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Studies On DNA Cleavage And Antimicrobial Screening Of Transition Metal Complexes

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Abstract: A new series of transition metal complexes of Fe(II), Co(II), Ni(II) and Cu(II), were synthesized from the ligand 3-acetyl-4-hydroxypyrano[3, 2-c]chromene-2,5-dione **(L^{I})** and 4-hydroxy-3-(3-oxobutyanoyl) pyrano [3, 2-c]chromen-2, 5-dione **(L^{II})**. The structural features were arrived from their elemental analyses, molar conductance, Mass, IR, UV-Vis. and 1H NMR spectral studies. The data show that the complexes have composition of $M[L(H_2O)]_2$ type. The *in vitro* antimicrobial screening tests were also recorded and gave good results in the presence of metal ion in the ligand system. The DNA cleavage ability of the complexes was monitored by the gel electrophoresis technique.
Key words: *in vitro,* antimicrobial, DNA cleavage, gel electrophoresis.

Introduction

Coumarin and its derivatives have created intense interest because of their antibiotic, antibacterial, antifungal, anticoagulating, plant regulating and anticancer activities [1-5]. The activities of nuclease is currently an attractive research area in molecular biology since artificial nucleases have potential applications as novel restriction enzymes and anticancer therapeutic agents [6]. Deoxyribonucleic acid (DNA) is the primary target molecule for most anticancer and antiviral therapies according to cell biologists. Among the different therapeutic strategies to eradicate cancer cells through DNA damage, the view of using small water soluble transition metal complexes, capable of oxidative or hydrolytic DNA cleavage as anticancer drugs is a challenging issue in bioinorganic chemistry [7,8]. Many transition metal complexes with vanadium [9], iron [10], copper [11,12], cobalt [13], lanthanides [14,15] and also actinides [16] have been reported as efficient DNA cleavage agents with or without sequence specificity, moreover the ligand or the metal in these complexes can be varied in an easily controlled manner to facilitate the individual applications [17]. Investigations of the interaction of DNA with small molecules are basic work in the design of new types of pharmaceutical molecules. Some types of metal complexes interacted with DNA could induce the breakage of DNA strands as shown by the gel electrophoresis technique. Thus, in cancer genes, after a cleavage of a DNA strand, the DNA double strands breaks. The replication ability of cancer gene is thereby destroyed.

In present work, we report synthesis and characterizations of ligand and their metal complexes using various spectroscopic techniques. The novel investigated compounds and their metal complexes were also evaluated for their antimicrobial activity against several bacterial strains and nuclease activity against calf thymus (CT) DNA.

Experimental:

Apparatus and reagents

All chemicals used were of analytical reagent (AR) grade and of the highest purity available. They include phosphorous oxychloride (Spectrochem), Phenol, malonic acid, zinc chloride, sodium carbonate, hydrochloric acid, ethanol, ammonia, agarose (Merck), and nutrient broth, glucose, yeast extract (Helini biomolecules). CT DNA was purchased from Genei chemical company, India. Solutions of CT DNA in Tris–HCl/NaCl (pH 7.2) buffer medium gave a ratio of A_{260}/A_{280}, of ca. 1.8–1.9, indicating that the DNA was sufficiently free from protein contamination [18]. Stock solutions were kept at 4°C and used within 4 days. Doubly distilled water was used to prepare the buffer.

All the melting points were determined in open glass capillaries in a liquid paraffin-bath and are uncorrected. The NMR Spectra were recorded on BRUKER NMR Spectrometer (300 MHz) and IR spectra on Shimadzu, 435-IR in frequency range of 4000-400 cm^{-1} using KBR pellet technique. The conductivity of metal complexes was determined using Systronic Conductivity Bridge. Elemental Analyses were performed on a Perkin- Elmer 2400 CHN elemental Analyzer Model 1106.

Synthesis of 4-hydroxy-2H-chromene-2-one *(1)*

It was prepared by the reported method given by shah & co-workers [19]. Phenol(0.1mole) and malonic acid(10.4g, 0.1mole) were added to a mixture of phosphorous oxychloride(40ml) and freshly fused zinc chloride(40ml).The reaction mixture was heated on water bath at 60-70^{o}C for 8-10 h and poured into ice. The solid residue is filtered out and treated with hot saturated sodium carbonate. The filtrate was slowly acidified with concentrated hydrochloric acid. At the point of neutralization, the precipitates are obtained are filtered and washed with water and further dried and recrystalised with methanol.

Synthesis of 4-hydroxy-2-methylenepyrano[3,2-c]chromene-5(2H)-one *(2)*

4-hydroxy -2H-chromene-2-one (0.1mole) and malonic acid (10.4g, 0.1mole) were added to a mixture of phosphorous oxychloride(40ml) and freshly fused zinc chloride(40g). The reaction mixture was heated on water bath at 60-70^{o}C for 16-18h and poured into ice. The solid residue is filtered out and treated with hot saturated sodium carbonate. The filtrate was slowly acidified with concentrated hydrochloric acid. At the point of neutralization, the precipitates are obtained are filtered and washed with water and further dried and recrystalised with methanol.

Synthesis of 3-acetyl-4-hydroxy-pyrano[3, 2-c]chromene-2,5-dione *(L^I)*

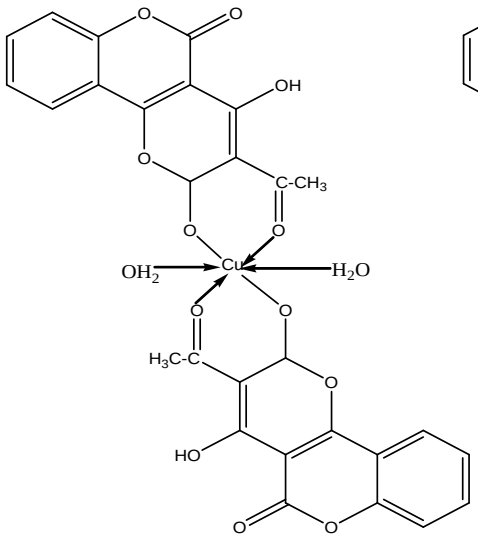
Acetyl derivatives are prepared [20, 21-25] by acetylation of different derivatives in presence of acetic acid and phosphorous oxychloride. 4-hydroxy-2-methylenepyrano [3,2-c]chromene-5(2H)-one (1g) was dissolved in glacial acetic acid(5ml) and phosphorous oxychloride (4ml). The reaction mixture was gently refluxed for 4-5hrs., then added to ice water. 3-acetyl-4-hydroxy-pyrano[3, 2-c]chromene-2,5-dione are solid mass, crystallized form glacial acetic acid.

Synthesis of 4-hydroxy-3-(3-oxobutanoyl)pyrano [3,2-c]chromene-2,5-dione *(L^{II})*

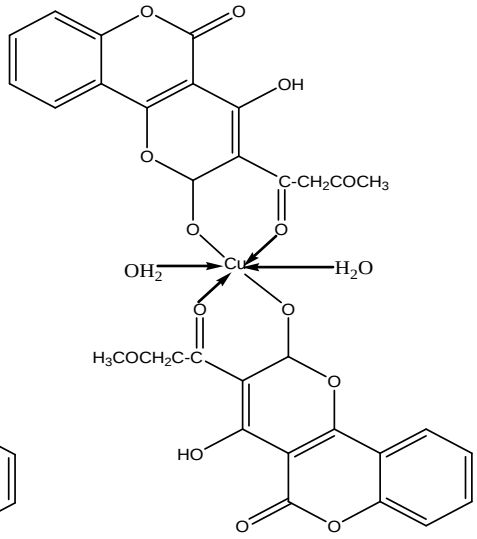
Acetyl derivatives, on its further reaction with ethyl acetate in presence of sodium give aceto acetyl derivatives [20, 21-25]. 3-acetyl-4-hydroxy-pyrano[3, 2-c]chromene-2,5-dione (1g) dissolved in ethyl acetate(25ml) was added to pulverized sodium(1g) and refluxed for 8h. It was then decomposed with ice and extracted with ether. The aqueous layer on acidification gave substituted 4-hydroxy-3-(3-oxobutyanoyl)pyrano[3,2-c]chromene-2,5-dione, which was then filtered out, dried and recrystalised with acetone.

Synthesis of metal complexes

The metal solutions (0.1M) were prepared by dissolving metal salt (ferrous ammonium sulphate and chloride of cobalt, nickel & copper) in distilled water and standardized with 0.1M EDTA solution. Reaction of standardized metal solution (10ml) was carried out with ligand solution ***(L^I & L^{II})*** (20ml) for two hours in water bath at 100^{o}C. Few drops of ammonium hydroxide were added to the reaction mixture to maintain the pH 10.5-11. Precipitates obtained were filtered, washed with water and alcohol, dried and recrystalised with DMSO.

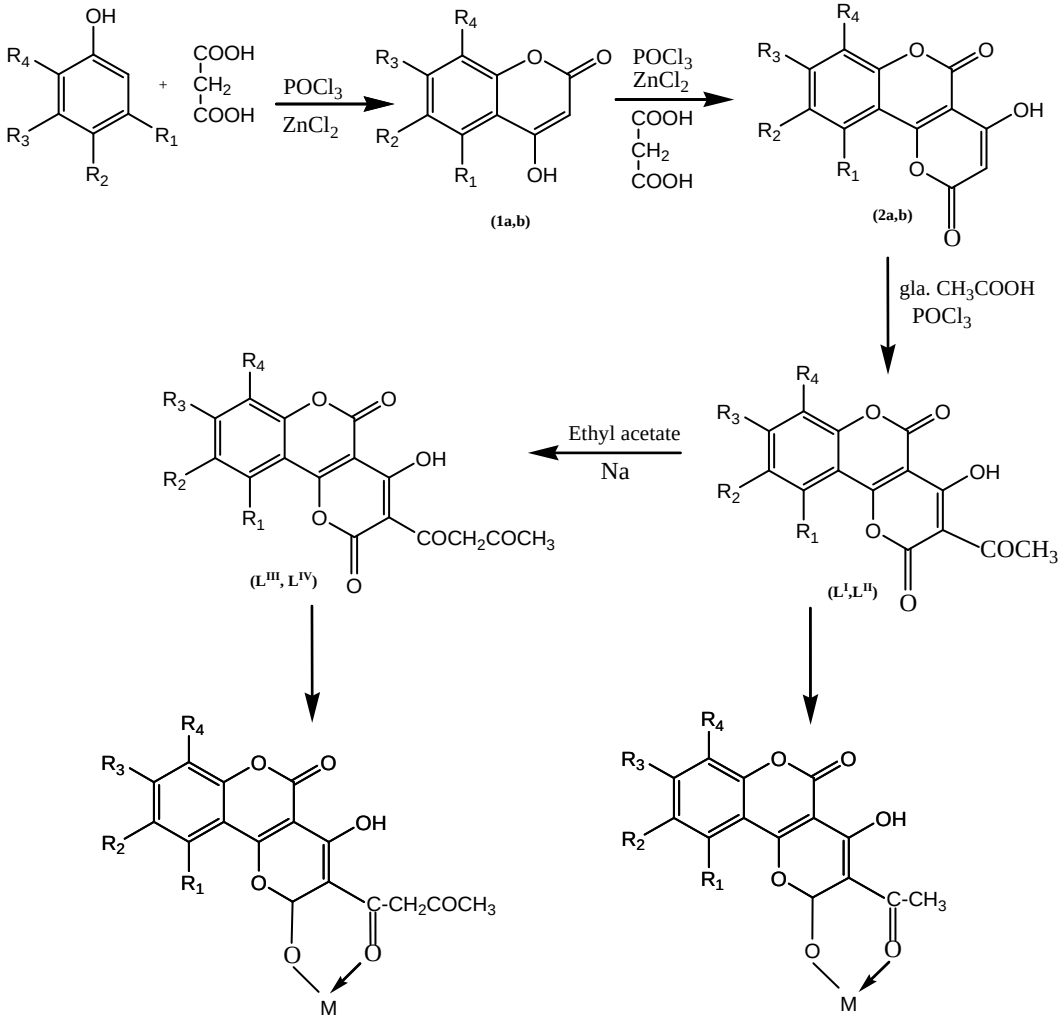


Structure-I



Structure-II

Reaction scheme



Where, R_1, R_2, R_3, R_4 = H or CH_3
M = Fe^{+2}, Co^{+2}, Ni^{+2} or Cu^{+2}

Conductivity

The conductivity of metal complexes was determined using Systronic Conductivity Bridge. It was dissolved in DMF and conductivity was measured. Conductivity of the DMF along was measured and solution of the complexes in DMF with different concentration was measured. The molar conductivity was calculated using the formula.

$$Molecular\ conductivity = \frac{1000 \times K}{C}$$

Where, K = Conductivity of the sol. of the complexes in DMF.

C = Concentration of the complexes (10^{-3} M).

The conductivity data are presented in (Table 2) and the data indicates that the complexes are non-electrolyte in nature [26].

Antimicrobial activity:

Quantitative antibacterial assay: Different concentrations of compounds were subjected to suitable broth for quantitative determination of antimicrobial activity. The lowest concentration, which completely inhibits visible microbial growth, was recorded as the minimum inhibitory concentration (**MIC**, mg/ml).

Qualitative antimicrobial assay: The *in vitro* biological screening effects of the investigated compounds were tested against the gram negative bacteria *E. coli* and gram positive bacteria *Bacillus megaterium* by the well diffusion method (agar cup method) using agar nutrient as the medium. While antifungal activity was carried out using glucose yeast extract media (GYE) against *Aspergillus niger* and *Candida albicans*. The stock solutions were prepared by dissolving the compounds in DMSO. In a typical procedure, a well was made with the help of borer on the nutrient medium plate which was previously inoculated with microorganisms. The well was filled with the different concentration of test solution using a micropipette and incubated at 37°C for 24 hrs (bacteria) and 48 hrs (fungi). DMSO was used as control. During incubation period, the test solution diffused and the growth of the inoculated microorganisms was affected. Antibacterial activity was indicated by the presence of clear zone of inhibition around the wells. The zone of inhibition was measured in mm.

Gel electrophoresis

The DNA cleavage experiment was conducted using CT DNA by gel electrophoresis with the corresponding metal complex in the presence of H_2O_2 as an oxidant. The reaction mixture was incubated before electrophoresis experiment at 35°C for 30minutes as follows: CT DNA 5µl (4×10^{-4} µg/µl), 12 µl each compound (1000 ppm), 2 µl H_2O_2 (35%). The samples were electrophoresed for 2 h at 50 V on 0.8% agarose gel using tris-acetic acid-EDTA buffer, Ph=8.3.After electrophoresis ,the gel was stained using,1 µg/cm3 ethidiumbromide (EB) and photographed under UV light using Sony camera.

Result And Discussion

All the compounds are stable at room temperature, insoluble in water, methanol, and ethanol, but soluble in DMF and DMSO. The analyses of the compounds are consistent with the stoichiometry proposed and are summarized in Table 1&2. Elemental analyses of metalcomplexes indicates that the metal: ligand (M:L) ratio is 1:2 for all the divalent metal ions. Because all metal complexes char above temperature 270°, their melting points were not recorded. All the complexes have low conductance values, which indicate that the complexes are nonelectrolytic[27] in nature.

Table:I Analytical and physical data of the synthesized compounds

Compd.	Molecular Formula	Molecular Weight (g)	Melting Point (°C)	Elemental analysis(%) Found/calcd. C	H	O
1	$C_9H_6O_3$	162	192°C	66.42/66.67	3.47/3.73	29.72/29.6
2	$C_{12}H_6O_5$	230	175°C	62.5/62.62	2.46/2.63	34.59/34.76
L^I	$C_{14}H_8O_6$	276	100°C	61.89/61.77	3.08/2.96	35.12/35.27
L^{II}	$C_{16}H_{10}O_7$	314	96°C	61.32/61.15	3.14/3.21	35.52/35.64

IR Spectra

The IR spectra of the ligands a strong broad band observed near ~3440 cm^{-1} due to the O-H stretching. The broad band suggests the existence of the compounds predominantly in the enolic form [28]. A strong C-O stretching band of alcohol is present at~1080 cm^{-1}. Attached methyl substituents gave C-H stretching band near 2932 cm^{-1}. Multiple bonds of aromatic C=C stretching is observed in between 1491-1560 cm^{-1} while Sharp bands of =C-H bending are observed between 730-100 cm^{-1}. A sharp band of cyclic ketone between 1726-1732 cm^{-1} observed while a sharp band appear between 1606-1616 cm^{-1} indicates presence of α,β-unsaturated acetyl ketone. Multiple weak bands C-O-C ether linkage observed between 1163-1294 cm^{-1}. Monodentate acetate usually shows two bands at ~1620 cm^{-1} and ~1310 cm^{-1} due to antisymmetric and symmetric stretching, respectively [29]. Since carbonyl absorption of the compounds also appeared in this region, the band at ~1620 cm^{-1} could not be located. However, a medium intensity band observed between 1294-1330 cm^{-1} suggests the monodentate coordination of the acetate groups.

In case of metal complexes bands of OH remains almost unaffected indicating that the enolic OH is not replaced during complex formation. In all the spectra of complexes both aromatic and acetyl ketone frequencies shifted by ~15–20 cm^{-1} to the lower energy region compared to the free ligand. This phenomenon appears may be due to the coordination of the carbonyl oxygen to the metal ion. That the carbonyl groups are involved in bonding with the metal is further supported by the appearance of a medium intensity bands in the region 476-486 cm^{-1} assignable to M-O vibrations [29].

Table II Physical characterization, analytical and molar conductance data of metal complexes

Complex	Molecular Formula	Molecular weight (g)	Elemental analysis(%) Found/Calcd. C	H	O	M	Molar conductance mho cm^2mol^{-1}
$Fe[L^I(H_2O)]_2$	$C_{28}H_{22}O_{14}Fe$	638	52.75/52.69	3.62/3.47	34.91/35.09	8.89/8.75	11.3
$Co[L^I(H_2O)]_2$	$C_{28}H_{22}O_{14}Co$	641	52.61/52.43	3.29/3.46	35.11/34.92	9.18/9.19	14.25
$Ni[L^I(H_2O)]_2$	$C_{28}H_{22}O_{14}Ni$	641	52.32/52.45	3.31/3.46	35.08/34.94	9.03/9.15	9.87
$Cu[L^I(H_2O)]_2$	$C_{28}H_{22}O_{14}Cu$	646	51.92/52.06	3.56/3.43	34.52/34.67	9.72/9.84	12.52
$Fe[L^{II}(H_2O)]_2$	$C_{32}H_{26}O_{16}Fe$	722	53.04/53.2	3.78/3.63	35.68/35.44	7.52/7.73	12.38
$Co[L^{II}(H_2O)]_2$	$C_{32}H_{26}O_{16}Co$	725	53.13/52.98	3.75/3.61	35.4/35.29	8.26/8.12	13.57
$Ni[L^{II}(H_2O)]_2$	$C_{32}H_{26}O_{16}Ni$	725	52.89/53	3.55/3.61	35.14/35.3	7.94/8.09	11.08
$Cu[L^{II}(H_2O)]_2$	$C_{32}H_{26}O_{16}Cu$	730	52.53/52.64	3.68/3.59	34.88/35.06	8.92/8.7	14.36

Antimicrobial activity

The antimicrobial activity of the ligand and its complexes was evaluated by the MIC method. The *in vitro* antimicrobial activity of the investigated compounds was tested against the microorganisms, such as *E. coli*, *Bacillus megaterium*, *Aspergillus*, and *Candida Albicans* by the serial dilution method. On the basis of result of MIC, zone of inhibition was measured (in millimeter) by disc diffusion technique [30-34] are summarized in Table 3. A comparative study of the ligand and its complexes, indicates that the complexes exhibit higher antimicrobial activity than the free ligand. Such increased activity of the complexes can be explained on the basis of Overtone's concept and Tweedy's chelation theory. According to Overtone's concept of cell

permeability, the lipid membrane that surrounds the cell favors the passage of only the lipid-soluble materials due to which liposolubility is an important factor, which controls the antimicrobial activity. On chelation, the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further, it increases the delocalization of π-electrons over the whole chelate ring and enhances the lipophilicity of the complexes. This increased lipophilicity enhances the penetration of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of the proteins that restricts further growth of the organism.

Table III Anti microbial data of ligands & their metal complexes
(Conc. in ppm & zone of inhibition in millimeter)

Compound	*Escheritia coli*		*Bacillus megaterium*		*Aspergillus niger*		*Candida albicans*	
	Conc.	Zone	Conc.	Zone	Conc.	Zone	Conc.	Zone
L^{I}	200	21	300	19	400	23	200	20
	300	22.5	400	20	500	26	300	21
	400	24	500	21.5	600	27	400	22.5
$Fe[L^{I}(H_2O)]_2$	300	21.5	300	18	200	20.5	200	21.5
	400	23	400	18	300	21	300	23
	500	23.5	500	20	400	25.5	400	25
$Co[L^{I}(H_2O)]_2$	100	20.5	100	19.5	300	22	200	19
	200	22	200	20	400	23.5	300	22
	300	23	300	21	500	26	400	23.5
$Ni[L^{I}(H_2O)]_2$	200	22.5	50	20	400	24	200	19
	300	24	100	22	500	26.5	300	21.5
	400	25	200	22.5	600	28	400	24
$Cu[L^{I}(H_2O)]_2$	200	24	200	23	400	24.5	200	22
	300	26	300	24	500	27	300	24
	400	27.5	400	26	600	29.5	400	26
L^{II}	100	17.5	200	18	400	18	200	0
	200	18	300	20	500	19.5	300	17
	300	20	400	21.5	600	22	400	19
$Fe[L^{II}(H_2O)]_2$	100	17	200	18	500	0	200	16
	200	17.5	300	19.5	600	19.5	300	16.5
	300	19	400	21	700	21	400	17
$Co[L^{II}(H_2O)]_2$	50	17.5	100	20	500	19.5	200	18
	100	18	200	20	600	21	300	18
	200	19.5	300	21.5	700	22	400	19
$Ni[L^{II}(H_2O)]_2$	200	18	200	21	500	19.5	200	17.5
	300	19	300	22	600	21	300	19
	400	21	400	22.5	700	22	400	23
$Cu[L^{II}(H_2O)]_2$	100	18.5	100	22	400	20	200	18
	200	21	200	23	500	22	300	20.5
	300	23	300	23.5	600	23.5	400	24

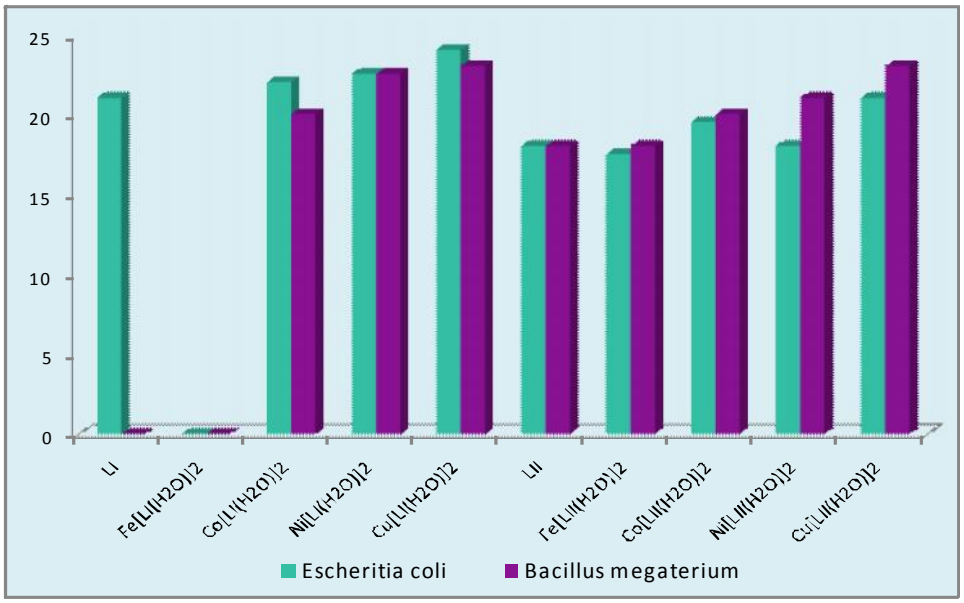


Figure: 1 Antibacterial data of the Ligands and their Complexes at 200ppm

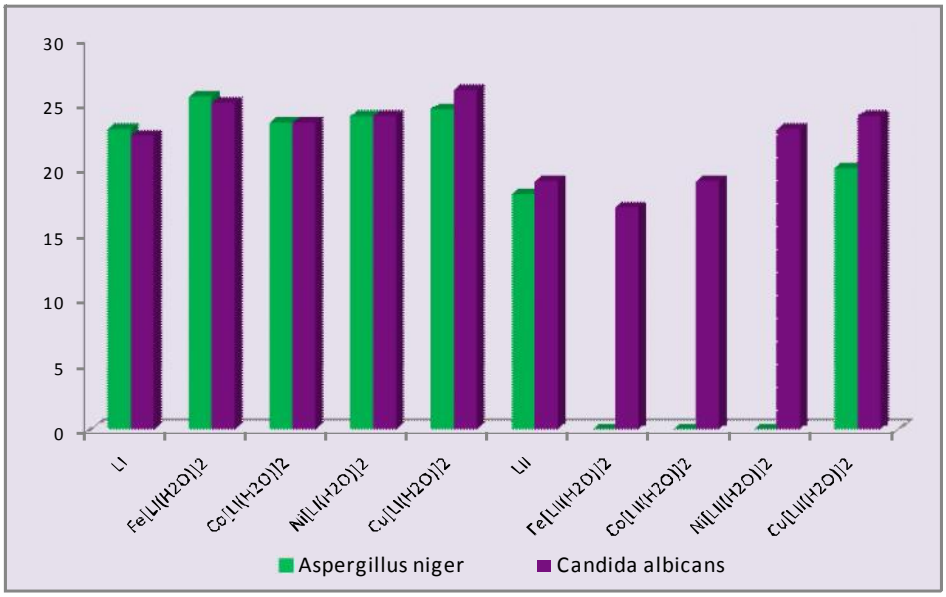


Figure: 2 Antifungal data of the Ligands and their Complexes at 400ppm conc.

DNA Cleavage Studies

The cleavage efficiency of the complexes compared with that of the control is due to their efficient DNA-binding ability. The metal complexes were able to degrade DNA.The general oxidative mechanisms proposed account for DNA cleavage by hydroxyl radicals *via.* abstraction of a hydrogen atom from sugar units and predict the release of specific residues arising from transformed sugars, depending on the position from which the hydrogen atom is removed [35]. The cleavage is inhibited by the free radical scavengers implying that hydroxyl radical or peroxy derivatives mediate the cleavage reaction. The reaction is modulated by a metallocomplexes bound hydroxyl radical or a peroxo species generated from the co-reactant H_2O_2. In the present study, the CT-DNA gel electrophoresis experiment was conducted at 35 °C using our synthesized complexes in the presence of H_2O_2 as an oxidant. It was found that, at very low concentrations, few complexes exhibit nuclease activity in the presence of H_2O_2. Control experiment using DNA alone does not show any significant cleavage of CT-DNA even on longer exposure time. Probably this may be due to the formation of redox couple of the metal ions and its behavior. The redox property of the metal complexes mediates oxidation of nucleic acids. In oxidative DNA cleavage mechanism, metal ions in the complexes react with H_2O_2 to generate the hydroxyl radical which

attacks at the C4' position of the sugar moiety and finally cleaves the DNA [36]. Due to the cleavage, intensity of DNA band decreases which can be observed in figures III & IV. A slight increase in the concentration over the optimal value (i.e., the value at which 100% cleavage efficiency was observed) led to extensive degradations, resulting in the disappearance of bands on agarose gel [37].

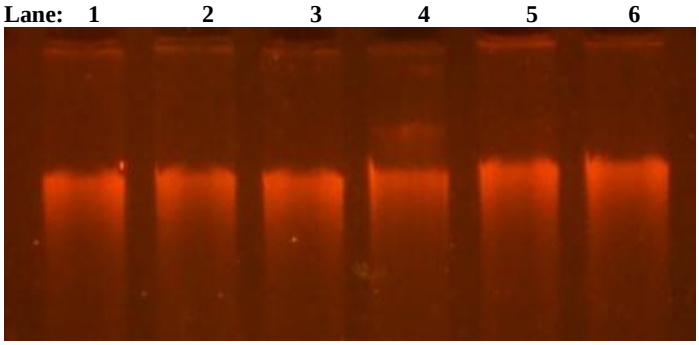


Figure 3. Agarose gel (0.8 %) showing the changes in the agarose gel electrophoretic pattern of CT DNA induced by H_2O_2.

Lane 1: DNA alone, **Lane 2:** DNA + L^I, **Lane 3:** DNA + $Fe[L^I(H_2O)]_2$, **Lane 4:** DNA + $Co[L^I(H_2O)]_2$, **Lane 5:** DNA + $Ni[L^I(H_2O)]_2$, **Lane 6:** DNA + $Cu[L^I(H_2O)]_2$

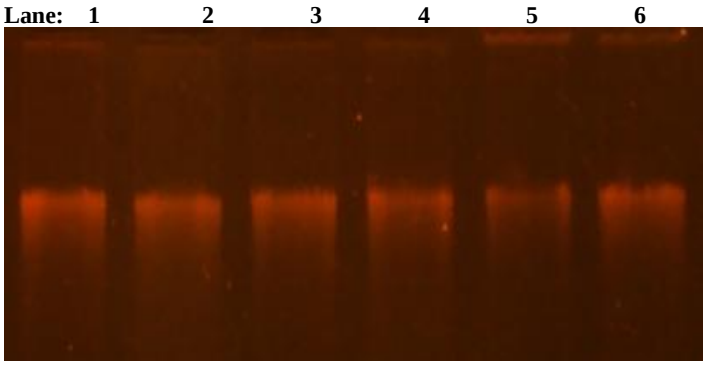


Figure 4. Agarose gel (0.8 %) showing the changes in the agarose gel electrophoretic pattern of CT DNA induced by H_2O_2.

Lane 1: DNA alone, **Lane 2:** DNA + L^{II}, **Lane 3:** DNA + $Fe[L^{II}(H_2O)]_2$, **Lane 4:** DNA + $Co[L^{II}(H_2O)]_2$, **Lane 5:** DNA + $Ni[L^{II}(H_2O)]_2$, **Lane 6:** DNA + $Cu[L^{II}(H_2O)]_2$

Conclusions

All metal complexes are non-electrolytes in DMF (molar conductance < 15 mho cm^2mol^{-1}; 10^{-3} M solution). Elemental analysis (Table I&II), IR and 1H NMR data of the complexes are in agreement with structure-1&2. Data of antimicrobial activity (Table III&IV) shows most the metal complexes are higher active than their ligands. Figure-1 indicates all the compounds are active against both the bacterial strains *E. coli* and *B. megaterium* at 200 ppm concentration accept L^I and it's Fe(II) complex while comparison of antifungal data at 400 ppm (figure-2) shows L^I higher active against both the fungus. From figures III it can be concluded that $Co[L^I(H_2O)]_2$ complex while from figure IV it can be concluded that $Co[L^{II}(H_2O)]_2$, $Ni[L^{II}(H_2O)]_2$ and $Cu[L^{II}(H_2O)]_2$ complexes cleave DNA in the presence of H_2O_2, whereas other complexes are not effective. Anong all the complexes, $Ni[L^{II}(H_2O)]_2$ shows lowest intensity band of DNA, it leads to the conclusion that $Ni[L^{II}(H_2O)]_2$ has highest DNA cleavage ability.

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