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Research Article

METAL (II) COMPLEXES OF BIOINORGANIC AND MEDICINAL RELEVANCE: ANTIBACTERIAL, ANTIOXIDANT AND DNA CLEAVAGE STUDIES OF TETRADENTATE COMPLEXES INVOLVING O, N-DONOR ENVIRONMENT OF 3, 3′-DIHYDROXYBENZIDINE-BASED SCHIFF BASES

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ABSTRACT

A new potentially tetradentate Schiff base ligand H_2L and their binuclear metal complexes have been prepared on the condensation of 3, 3′-dihydroxybenzidine, o- phthalaldehyde and 2-aminophenol in the molar ratio of 1:2:2. The Schiff base, H_2L, ligand acts as dibasic with two N_2O_2-tetradentate sites and can coordinate with two metal ions to form binuclear complexes after the deprotonation of the hydrogen atoms of the phenolic groups in all the complexes. All the metal complexes have been fully characterized with the help of elemental analyses, molecular weights, molar conductance values, magnetic moments and Spectral studies. The analytical data helped to elucidate the structure of the metal complexes. All the complexes are non-electrolyte in DMF due to their low molar conductance value. The IR spectral data suggest the involvement of azomethine nitrogen in co-ordination to the central metal ion. Electronic, ESR and magnetic data proposed the square planar geometry for Cu(II), Ni(II) and square pyramidal for VO(II) complexes under investigations. Cyclic voltammetry and controlled electrolysis studies indicate that the metal ion in the binuclear complexes undergo quasireversible one electron reduction and oxidation. The nucleolytic cleavage activities of the complexes were assayed on pUC18 plasmid DNA using gel electrophoresis in the presence and absence of H_2O_2 and the complexes show promising nuclease activity. The complexes show significant growth inhibitory activity against the bacteria like *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Klebsilla pneumonia* than the free ligands. In addition, the antioxidant activities of the complexes were also investigated through scavenging effect on DPPH radicals. The obtained IC_{50} value of the DPPH activity for the copper complex (IC_{50} = 5.46 mg/ml) was higher than other compounds.

Keywords: 3, 3′-dihydroxybenzidine, Binuclear tetradentate Schiff base, Spectroscopic, Nucleolytic cleavage.

INTRODUCTION

Schiff bases have been often used as chelating ligands in coordination chemistry [1], in catalysis, anti-oxidative activity, anti-bacterial activity, medicine as antibiotics, anti-inflammatory agents, in industry for anti-corrosion properties [2]. On the industrial scale, they have wide range of applications such as dyes and pigments [3]. The Schiff base metal complexes are also very useful model systems in chelate chemistry. The metal ion in such complexes can be coordinated by the imine nitrogen atom and also by the other active centres present in the molecule. This can lead to many interesting catalytic and potential properties [4, 5]. In recent decades, metallocomplexes have received much attention in chemistry, biochemistry, and pharmacy as promising compounds for the creation of novel drugs. On the one hand, using such complexes, it is possible to avoid some negative effects inherent in drug compositions containing inorganic salts, which can dissociate with the formation of metal ions. On the other hand, metal ions can modify both magnitude and direction of the pharmacological activity of the initial organic compounds (ligands) as a result of changes in their size, shape, charge density distribution, and redox potentials [6]. During recent years coordination compounds of biologically active Schiff base ligands have received much attention and it has been reported that chelation causes drastic change in the biological properties of the ligands and also the metal moiety, moreover, many drugs possess modified pharmacological and toxicological properties when administered in the form of transition metal complexes [7].

Presently, there is a growing interest in the design and synthesis of coordination compounds of Schiff base ligands with tetradentate substituents are increasing, due to their potential applications in the areas such as MRI, imaging with isotopes and radiotherapy, luminescent probes and DNA cleaver [8]. Tetradentate Schiff base complexes are well known to form stable complexes, where the coordination takes place through the N_2O_2 type donor set [9]. Especially dinucleating metal complexes with tetradentate substituents with N and O donar atoms have been a fascinating area of research, in view of their significance as biomimetric catalysts in the process of oxygenation. Discoveries of dinuclear cores at the active sites of some metalloproteins have aroused interest in the investigation of multimetallic systems [10]. Dinuclear copper complexes containing two metal centers in close proximity are the subject of recent extensive investigation since this structural unit is involved in a variety of important biochemical processes, such as oxygen transport and oxygen activation by oxidase and monooxygenase enzymes [11]. The nickel complexes of Schiff base derivatives show pronounced antibacterial activities, against human pathogenic bacteria, fungal activities and cytotoxic activity. It has so far been reported that oxovanadium complexes perform various reactions such as epoxidation of alkenes, oxidation of alcohols and inorganic compounds like halides and sulfur oxide, and conversion of sulfides to sulfoxides and sulfones [12]. The potential of these metal complexes to act as therapeutic agents is already well established and notable are Copper, Nickel and Oxovanadium metal ions. These enormous biological applications have prompted to synthesize novel binuclear Schiff base metal complexes with tetradentate arm.

In this paper, we report a synthetic approach to and structural characterization of a Schiff base derived from condensation of 3, 3′-dihydroxybenzidine, o-phthalaldehyde and 2-aminophenol and their complexes with Cu(II), Ni(II) and VO(II). The synthesised binuclear Schiff base complexes have been characterized by elemental analysis, molar conductance, infrared, electronic spectra, cyclic voltammetry, magnetic, ^1H NMR and EPR studies. The Schiff base ligand and its complexes were investigated for their DNA cleavage and anti-bacterial properties.

MATERIALS AND METHODS

Measurements

Elemental analysis was carried out on a Carlo Erba Model 1106 elemental analyzer. Electronic spectral studies were carried out on a Perkin Elmer Lambda-25 spectrophotometer in the range 200– 800 nm. IR spectra were recorded on a SHIMADZU model spectrophotometer on KBr disks in the range 4000– 400 cm^{-1}. Molar conductivity was measured by using an ELICO CM 185 conductivity Bridge using freshly prepared solution of the complexes in DMF solution. Cyclic Voltammograms were obtained on CHI760 electrochemical analyzer using DMF. The measurements were carried out using a three electrode cell in which glassy carbon electrode was working electrode, saturated Ag/AgCl electrode was reference electrode and platinum wire was used as auxiliary electrode. Glassy carbon electrode surface was pretreated by metallographic polishing with alumina on a velvet cloth (abraded with fine grade of emery paper), followed by ultrasonic cleaning in double distilled water and washing with small amount of highly diluted acid and DMF. The magnetic studies were carried out at room temperature on a Gouy balance calibrated with $Hg[Co(SCN)_4]$. EPR spectra were recorded on a Varian JEOL-JES-TE100 ESR spectrophotometer at X-band microwave frequencies for powdered samples.

Materials

The reagents and chemicals were obtained from commercial sources (Sigma–Aldrich, USA, Merck and Loba chemicals, India,). Metal salts, 3, 3′-dihydroxybenzidine, o- phthalaldehyde and 2-aminophenol were obtained from Aldrich and used as received. Ethanol, DMSO and DMF were used as solvents purchased from Merck and Loba chemicals. TBAP used as supporting electrolyte in electrochemical measurement was also purchased from Sigma–Aldrich. (Caution! TBAP is potentially explosive; hence care should be taken in handling the compound). The commercial solvents were distilled and then used for preparation of complexes.

Preparation of 4, 4′-Bis-({2-[(2-hydroxy-phenylimino)-methyl]-benzylidene}-amino)-biphenyl-3, 3′-diol

3, 3′-dihydroxybenzidine (1 mM) in 10 mL of ethanol, o-phthalaldehyde (2 mM) and 2-aminophenol (2 mM) in 20 mL of ethanol were mixed and heated at reflux for 2 hrs as shown in Figure 1. The resulting yellow color solution was allowed to cool. The yellow color product was obtained and dried in desiccator using silica gel as drying agent.

Synthesis of binuclear Schiff base complexes

Metal(II) acetates of [Cu(II), Ni(II)] and [VO(II)] sulphate (2 mM) and the potential binucleating Schiff base ligand (1 mM) were dissolved in DMF (20 ml) and the mixture was heated to reflux for 4 hrs. After partial evaporation of the solvent, solid (70– 80%) metal (II) Schiff base complexes (Figure 1) were separated and dried in vacuum. The analysis results are in good consistency with proposed formulas in Table 1.

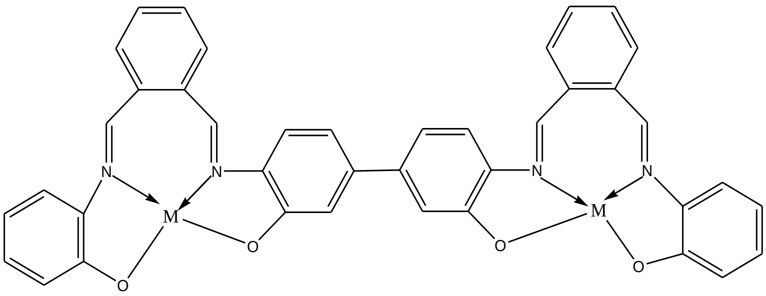

Fig. 1: Proposed structure of the synthesized complexes

Where, M= Cu(II), Ni(II) and VO(II)

Anti-bacterial activity

The Schiff base ligand and its complexes were investigated for anti-bacterial against the bacteria like *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Klebsilla pneumonia* by using disc-agar diffusion method. All complexes exhibit anti-bacterial and activities against these organisms and are found to be more effective than the free ligand [13].

The anti-bacterial activity was carried out at Progen Lab at Salem, Tamilnadu (India). The standard disc-agar diffusion method [14] was followed to determine the activity of the synthesized compounds against the sensitive organisms. DMF was used as a control and *Streptomycin* as a standard. The tested compounds were dissolved in DMF (which have no inhibition activity), to get concentration of 100 lg/mL. The test was performed on medium potato dextrose agar contains infusion of 200 g potatoes, 6 g dextrose and 15 g agar. Uniform size filter paper disks (three disks per compound) were impregnated by equal volume from the specific concentration of dissolved tested compoundsand carefully placed on incubated agar surface. After incubation for 36 hrs at 27°C inhibition of the organism which evidenced by clear zone surround each disk was measured and used to calculate mean of inhibition zones.

Antioxidant activity

DPPH radical scavenging activity

DPPH (2, 2-diphenyl-1-picryl-hydrazyl) radical scavenging activity (RSA) evaluation is a standard assay in antioxidant activity studies. It is a rapid technique for screening the radical scavenging activity of specific compounds [15]. The free radical scavenging effects of all the compounds and ligand with the DPPH radical were evaluated with various concentrations (200, 400, 600, 800, 1000 µg/ mL) of the test compound in 1mL DMF were added to a 3 mL of 0.003 % (w/v) methanol solution of DPPH. After 30 min incubation period at room temperature, the scavenging ability determines the antiradical power of an antioxidant by measuring the decrease in the absorbance of DPPH at 517nm. Resulting from a colour change, the absorbance decreased when the DPPH is scavenged by an antioxidant, through donation of hydrogen to form a stable DPPH molecule. All tests and analyses were performed with three replicates and the results were averaged. The percent of inhibition (I %) of free radical production from DPPH was calculated by using the following equation.

$$\text{DPPH}^{o}\text{ scavenging effect (\%)} = \left[\frac{A_0\text{-}A_1}{A_0} \right] \text{ X } 100$$

Where A_0 is the absorbance of the control solution, and A_1 is the absorbance in the presence of sample solutions or standards for positive control.

Gel electrophoresis

The cleavage of pUC18 DNA was determined by agarose gel electrophoresis. The gel electrophoresis experiments were performed by incubation of the samples containing 40 µM pUC18 DNA, 50 µM metal complexes and 50 µM H_2O_2 in tris-HCl buffer (pH 7.2) at 37°C for 2 h. After incubation, the samples were electrophoresed for 2 h at 50 V on 1% agarose gel using tris-acetic acid-EDTA buffer (pH 7.2). The gel was then stained using 1 µg cm^{-3} ethidium bromide (EB) and photographed under ultraviolet light at 360 nm. All the experiments were performed at room temperature [16].

RESULTS AND DISCUSSION

Schiff's base condensation of the aldehyde compounds with diamines in the presence of metal ion results into binuclear Schiff base complexes. Figure 1 represents the structure of binuclear metal(II) complexes. It is proposed that the square planar geometry for Cu(II), Ni(II) and square pyramidal for VO(II) complexes. Extensive physico-chemical and biological studies of the tetradentate binuclear Schiff base complexes were carried out and discussed. The yields of the complexes fall in the range 70- 80%. The complexes are soluble in DMF and DMSO and slightly soluble in methanol and dichloromethane. The analytical and spectral data are completely consistent with the proposed formulation as represented in the Table 1.

Table 1: Colour, conductivity and analytical data of the Schiff base ligand and its binuclear metal complexes

Compounds	Molecular Formula	Color	Yield %	Melting Point (º)	Calculated (Found) (%)				Λ_m (ohm^{-1}cm^2 mol^{-1})
					C	H	N	Metal	
L	$C_{40}H_{30}N_4O_4$	Yellow	80	136	76.10 (76.08)	4.75 (4.74)	8.87 (8.85)	--	--
[Cu_2L]	[Cu_2 ($C_{40}H_{26}N_4O_4$)]	Dark green	75	>200	63.68 (63.69)	3.44 (3.43)	7.42 (7.41)	16.84 (16.80)	13.9
[Ni_2L]	[Ni_2 ($C_{40}H_{26}N_4O_4$)]	Brown	75	>200	64.51 (64.50)	3.49 (3.47)	7.52 (7.50)	15.77 (15.73)	11.2
[VO_2L]	[VO_2($C_{40}H_{26}N_4O_4$)]	Black	70	>200	63.11 (63.08)	3.41 (3.39)	7.36 (7.35)	17.59 (17.57)	15.3

IR Spectra and mode of bonding

The IR spectra of the complexes show a sharp band in the range 1608–1602 cm^{-1}, attributed to (–C=N), which is shifted to lower frequency on going from the free ligand (at 1610 cm^{-1}) to the complexes. Deprotonation of all phenolic functions is confirmed by the lack of O-H stretching bands in the IR region 3400–3300 cm^{-1} for all complexes [17, 18]. The band at 1179 cm^{-1} for [H_2L] is ascribed to the phenolic C-O stretching vibrations. The various absorption bands in the region 1472-1494 cm^{-1} may be assigned due to υ(C=C) aromatic stretching vibrations of the 3, 3′-dihydroxybenzidine, o-pthalaldehyde and 2-aminophenol. It has been reported that oxovanadium complexes with coordination numbers 5 and 6 have υ (V=O) values higher than and lower than about 990 cm^{-1}, respectively, and this figure can serve as a criterion for discriminating the coordination number of VO^{2+} complexes [19]. If this criterion is accepted, the three complexes 1–3, whose υ (V=O) values are around 980 cm^{-1}, are penta-coordinated to exhibit square-pyramidal geometry.

Assignment of the proposed coordination site is further supported by the appearance of medium bands at 458–478 cm^{-1} which could be attributed to υ(M–N), respectively [20, 21] and also presence of bands at 515-534 cm^{-1} indicates that υ(M-O) vibrations [22]. The IR spectral data of all the complexes were given in Table 2.

Table 2: Infrared spectral data of the ligand and its metal complexes

Compounds	Free-OH	(C=N) (cm^{-1})	(C=C) (cm^{-1})	(M–N) (cm^{-1})	(M–O) (cm^{-1})
$C_{40}H_{30}N_4O_4$	3380	1610	1480	--	--
[Cu_2 ($C_{40}H_{26}N_4O_4$)]	--	1608	1472	458	515
[Ni_2 ($C_{40}H_{26}N_4O_4$)]	--	1602	1490	474	518
[VO_2($C_{40}H_{26}N_4O_4$)]	--	1605	1494	478	534

Electronic absorption spectroscopy

The electronic spectra of the ligands and their metal complexes were recorded in DMF medium. The bands below 378 nm are attributable to intraligand π—π* and n—π* transitions. In the electronic spectra of the complexes, the intraligand transitions are slightly shifted as a result of coordination in the region at 465- 485 nm. The complexes of the Cu(II), Ni(II) and VO(II) show the low intensity bands at 520–642 nm, which are assigned as d-d transition of the metal ions. The electronic spectra of the Cu(II) complex show an absorption band at 593 nm attributed to the $^2B_{1g} \rightarrow {}^2A_{1g}$ transition, characteristic for square planar geometry. The electronic spectrum of Ni(II) complex exhibited two absorption bands at 564 and 642 nm which may be assigned to two spin allowed transitions, $^1A_{1g} \rightarrow {}^1A_{2g}$, $^1A_{1g} \rightarrow {}^1B_{1g}$, respectively characteristic of square planar geometry around Ni(II) ion [23]. The d-d bands of VO(II)complexes in the visible region at 520-620 nm, which according to the scheme of Ballhausen and Gray are assigned to $d_{xy} \rightarrow d_{xz}$, d_{yz}, $d_{xy} \rightarrow d_{x^2}-d_{z^2}$ and $d_{xy} \rightarrow d_{z^2}$ [24]. The observed results (Table 3) indicate that the present oxovandium(IV) complexes are square-pyramidal geometry.

Table 3: UV–Visible data of Schiff base and its binuclear metal complexes.

Compounds	Absorption nm (cm^{-1})				Geometry of the complex
	π→ π*	n→ π*	L→ M CT	d-d	
$C_{40}H_{30}N_4O_4$	273	360	--	--	--
[Cu_2 ($C_{40}H_{26}N_4O_4$)]	271	362	470	593	
[Ni_2 ($C_{40}H_{26}N_4O_4$)]	282	371	465	564, 642	Square planar
[VO_2($C_{40}H_{26}N_4O_4$)]	275	378	485	520, 580, 620	Square pyramidal

Molar conductivity

The molar conductivity (Λ_m) data (11.2–15.3 ohm^{-1}cm^{2}mol^{-1}) measured at 30°C using 10^{-3} M solutions of the complexes in DMF solvent. These low values indicate that all their complexes are non-electrolytes due to the absence of any counter ions in their structures [25]. The molar conductance values of these complexes (Table 1) indicate that the [H_2L] Schiff base ligand is coordinated to the Cu(II), Ni(II) and VO(II) ions as a doubly negatively charged anions. Therefore, it seems that two phenolic OH have been deprotonated and bonded to the metal ions as oxygen anion [26].

Magnetic moment

Magnetic moment of the complexes was observed at room temperature. The nickel complexes are diamagnetic in nature due to square planar [27] geometry around the metal ion. Copper complex show μ_{eff} value in the range 1.72 B.M. which is close to the spin only value of 1.73 B.M. The lower value of magnetic moment at room temperature is consistent with square planar geometry around the metal ions [28]. The room temperature value of VO(II) ion for the complex is 1.70 B.M. It is possible that the oxovanadium(IV) complexes have square-pyramidal geometry [29]. The values are almost equal spin only value. This indicates that the two metal centers are equivalent and there is no interaction between the two metal centers.

^{1}H NMR spectra

The ^{1}H NMR spectra of the (4, 4′-Bis-({2-[(2-hydroxy-phenylimino)-methyl]-benzylidene}-amino)-biphenyl-3, 3′-diol) ligand were recorded using $CDCl_3$ at room temperature respectively. The signals appearing in the region 6.91–7.52 ppm have been assigned to the hydrogen atoms of the aromatic rings of the free Schiff base ligand. The azomethine group in the Schiff base ligand shows up at 8.14 ppm as a single signal [30] and the resonance exhibits due to phenolic –OH protons around 10.26 ppm.

ESR spectra

The ESR spectrum of Cu(II) complex provides information about hyperfine and super hyperfine structures which are important in studying the metal ion environment, i.e. the geometry, nature of the ligation sites from the Schiff bases to the metal and the degree of covalency of the metal ligand bonds. The solid state ESR spectrum of $[Cu_2 (C_{40}H_{26}N_4O_4)]$complex is displayed at room temperature on X-band at frequency 9.3 GHz under the magnetic field strength 4000 G. ESR spectrum of the complex shows only an intense and broad signal without hyperfine splitting (g_{iso} = 2.075). The shape of the spectrum is consistent with square planar environment around Cu(II) ion and the higher g value for the investigated complex, when compared to that of free electron (g = 2.0023) revealing an appreciable covalency of metal ligand bonding characteristic of square planar stereochemistry[31].

Electrochemistry

Electrochemical properties of complexes were probed by cyclic voltammetry in DMF. The electrochemical data are given in Table 4. (Reduction) and 5(Oxidation). All the complexes show a double redox wave at negative potential in the range −0.64 to −1.21 V. At positive potential region the complexes show a double redox wave in the range +0.33 to +1.01 V. Generally the electrochemical properties of the complexes depend on a number of factors such as chelate ring size , axial ligation degree and distribution of unsaturation and substitution pattern in the chelate ring, charge type and coordination number [32].

Table 4: Electrochemical data of binuclear Schiff base metal complexes in DMF medium (reduction)

Complexes	E_{pc} (V)	E_{pa} (V)	$E_{1/2}$ (V)	ΔE (mV)
$[Cu_2 (C_{40}H_{26}N_4O_4)]$	−1.14	−0.98	−1.06	160
	−0.72	−0.56	−0.64	160
$[Ni_2 (C_{40}H_{26}N_4O_4)]$	−1.31	−1.12	−1.21	190
	−0.78	−0.59	−0.68	190
$[VO_2(C_{40}H_{26}N_4O_4)]$	−1.26	−1.08	−1.17	180
	−0.75	−0.61	−0.68	140

Reduction and Oxidation at negative and positive potential is the usual trend observed for the phenoxide oxygen donor [33] complexes. This is due to the ‘hard’ nature and negative influence of the phenoxide ligand. The cyclic voltammograms of copper complexes at negative potential show a single electron transfer. The redox process is quasi-reversible in nature as is evident from the criteria: ΔEp is always greater than 60 mV. Controlled potential electrolysis was carried out at 100 mVs^{-1} and the experiment reports that each couple correspond to one electron transfer process. Thus a quasi-reversible electron transfer process occurs are assigned as follows.

$Cu^{II}Cu^{II} \leftrightarrows Cu^{I}Cu^{II} \leftrightarrows Cu^{I}Cu^{I}$

The Cu(II) complexes show a quasireversible oxidation waves, which is assigned as a Cu(II)/ Cu(III) couple. The ΔEp values are suggest the each couple was quasireversible. The $E_{1/2}$ values indicate that each couple corresponds to one electron transfer process.

Table 5: Electrochemical data of binuclear Schiff base metal complexes in DMF medium (oxidation)

Complexes	E_{pc} (V)	E_{pa} (V)	$E_{1/2}$ (V)	ΔE (mV)
$[Cu_2 (C_{40}H_{26}N_4O_4)]$	0.42	0.70	0.56	500
	0.91	1.12	1.01	210
$[Ni_2 (C_{40}H_{26}N_4O_4)]$	0.14	0.52	0.33	380
	0.82	1.01	0.91	190
$[VO_2(C_{40}H_{26}N_4O_4)]$	0.43	0.79	0.61	360
	0.91	0.71	0.81	200

Cyclic voltammograms of the nickel complexes at negative potential show a double redox wave. The redox process is quasi-reversible in nature with ΔEp values greater than 60 mV. The data are summarised in Table 4. Controlled potential electrolysis was also carried out and the experimental reports that each couple correspond to one electron transfer process, as follows [34]. Similarly positive potential is also quasireversible in nature.

$$Ni^{II}Ni^{II} \leftrightarrows Ni^{I}Ni^{II} \leftrightarrows Ni^{I}Ni^{I}$$

Electrochemical measurements of oxovanadium(IV) complex with tetradentate dibasic Schiff base complexes are reported to show oxidation to V^{V} and reduction to V^{III} *vs* SCE, respectively. It is observed that the complex show quasireversible waves at negative and positive potential.

Anti-bacterial screening

The antibacterial activity of the ligand and complexes were screened using the disc diffusion method [35]. The microorganisms used in the present investigations included *Staphylococcus aureus, Escherichia coli, Bacillus subtilis* and *Klebsilla pneumonia*. *Streptomycin* was used as standard antibiotics. The results of the bactericidal study of the synthesized compounds are displayed in Table 6. The results showed that all the complexes are more toxic against the pathogenic bacteria species than the free ligands. The toxicity of the complexes increases with increase in the concentration of the solution. Copper complex was found to be more toxic than the other complexes which mean $[Cu_2L]$ complex show higher bactericidal activity than $[Ni_2L]$ and $[VO_2L]$ complexes as shown in the figures 2, 3, 4 and 5. The variation in the effectiveness of different compounds against different organisms depends either on the impermeability of the cells of the microbes or differences in ribosomes of microbial cells [36].

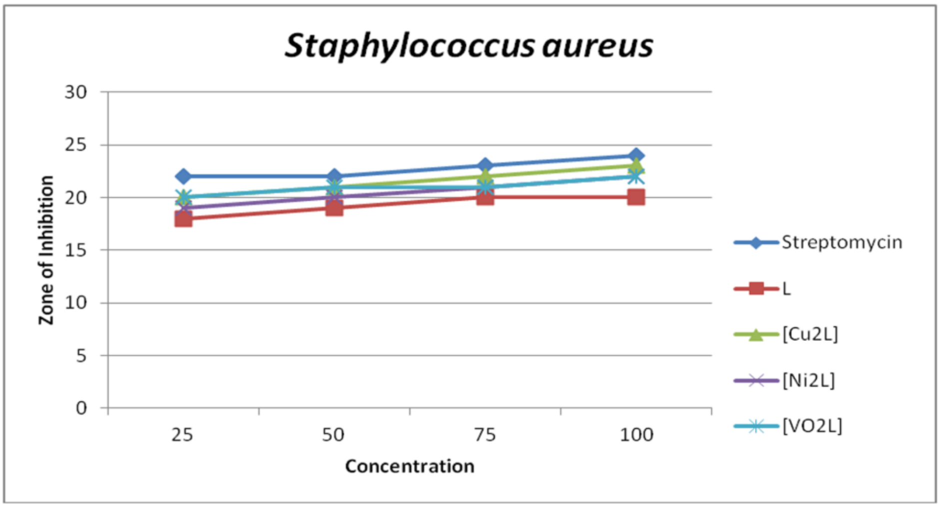

Fig. 2: Difference between the anti-bacterial activities of the Schiff base and its binuclear metal complexes against *Staphylococcus aureus*.

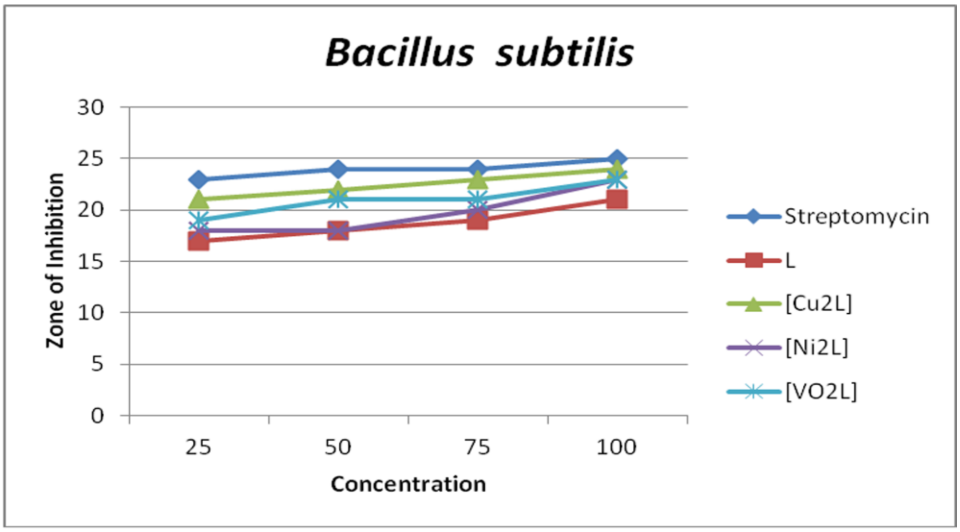

Fig. 3: Difference between the anti-bacterial activities of the Schiff base and its binuclear metal complexes against *Bacillus subtilis.*

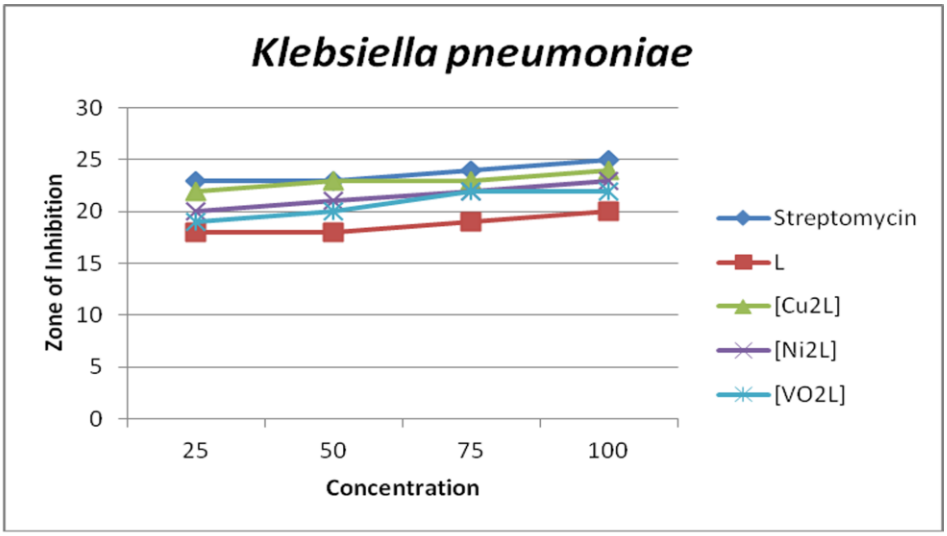

Fig. 4: Difference between the anti-bacterial activities of the Schiff base and its binuclear metal complexes against *Klebsilla pneumonia.*

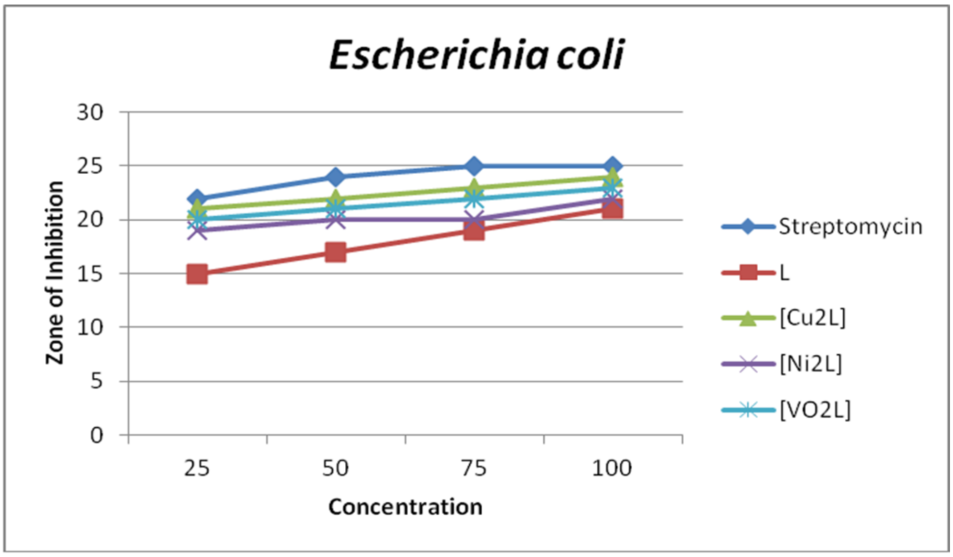

Fig. 5: Difference between the anti-bacterial activities of the Schiff base and its binuclear metal complexes against *Escherichia coli.*

This higher antibacterial activity of the metal complexes, compared with that of Schiff bases, is perhaps due to change in structure due to coordination, and chelating tends to make metal complexes act as more powerful and potent bacteriostatic agents, thus inhibiting the growth of the microorganisms. Such increased activity of the metal chelates can be explained on the basis of chelation theory. 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 p-electrons over the whole chelate ring and 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 proteins, which restricts further growth of the organism [37]. In general metal complexes are more active than the ligands because metal complexes may serve as a vehicle for activation of ligands as the principle cytotoxic species [38]. The studies showed that the newly synthesized compounds possess higher antifungal activity than the antibacterial property.

Table 6: Anti-biogram assay of the Schiff base and its binuclear metal complexes.

Samples	**Diameter of inhibition zone (mm)**															
	Gram(+ve)								**Gram(-ve)**							
	***Staphylococcus aureus* mg/ml**				***Bacillus subtilis* mg/ml**				***Klebsiella pneumoniae* mg/ml**				***Escherichia coli* mg/ml**			
	25	50	75	100	25	50	75	100	25	50	75	100	25	50	75	100
Streptomycin (standard)	22	22	23	24	23	24	24	25	23	23	24	25	22	24	25	25
L	18	19	20	20	17	18	19	21	18	18	19	20	15	17	19	21
[Cu_2L]	20	21	22	23	21	22	23	24	22	23	23	24	21	22	23	24
[Ni_2L]	19	20	21	22	18	18	20	23	20	21	22	23	19	20	20	22
[VO_2L]	20	21	21	22	19	21	21	23	19	20	22	22	20	21	22	23

Antioxidant activity of ligand and complexes

The antioxidant activity of ligand and its metal complexes have attracted a lot of interests and have been investigated mainly in the *in vitro* method [39, 40]. The antioxidant activities of 4,4'-Bis-({2-[(2-hydroxy-phenylimino)-methyl]-benzylidene}-amino)-biphenyl-3,3'-diol and its metal complexes were evaluated by *in vitro* methods in order to compare the results and to establish some structure antioxidant–activity relationship. The evaluation study was carried out with different concentrations of ligand and metal complexes and Ascorbic acid was used as a standard.

DPPH radical scavenging activity

The methanolic solutions of 4, 4'-Bis-({2-[(2-hydroxy-phenylimino)-methyl]-benzylidene}-amino)-biphenyl-3, 3'-diol and its metal complexes were studied and compared with the standard (Figure 6). Initially free ligand showed negligible DPPH activity, however upon complexation with metal ions (Cu, Ni and VO) the activity was enhanced significantly. All the metal complexes showed comparable or slight less activity to that of standard (Ascorbic acid). The copper complex showed significantly higher DPPH activity followed by Nickel and vanadium complexes. IC_{50} value of ligand on DPPH radical is 7.67 mg/ml whereas, [Cu_2L], [Ni_2L] and [VO_2L] complexes showed their IC_{50} values at 5.46, 6.26 and 6.28, respectively. From the above results, it can be concluded that the scavenging effects of the free ligand are less when compared to that of their corresponding complexes which is due to the chelation of the organic molecules with the metal ions.

Electrophoretic separation of metal complexes

The [Cu_2L], [Ni_2L] and [VO_2L] complexes were studied for their DNA cleavage activity by agarose gel electrophoresis. It is an effective method for the determination of DNA damage to the double helix, allowing for the determination of strand breaks [41]. 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 pUC18 DNA 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.

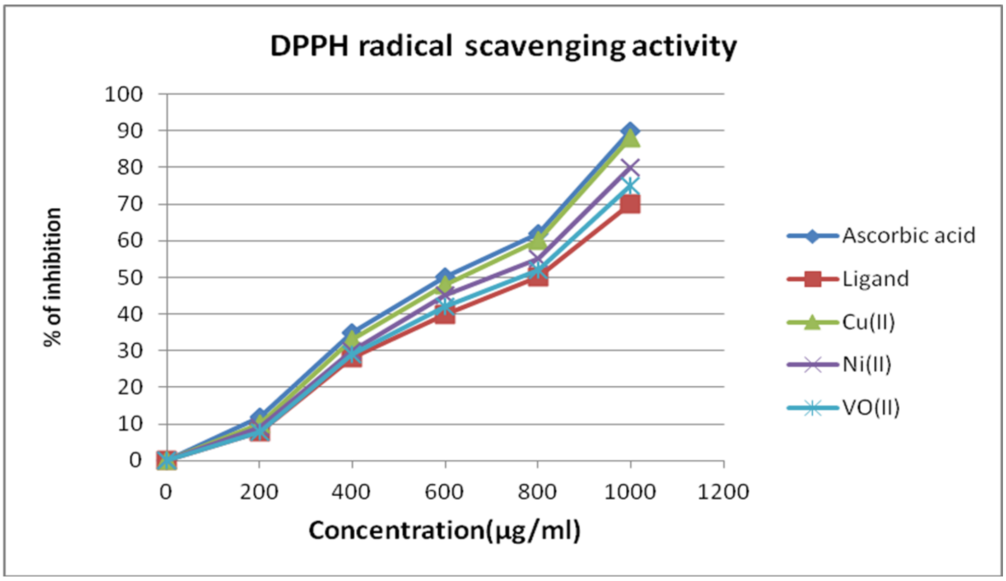

Fig. 6: DPPH radical scavenging activity spectrophotometric assay of various concentration of metal complexes.

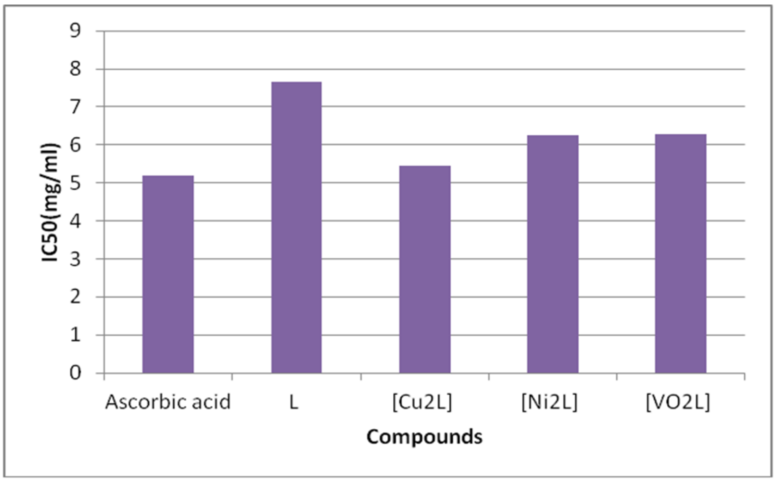

Fig. 7: DPPH scavenging capacities (IC_{50}) of metal complexes

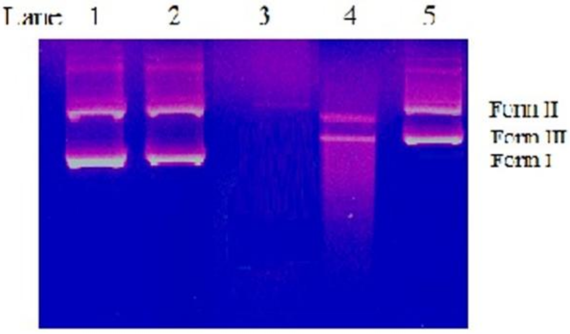

Fig. 8: Changes in the agarose gel electrophoretic pattern of pUC18DNA induced by H_2O_2 and metal complexes: Lane 1, DNA alone; Lane 2, DNA alone + H_2O_2; Lane 3, DNA + [$Cu_2(C_{40}H_{26}N_4O_4)$] complex + H_2O_2; Lane 4, DNA + [$Ni_2(C_{40}H_{26}N_4O_4)$] complexH_2O_2; Lane 5, DNA + [$VO_2(C_{40}H_{26}N_4O_4)$] complex + H_2O_2.

The results (Figure. 8) of gel electrophoretic separations of pUC18 DNA induced by metal complexes (lane 3–5) in the presence of H_2O_2. Under the same conditions, lane 1 and 2 did not show any apparent cleavage. [Cu_2L] complex (lane 3) shows more cleavage activity in the presence of H_2O_2 at higher concentration (50 µM). Lane 4 and 5 reveals that [Ni_2L] and [VO_2L] complex in the presence of H_2O_2 at higher concentration (50 µM) shows the conversion of supercoiled form (Form-I) into linear form (Form-III). From the observed results we concluded that metal complexes behave as efficient chemical nuclease for double strand cleavage of DNA[42]. This may be attributed to the formation of hydroxyl free radicals. All the complexes show pronounced nuclease activity in the presence of oxidant H_2O_2 which may be due to the increased production of hydroxyl 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 [Cu_2L] complex show more activity than the other complexes.

CONCLUSION

Taken together , the synthesis and spectroscopic characterization of a series of [Cu_2L], [Ni_2L] and [VO_2L] complexes with a new Schiff base ligand derived from 3, 3′-dihydroxybenzidine, o-phthalaldehyde and 2-aminophenol. These complexes were characterized by using different physico-chemical techniques. These complexes are all neutral and found to square planar geometry for [Cu_2L], [Ni_2L] and square pyramidal for [VO_2L] complexes. 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 complexes showed that significant antibacterial activity. DNA cleavage studies show that Cu(II) complex cleave the DNA molecule completely. Further, the results obtained against the DPPH radical confirmed that the complexes are more effective to arrest the formation of the DPPH radicals and the lower IC_{50} values observed in antioxidant assays did demonstrate that these complexes exhibited differential and selective effects to scavenge radicals and hence the potential as drugs to eliminate the radicals.

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