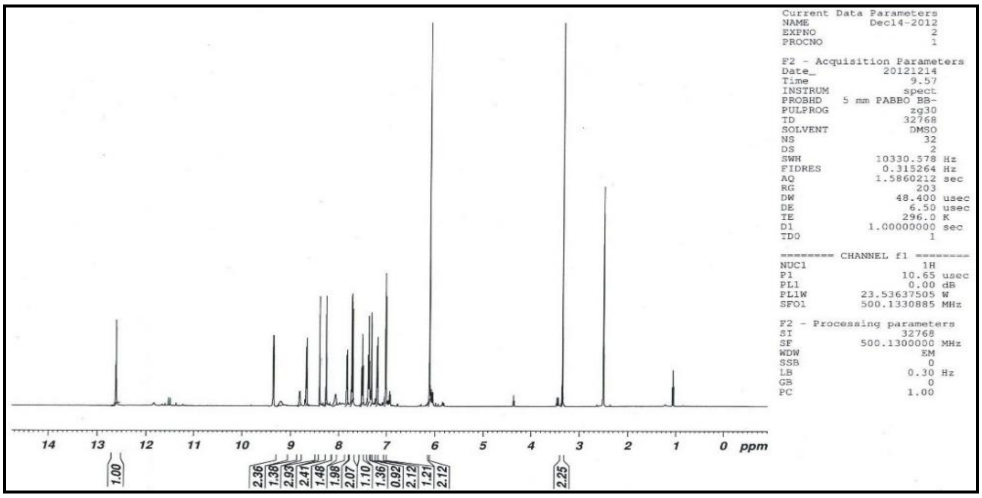


Fig. 4 ^{1}H NMR spectrum of the ligand



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PROCNO                   1

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PULPROG               zg30
TD                   32768
SOLVENT               DMSO
NS                      32
DS                       2
SWH              10330.578 Hz
FIDRES            0.315264 Hz
AQ               1.5860212 sec
RG                     203
DW                  48.400 usec
DE                    6.50 usec
TE                   296.0 K
D1              1.00000000 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                    1H
P1                   10.65 usec
PL1                   0.00 dB
PL1W           23.53637505 W
SFO1           500.1330885 MHz

F2 - Processing parameters
SI                   32768
SF             500.1300000 MHz
WDW                     EM
SSB                      0
LB                    0.30 Hz
GB                       0
PC                    1.00
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Fig. 5 ^{1}H-NMR spectrum of the Cd(II) complex

4.4 Mass Studies

The GC-Mass spectrum of BMBFC (**Fig. 6**) shows the M$^+$ peak found at m/z 308.

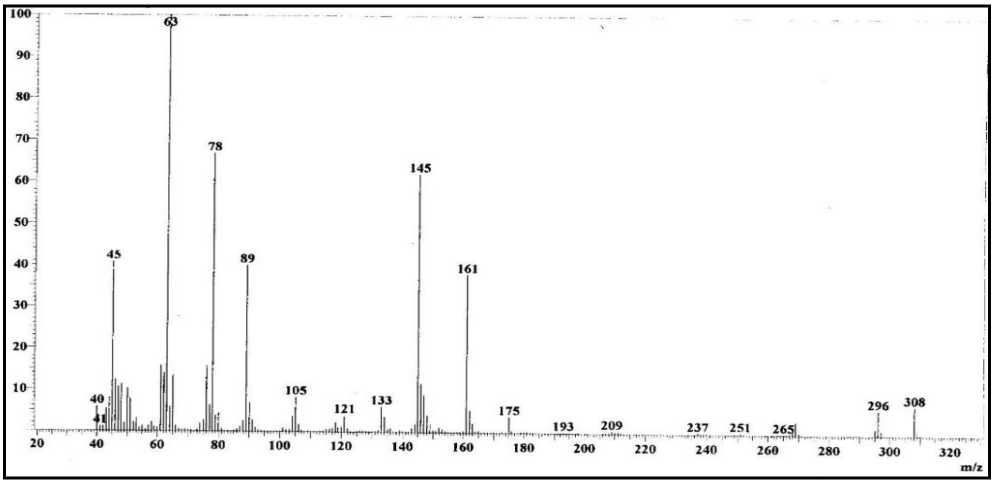

Fig 6 GC-Mass spectrum of the ligand

4.5 Electronic studies

The data is summarized in **Table 1**. The two bands at 15,762 and 21,136 cm^{-1}, was obserbed for the Co^{+2} complex [9]. The lowest band, ν_1 was estimated using the band fitting method [10].

Two absorption bands were displayed for the Ni^{+2} complex at 15580 and 26173 cm^{-1} indicates the geometry, octahedral. The ν_1 is computed using reported procedure [10].

Broad asymmetric band observed for Cu^{+2} complex lies between 13362- 17460 cm^{-1}. Hence, the geometry of distorted octahedral is suggested for Cu^{+2} ion.

The value of β for the synthesized Nickel complex is lower than the Cobalt complex. This suggests a greater degree of covalency for Metal–Ligand bond [11].

Table . 1 Electronic Spectroscopic Values

Complex	**Transitions, cm^{-1}**			**Dq values cm^{-1}**	**B$^{\text{l}}$ cm^{-1}**	**B value**	**B Per**	**ν_2/ ν_1**	**L.F.S.E kcal**
	ν_1^*	ν_2	ν_3						
1	7312	15762	21136	845	997	0.958	2.670	2.155	14.845
2	9560	15580	26173	956	871	0.837	16.251	1.629	32.777
3	13362 – 17460			1540	-	-	-	-	26.457

4.6 ESR spectral studies

The $g_{\perp}$ with $g_{\parallel}$ figures are 2.029 and 2.150 (**Table 2**). Axial symmetry parameter G value is 5.557. g_{av} value was computed as 2.069. The Cu^{+2} complex exhibited asymmetric EPR bands with $g_{\parallel} > g_{\perp} > 2.00277$ [12].

Table 2 ESR studies

Complex	$g_{\parallel}$	$g_{\perp}$	g_{av}	g_{iso}	G value
1	2.150	2.029	2.069	2.1104	5.557

4.7 Redox study

Reduction peak found at E_{pc}= 0.101Volts and an oxidation peak found at E_{pa}= 0.006 Volts is observed as shown in **Fig. 7**. The ΔE_p value is 0.095V. The redox process is observed for the voltammogram which corresponnds to the Cu^{+2} / Cu^{+2} redox couple [13].

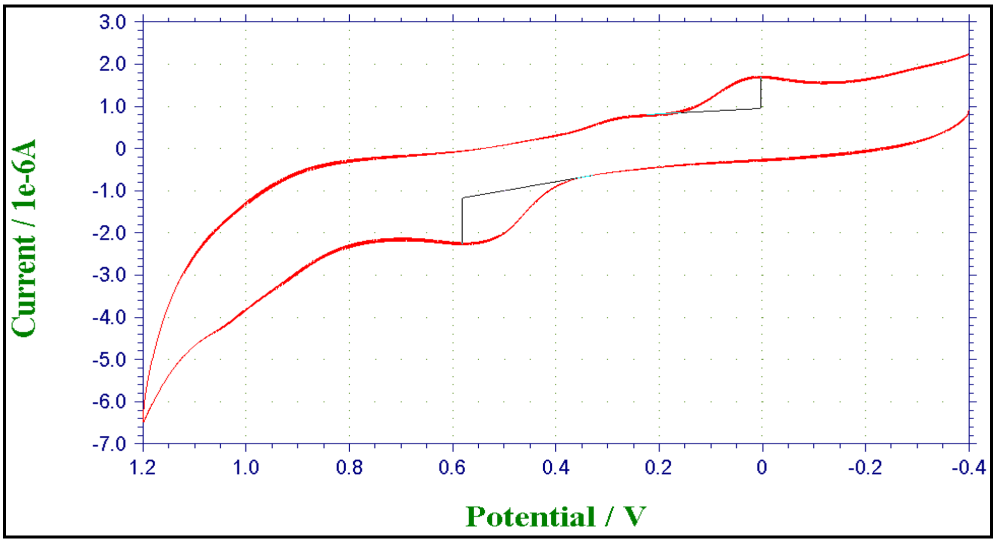

Fig. 7 Cyclic voltammogram of the Cu^{+2} complex

4.8 Pharmacology

4.8.1 Antifungal and Antibacterial bioassays: The bacterial strains tested included *Staphylococcus aureus*, *Bacillus subtilis*, *E. coli*, and *Pseudomonas aeruginosa*, while the fungal species examined were *Aspergillus niger*, *Aspergillus flavus*, *Cladosporium oxysporum*, and *C. Albicans*. The valuse are tabulated in **Table 3**.

Table 3 Minimum Inhibitory Concentration (MIC in µg/mL)

Compound	**E.coli**	**S.aureus**	**B.subtilis**	**P.aeruginosa**	**A.niger**	**A.flavus**	**C.oxysporum**	**C.albicans**
BMBFC	75	75	50	50	75	75	50	75
Phen	-	75	-	-	75	-	-	-
Complex **1**	25	25	25	12.50	50	12.50	25	50
Complex **2**	25	50	12.50	12.50	50	50	12.50	25
Complex **3**	50	50	25	25	25	50	25	50
Complex **4**	25	12.50	25	12.50	12.50	25	25	50
Complex **5**	12.5	12.50	12.50	25	25	12.50	12.50	12.50
Complex **6**	12.5	12.50	25	25	12.50	12.50	25	25
Gentamycine	12.5	12.50	12.50	12.50	-	-	-	-
Amphotericin	-	-	-	-	12.50	12.50	12.50	12.50

The synthesized complexes such as Co^{+2}, Ni^{+2}, and Cu^{+2} demonstrated moderate antibacterial and antifungal effects, while the Cd^{+2}, Zn^{+2}, and Hg^{+2} complexes displayed significantly stronger activity in comparison. Overall, the antifungal activity was greater than the antibacterial effects. The observed enhancement in biological activity can be interpreted using the reported theory [14].

4.8.2 DNA cleavage efficiency

Using the gel electrophoresis method the cleavage of calf thymus DNA of **1–6** were monitored (**Fig. 8**).

The agarose gel was qualitatively analyzed by observing DNA bands that migrated either more slowly or further than the control DNA. Treatment of DNA with the complexes (lanes S6–S1) showed a difference in molecular weight indicating that all complexes interacted with the DNA. This difference was evident in the lanes S6–S1. From the DNA cleavage studies the compounds inhibit the development of pathogenic organisms through DNA cleavage [15].

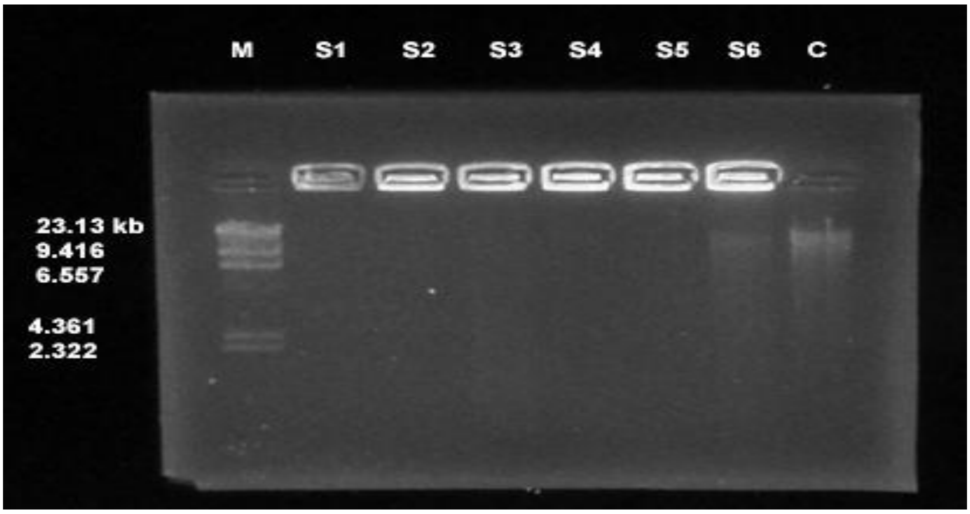

Fig 8 The study of DNA cleavage M: Standard molecular weight marker, C: Control DNA, Lanes S1-S6: Complexes, **1-6**

5. Conclusion

The spectral data (IR, ^{1}H-NMR, UV-Vis, mass spectrometry, ESR) and elemental analysis supports the octahedral geometry for all synthesized complexes. Antimicrobial reports suggest that the Cd^{+2}, Zn^{+2}, and Hg^{+2} complexes exhibit greater effectiveness compared to the Ni^{+2}, Co^{+2} and Cu^{+2} complexes and the ligand. The findings also demonstrate that the biological activity of the compounds are enhanced by complexation. The Cu^{+2} complex displays a redox couple which is quasi-reversible involving a single-electron transfer in its electrochemical behavior. According to the electrophoresis studies, all metal complexes are proficient in DNA cleavage. Current research indicates that it is possible to synthesize and characterize a variety of other benzofuran derivatives and their metal complexes.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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