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Antioxidant, *In-vitro* Antibacterial and DNA-cleavage activities of Nickel (II) Complex derived from N, N'-(1, 2 Phenylenebis (Methanylylidene)) bis(4-Nitroaniline)

T. Sujeshwari [1], E. Akila [2] & *P. Maheswaran [3*]

[1] Research Scholar, Department of Chemistry, P.G.P College of Arts and Science, Namakkal, Tamil Nadu, India

[2] Department of Chemistry, Sri Sarada College for Women (Autonomous), Salem-16, Tamil Nadu, India

[3*] Principal and Head, Department of Chemistry, P.G.P College of Arts and Science, Namakkal, Tamil Nadu, India

ABSTRACT

New mononuclear Nickel (II) complex with Schiff base derived from the condensation of o-phthalaldehyde with p-nitroaniline were synthesized. The ligand and its complex were characterized by elemental analysis, molar conductivity, magnetic moment, IR, ^{1}H NMR, and electronic spectra. The conductivity data confirmed the non-electrolytic nature of the complex. The spectral and analytical data suggested a distorted octahedral geometry for Ni(II) complex. The synthesized complex with ligand have been screened for antimicrobial activity against two gram-positive bacteria (Staphylococcus aureus and Bacillus subtilis) and two gram-negative bacteria (Escherichia coli and klebsiella pneumoniae). The obtained results showed that Ni(II) complex is more potent than free ligand. DNA cleavage studies of Ni(II) complex have been investigated by gel electrophoresis using super coiled pUC18DNA. The experimental results revealed that the Ni(II) complex cleaved pUC18DNA. in an efficient manner than the parent ligand. In addition, the antioxidant activity of the ligand and its metal complex were investigated through scavenging effects for DPPH radical indicating that the compound show stronger antioxidant activity than free ligand but lower when compared to standard oxidants such as vitamin C.

Keywords: Mononuclear, Antimicrobial studies and DNA cleavage.

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***Address for Correspondence:**

T. Sujeshwari, Research Scholar, Department of Chemistry, P.G.P College of Arts and Science, Namakkal, Tamil Nadu, India

INTRODUCTION

The Schiff bases ligand has been found extensive use in the field of research and field of interest now a day. The Schiff bases compounds are recognized due to the presence of azomethine group (-CH=N) which is formed by reacting either by aldehyde or ketone with compounds containing amine group [1]. As the Schiff bases can be synthesized by easy and simple condensation reaction and has been found its extensive use in the field of coordination chemistry due to its property of donating electrons as a ligand. Schiff base mononuclear transition metal complexes due to its synthetic accessibility and its impact in coordination chemistry, as well as the relevance to structural characterization has received a great deal of interest in the field of research [2]. Besides, the DNA interaction of Schiff base metal complexes has also gained much interest towards their applications in biotechnology and medicine. The scientific community has showed that metal coordination compounds have shown some outstanding DNA cleavage properties, as well as antibacterial and antioxidant activities [3-4]. It is seen that transition metals are particularly suitable for this purpose, because they can adopt a wide variety of oxidation states, coordination numbers and geometries, in comparison to other main group elements.

Over the past decades, considerable interest in the synthesis and characterization of transition metal complexes with Schiff Base ligands had been developed to check the role of metal active sites in several catalytic biological processes. In the present study, the synthesis and characterization of mononuclear Ni(II) complex of a Schiff base ligand derived from o-phthalaldehyde and p-nitroaniline has been reported. Schiff base ligand and its metal complex were characterize by the elemental analysis, magnetic susceptibility, molar conductivity measurements, electronic spectra, IR, ^{1}H NMR spectrum to determine the mode of co-ordination and geometry. In order to establish the biological role of metals, the Schiff base and its Ni(II) complex have been screened for their antimicrobial activity against two gram-positive bacteria such as S. aureus and B. subtilis and two gram-negative bacteria such as E. coli and K. pneumonia. The oxidative damage caused by ROS on lipids, proteins, and nucleic acids plays a significant role in the development of life limiting chronic diseases such as cancer, hypertension, cardiac infarction, arteriosclerosis, rheumatism, and cataracts [5]. Thus, the percentage of antioxidant activities of Schiff base and its Ni(II) complex were also determined.

MATERIALS AND INSTRUMENTS

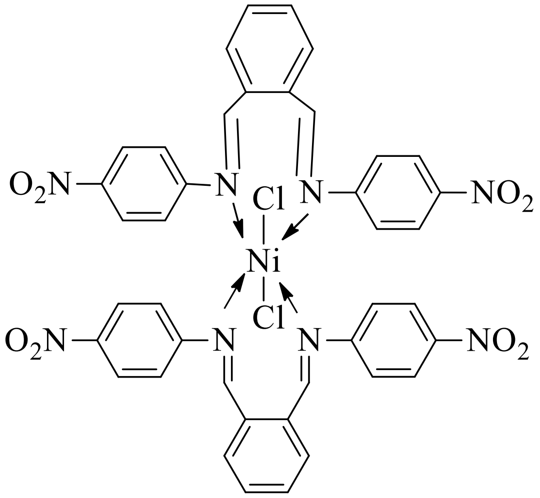
All chemicals and reagents used were of analytical grade and were used as received without any further purification. All the solvents were purified according to standard procedures. The elemental analysis (Carbon, Hydrogen and nitrogen) were performed using a Carlo Eraba 1106 instrument. The molar conductance of the freshly prepared Schiff base transition metal complex (10^{-3} M) was recorded in DMF at room temperature using ELICO CM 185 Conductivity Bridge. The Infrared spectra were recorded on the Perkin Elmer FT-IR-8300 Model spectrometer using KBr disc in the region 4000-200 cm^{-1}. Electronic absorption spectra in the UV-Visible range were recorded on Double beam spectrometer Cyber lab between 200-800 nm by using DMF as the solvent. ^1H-NMR spectra of the ligand were recorded on Bruker Spectrometer at 400 MHZ in DMSO-d_6.

Synthesis of Schiff base ligand

The Schiff base ligand was synthesized by adding 1, 2-benzene dicarboxaldehyde (1 mM) in 20 ml of ethanol, and p-nitroaniline (2 mM) in 20 ml of ethanol. The mixture was refluxed for 2½ hrs. Then the solution of the ligand was kept for slow evaporation and collected and dried in air [6].

Synthesis of mononuclear Ni(II) complex

The ethanolic solution of synthesized ligand N, N'–(1, 2 phenylenebis(methanylylidene) bis(4-nitroaniline) (2 mM) was added drop wise and strirring to an ethanolic solution of the metal salt $NiCl_2.6H_2O$ (1 mM) with constant stirring, and the mixture was boiled under reflux for 4½ hrs. Then the volume of the reaction mixture was reduced by evaporation. The resultant product was filtered, washed with ethanol and dried [7].



Scheme 1: Mononuclear Schiff base Ni(II) complex

Antibacterial studies

The synthesized complex and the Schiff base ligand were tested for their antimicrobial studies using bacterial strains such as *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Bacillus subtilis*. Antibacterial test was performed by using the disc diffusion method. The bacterial strains were subcultured individually in nutrient agar medium. The compounds were dissolved in DMF. The plates were incubated aerobically at 37º C for 24 hours and the antimicrobial activity was assessed by measuring the inhibition of microbial growth around the well. This method involves the exposure of the zone inhibition toward the diffusion of micro-organism on agar plate [8].

Total antioxidant capacity assay

- Different concentrations (10 µl, 20 µl, 30 µl, 40 µl and 50 µl) of synthesized compounds were taken and 1ml of reagent solution was added. [Reagent solution: 0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM Ammonium molybdate.
- The tubes were capped and incubated in thermal block at 95º C for 90 minutes.
- After the time interval the tubes were cool down at room temperature.
- The absorbance was recorded at 695 nm using shimadzu UV 1800 spectrophotometer [9].

Calculation of 50% Inhibitory Concentration (IC_{50})

The concentration (mg/ ml) of the fractions that was required to scavenge 50% of the radicals was calculated by using the percentage scavenging activities at five different concentrations of the fractions.

Percentage inhibition (I %) was calculated using the formula,

I % = (Ac-As) / Ac x 100

Where, Ac = absorbance of the control and

As = absorbance of the sample.

TAC = Total Antioxidant Capacity assay

DNA cleavage studies

The DNA cleavage studies were done by gel electrophoresis experiment for which pUC18 was used as the plasmid DNA. The DNA cleavage activity was evaluated by monitoring the conversion of supercoiled plasmid DNA to nicked circular DNA and linear DNA. The reaction mixture was prepared by adding 40µl Puc18 DNA, 50 mM Tris-HCl, pH 7.4, 50 mM NaCl, 10 mM H_2O_2 in a different volume, followed by adding Millipore water for final volume. Samples were typically incubated for 1 hr at 37 ºC [10].

RESULTS AND DISCUSSION

The synthesized transition Ni(II) complex are found to be freely soluble in DMSO**,** DMF and ethanol. The analytical data of the synthesized ligand and its mononuclear metal complex are shown in Table 1. The lower molar conductivity value (7.60 Ω^{-1} cm^2 mol^{-1}) for Ni(II) complex (10^{-3} M) in DMSO at room temperature indicates their non-electrolytic nature [11-12].

Table 1: Analytical data of the Schiff base ligand and its mononuclear metal complex

Compound	Molecular formula	Colour	Yield %	Melting point (C)	% of Nitrogen		% of Metal	
					Cal	Exp	Cal	Exp
L	$C_{20}H_{14}N_4O_4$	Yellowish orange	70	145	15.0	14.8	-	-
ML_2X_2	$Ni(C_{40}H_{28}N_8O_8Cl_2)$	Dark Green	60	>200	12.7	12.5	6.7	6.4

IR Spectral analysis

The FT-IR spectral analysis of Schiff base and its metal complex indicated the presence of all expected functionalities are listed in Table 2. The metal complex exhibited the absorptions of azomethine region at lower frequencies than free ligand. The infrared spectrum of the Schiff base ligand showed a medium absorption at 1655.34 cm^{-1} assigned to the C=N stretching vibrations, indicating the formation of the Schiff base linkage. In the complex, this band is shifted to lower frequencies in the 1649.42 cm^{-1} upon complexation with the metal [13], which can be attributed to the coordination of the imine nitrogen to the metal centre. The characteristic bands for the entire complex in the region 691.10 cm^{-1} may assigned for the vibration of aromatic rings and C-H stretching respectively. The other absorption characteristic appeared in the region 484.34 and 315.10 cm^{-1} may be assigned for M-N and M-Cl vibrations [14].

Table 2: IR spectral data (4000–200 cm^{-1}) of Schiff base and its metal complex

Compounds	υ(C=N) (cm^{-1})	υ(M-Cl) (cm^{-1})	υ(M-N) (cm^{-1})
$C_{20}H_{14}N_4O_4$	1655.34	--	--
$Ni(C_{40}H_{28}N_8O_8Cl_2)$	1649.42	315.10	484.34

Electronic spectra and magnetic moment studies

The electronic absorption spectra of the Ni(II) complex were recorded in freshly prepared solution in DMF at room temperature. The electronic spectra of the Schiff base ligand and its complex have been measured in DMF solution 200–800 nm at room temperature [15]. The data are represented in Table 3. In the electronic spectra of the Schiff base ligand, an intense absorption at 365 nm were assigned to a benzene π→π* transition and the band at 382 nm were assigned due to n→π* transition associated with the azomethine chromophore (-C=N). The dark green Ni(II) complex showed three bands at at 562, 585, and 613 nm which are assigned to ^3A_2g (F) →^3T_2g (F) (ν_1), ^3A_2g (F) →^3T_1g (F) (ν_2) and ^3A_2g (F) →^3T_1g (P) (ν_3) transitions respectively, indicating an distorted octahedral geometry. The magnetic moment of 3.41 B.M. falls within the range normally observed for octahedral Ni(II) complex.

Table 3: Electronic Spectral data of Schiff base ligand and its Ni(II) complex.

Compound	Electronic spectra (nm)				Geometry of the complex
	π→π*	n→π*	L→M	d-d	
$C_{20}H_{14}N_4O_4$	365	382	-	-	-
$Ni(C_{40}H_{28}N_8O_8Cl_2)$	298	365	420	562, 585, 613	Distorted Octahedral

^{1}H – NMR Spectral studies

The ^{1}H-NMR spectra of the Schiff base ligand was measured at room temperature in DMSO- d_6. The ^{1}H NMR spectra of the ligand exhibits well resolved signals and significant differences in their chemical shifts. The spectrum of the Schiff base ligand exhibits a singlet signal in the 8.48 ppm, which is attributed to the azomethine group (–CH=N–) proton and the multiplets around 6.6-7.9 ppm are assigned due to aromatic protons [16]. The spectra of the ligand is shown in Fig 1.

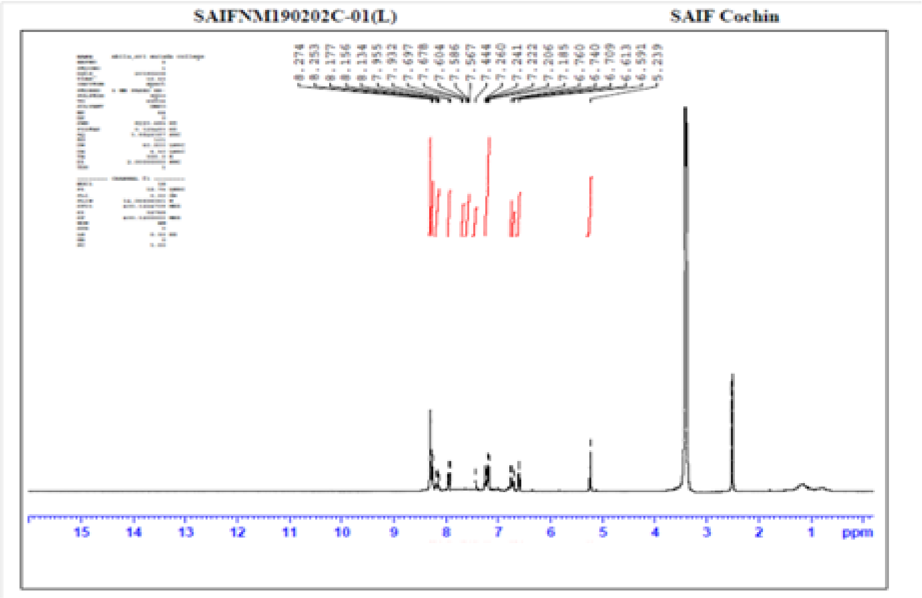


Figure 1: ^1H NMR spectrum of $C_{20}H_{14}N_4O_4$

Antibacterial activity

The synthesized ligand and its complex were screened for their antibacterial activity against *Staphylococcus aureus, Bacillus subtilis* while *Escherichia coli* and *Klebsilla pneumonia*. The standard drug streptomycin was also tested for their antibacterial activities at the same concentration and conditions similar to tested compound concentration. From the antibacterial studies, it is inferred that the Schiff bases and its Ni(II) complex were found to be potentially active against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Klebsilla pneumonia* [17]. The antibacterial activity of Schiff base and its metal complex against different bacteria are shown in Fig 2, 3, 4, and 5.

From the obtained results, it can be observed that the Ni(II) complex showed greater activity as compared to the Schiff base. The improved activities of the metal complex as compared to the ligand can be explained on the basis of chelation theory.

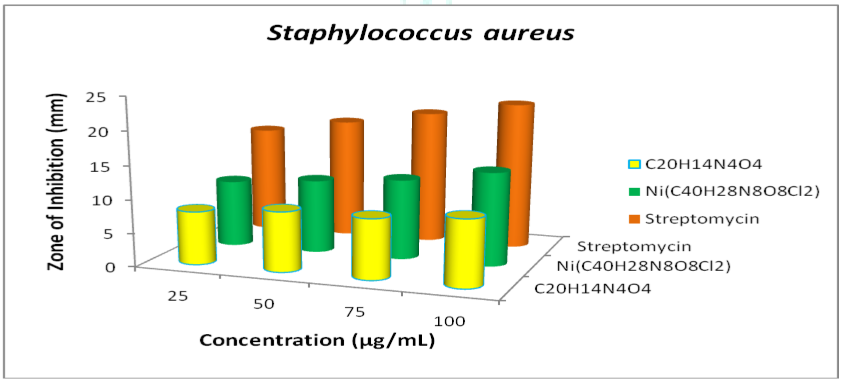


Figure 2: Antibacterial activity of Schiff base ligand and its mononuclear Schiff base metal complex against *Staphylococcus aureus*

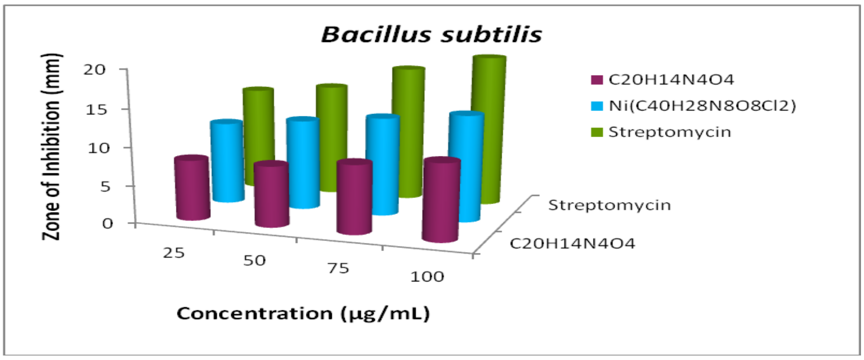


Figure 3: Antibacterial activity of Schiff base ligand and its mononuclear Schiff base metal complex against *Bacillus subtilis*

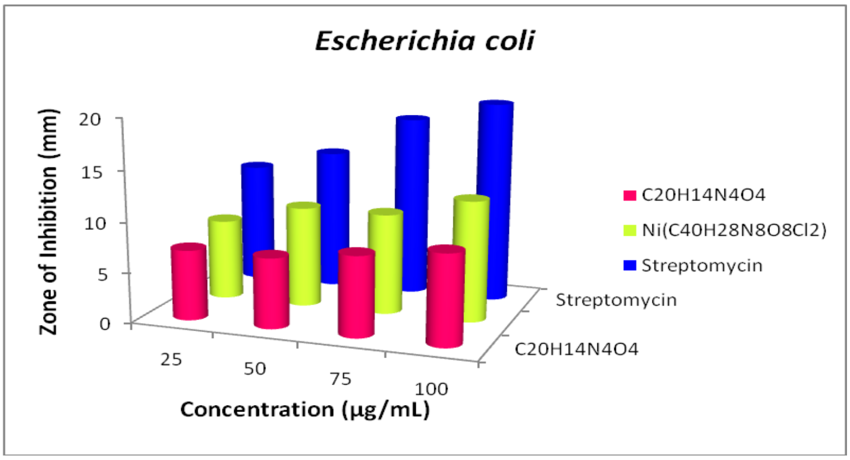


Figure 4: Antibacterial activity of Schiff base ligand and its mononuclear Schiff base metal complexes against *Escherichia coli*

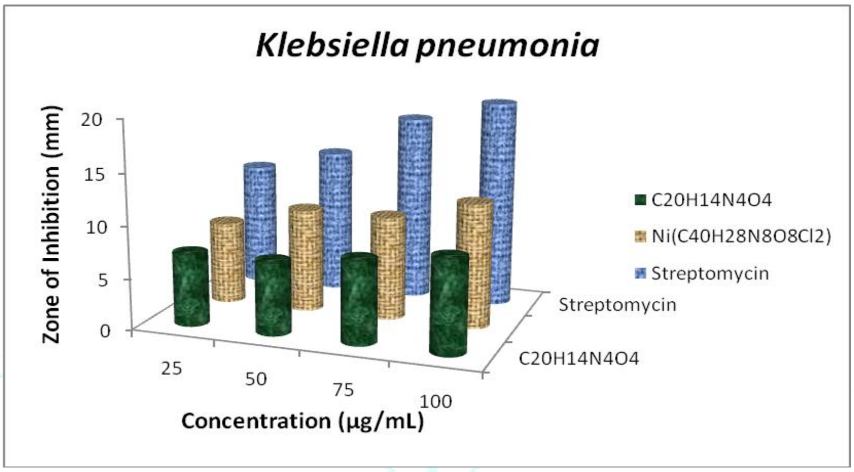


Figure 5: Antibacterial activity of Schiff base ligand and its mononuclear Schiff base metal complex against *Klebsiella pneumonia*

Antioxidant activity

The antioxidant assay was carried out using different concentrations of the Schiff base and its Ni(II) metal complex with radical, while ascorbic acid (vitamin C) was used as standard.

Total Antioxidant Capacity assay

The total antioxidant capacity of Ni($C_{40}H_{28}N_8O_8Cl_2$) is significantly higher than that of free ligand, indicating that this complex is a much better antioxidant than ligand but lower when compared to ascorbic acid (vitamin C) as standard [18]. Total antioxidant capacity of $C_{20}H_{14}N_4O_4$ and Ni($C_{40}H_{28}N_8O_8Cl_2$) as well as the standard was increased in a dose-dependent manner, antioxidant ability of Schiff base, increased significantly after chelation of transition metal ion (Fig 6 and 7). The Ni($C_{40}H_{28}N_8O_8Cl_2$) complex possess higher antioxidant potential (IC_{50}) than vitamin C (standard drugs) and lower than $C_{20}H_{14}N_4O_4$ ligand.

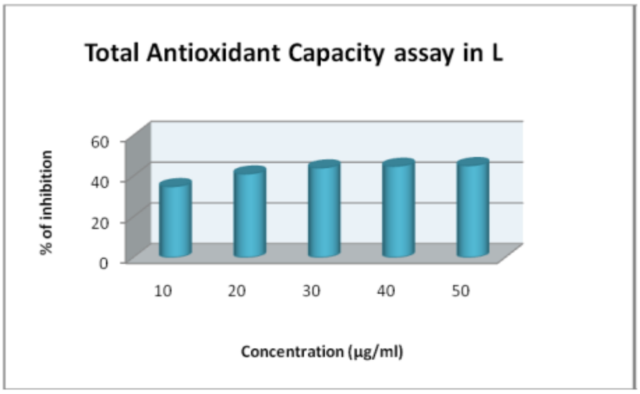


Figure 6: Total Antioxidant Capacity of $C_{20}H_{14}N_4O_4$

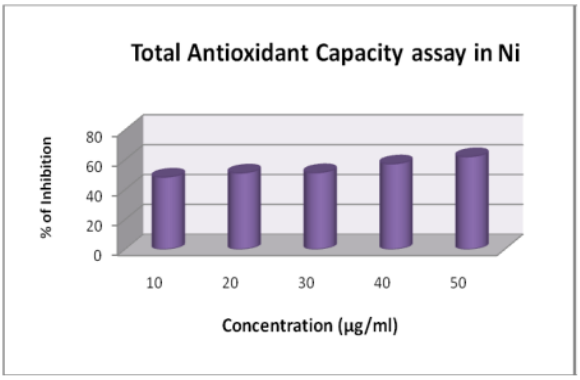


Figure 7:. Total Antioxidant Capacity of Ni($C_{40}H_{28}N_8O_8Cl_2$)

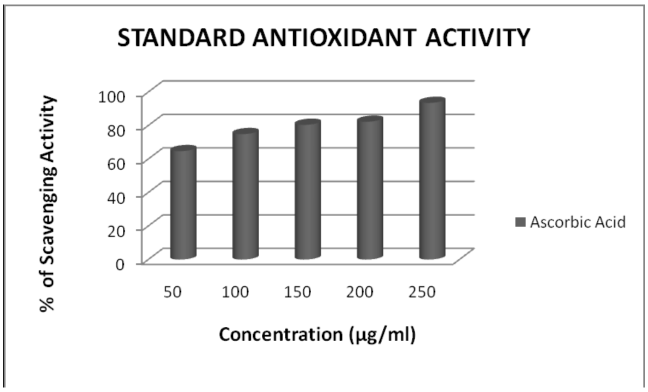


Figure 8: Total Antioxidant Capacity of Ascorbic acid

DNA cleavage activity

The Schiff base and its Ni($C_{40}H_{28}N_8O_8Cl_2$) complex were studied for their DNA cleavage activity by agarose gel electrophoresis. Gel electrophoresis is used in DNA typing, where DNA is fragmented at particular sequence points on the genome and compared to a control sample. Hence, it can be used in the detection of DNA damage also. DNA cleavage is controlled by relaxation of supercoiled circular conformation of pUC18DNA to nicked circular conformation and linear conformation. This type of damage will only give an indication to the extent of strand breakage in a sample of damaged DNA.

The results (Fig 9.) of gel electrophoretic separations of pUC18DNA induced by metal complex (lane 3) and ligand (lane 4) in the presence of H_2O_2. Under the same conditions, lane 1 and 2 did not show any apparent cleavage. Ni($C_{40}H_{28}N_8O_8Cl_2$) complex (lane 3) shows more cleavage activity in the presence of H_2O_2 at higher concentration (40 µM). $C_{20}H_{14}N_4O_4$ (lane 4) at higher concentration (40 µM) also shows the conversion of supercoiled form (Form-I) into linear form (Form-III).

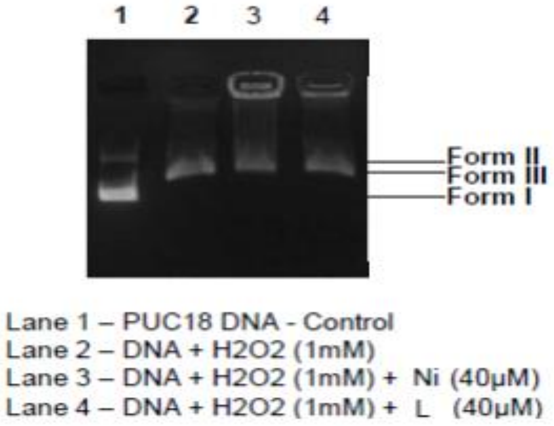


Figure 9: Agarose gel electrophoresis pattern showing effect of pUC18 DNA induced by H_2O_2 and metal complex. Lane 1-DNA alone; Lane 2- DNA alone + H_2O_2; Lane 3-DNA + Ni($C_{40}H_{28}N_8O_8Cl_2$) + H_2O_2; Lane 4-DNA + $C_{20}H_{14}N_4O_4$ + H_2O_2.

From the observed results we concluded that ligand and its metal complex behave as efficient chemical nuclease for double strand cleavage of DNA [19]. This may be attributed to the formation of hydroxyl free radicals. Control experiments using DNA alone do not show any significant cleavage of pUC18 DNA even on longer exposure time. From the observed results, it is concluded that Schiff base and Ni($C_{40}H_{28}N_8O_8Cl_2$) complex have better cleavage activity.

CONCLUSION

In this report, the mononuclear Schiff base Ni($C_{40}H_{28}N_8O_8Cl_2$) complex derived from N, N′ – (1, 2–phenylenebis (methanylylidene)bis(4-nitroaniline), have been synthesized and characterized by using various physico-chemical and spectral analyses techniques. The molar conductance value indicates that the complex is non-electrolyte in nature. The IR spectra of synthesized compounds confirmed the coordination of ligand to the metal ion. The UV-Vis and magnetic moment data of the complex suggest a distorted octahedral geometry. The synthesized compounds have antibacterial activity against the two Gram-positive bacteria: *Staphylococcus aureus* and *Bacillus subtilis* and also against the two Gram-negative bacteria: *Escherichia coli* and *Klebsilla pneumonia*. Unlike the parent Schiff base ligand, the complex showed significant antibacterial activity. DNA cleavage studies show that Ni($C_{40}H_{28}N_8O_8Cl_2$) complex cleave the DNA molecule completely. Further, the results obtained in antioxidant activity confirmed that the complex is more effective to arrest the formation of radicals and the lower IC_{50} value observed in antioxidant assay did demonstrate that the complex exhibited differential and selective effects to scavenge radicals and hence the potential as drugs to eliminate the radicals.

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