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Cu(II) and Ni(II) Complexes of Bidentate E-*N*-(4-Isopropyl benzylidene)isonicotinohydrazide: Synthesis, Characterization, DNA Cleavage and Antioxidant Activity

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The reaction of $Cu(NO_3)_2\cdot 3H_2O$ and $Ni(NO_3)_2\cdot 6H_2O$ with E-*N*-(4-isopropylbenzylidine)isonicotinohydrazide derived from isonicotinic acid hydrazide with 4-isopropyl benzaldehyde were synthesized and characterized structurally by physico-chemical and spectral techniques. The metal-ligand ratio in metal complexes is in the order of metal:ligand$_1$:ligand$_2$ (1:1:1) indicating the formulae of the complexes as $[M(SB)_2(NO_2)_2]$ (where $M = Cu^{2+}$ & Ni^{2+}, $SB = C_{16}H_{17}N_3O$). The magnetic moment and electronic spectral data suggest tetragonal geometry for Cu(II) complex and octahedral geometry for Ni(II) complex around the central metal ions. The FT-IR and Far-IR spectroscopic data suggest the bidentate nature of ligand. The geometry of the Cu(II) complex and its covalence is confirmed by EPR spectral data. The Thermal decomposition of metal complexes has been identified using thermogravimetric analysis. The conductometric measurements proved the non-electrolytic behaviour of all the compounds. With the help of the agarose gel electrophoresis method, the DNA cleavage studies of Schiff base metal complexes were predicted. Also, the *in-vitro* antioxidant activity of Cu(II) complex was determined by a free radical scavenging assay.

Keywords: Isonicotinicacidhydrazide, 4-Isopropylbenzaldehyde, Schiff base, Cyclic voltammetry, DNA cleavage.

INTRODUCTION

Transition metals are commonly used as catalysts particularly in reducing reactions such as hydrogenation [1]. Transition metal complexes easily interact with DNA rather than cleavage [2]. DNA cleavage plays a powerful tool in many biological processes *e.g.* DNA replication, transcription, gene editing, therapeutics and biosensor design [3]. Transition metal complexes based on a variety of Schiff bases are withdrawing attention because of their diverse structural features and coordinating ability. Many Schiff base metal complexes show high catalytic activity [4].

Isoniazid (INH) is one of the most effective and specific agents for the treatment of an airborne infectious disease caused by *Mycobacterium tuberculosis* (Mtb) [5]. To this day, INH remains a cornerstone of modern short-course tuberculosis (TB) chemotherapy. Isoniazid is a structural analog of ethionamide (ETH) and is a pro-drug involved in inhibiting the biosynthesis of mycolic acid, an essential cell wall component of Mtb [6,7]. Cuminaldehyde (*p*-isopropylbenzaldehyde) is a culinary and flavouring agent due to aromatic monoterpenoid, known as a major constituent of essential oil in cumin seeds which has the characteristic aroma and therefore widely used as traditional medicine for the treatment of some diseases [8,9]. Despite these benefits, like other natural essential oil bioactives, cuminaldehyde is a highly volatile compound that has been used for thousands of years in Ayurvedic food processing [10]. A new Schiff base ligand was derived from isonicotinic acid hydrazide with 4-isopropyl benzaldehyde. The synthesized ligand was used further to synthesize metal complexes with copper and nickel metal ions and investigate their properties.

EXPERIMENTAL

The metal(II) salts, cuminaldehyde, isonicotinic acid hydrazide and the solvents were purchased from the Sigma-Aldrich, USA and of AnalaR grade.

Synthesis of E-*N*-(4-isopropylbenzyledine)isonicotino hydrazide: Isonicotinic acid hydrazide (0.5129 g, 3.73 mmol) dissolved in a 1:1 ratio of water and methanol was mixed with

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4-isopropyl benzaldehyde (0.5427 g, 3.66 mmol) containing 10 mL of ethanol. To this mixture, a 10% NaOH was added as a catalyst and the flask was shaken vigorously for 20-30 min, until the white precipitate appeared. The precipitate was filtered and dried. Finally, the mixture was recrystallized by using ethanol.

Synthesis of Cu(II) complex: A methanolic solution of $Cu(NO_3)_2 \cdot 3H_2O$ (1 g, 4.13 mmol) was added dropwise to an ethanolic solution (35 mL) of *E*-*N*-(4-isopropyl benzyledine)-isonicotino hydrazide (1.10649 g, 4.13 mmol). After that, $NaNO_2$ (0.5711 g, 8.27 mmol) was added and the mixture was shaken vigorously over a period of 30 min accompanied. The green-colored precipitate obtained was washed with distilled water, dried in desiccators and kept in a glass container.

Synthesis of Ni(II) complex: A methanolic solution of $Ni(NO_3)_2 \cdot 6H_2O$ (1 g, 3.43 mmol) was added to E-*N*-(4-isopropyl benzyledine)isonicotino hydrazide (1.8385 g, 6.87 mmol) followed by addition of $NaNO_2$ (0.4745 g, 6.87 mmol). The mixture was shaken vigorously over a period of 30 min. The green-colored precipitate obtained was washed with distilled water, dried in desiccators and kept in a glass container.

Characterization: Elemental analysis (CHNS(O)) was performed on an Elemental Vario make EL III model (Thermo Finnegan) instrument at 950-1200 ºC. For the FT-IR spectra of complexes predicted using Shimadzu FT-IR spectrometer. Magnetic susceptibility measurements were measured using a lakeshore 7410 vibrating sample magnetometer (VSM) at room temperature. Melting points of the compounds were obtained by the Ajay melting point apparatus (in ºC) versa stat model, Princeton makes electrochemical work station used to conduct the electrochemical behaviour of the complexes. Conductivity measurements of the compounds were measured in 10^{-3} M acetonitrile solution using Systronic Conductivity Bridge.

***in vitro* antioxidant study:** Antioxidant activity of the metal(II) complexes and Schiff base were carried out by DPPH free radical scavenging method with 517 nm absorbance. The reaction mixture was prepared by the addition of 1 mL of 0.1 mM DPPH in CH_3OH to the metal(II) complexes at 10, 100, 500, and 1000 µg/mL concentrations, respectively using L-ascorbic acid as reference [11]. The % scavenging activity was measured using the following equation:

$$\text{Radical scavenging activity } (\%) = \frac{A_0 - A_1}{A_0} \times 100$$

DNA cleavage activity: The DNA cleavage studies with pUC 57 plasmid DNA by using the reaction mixture 20 µL of pUC57 DNA, 50 Mm Tris-HCl, 50 Mm NaCl, 1 mM H_2O_2 added to the complexes in DMSO. After the addition of Millipore water for the final volume, the mixture was incubated at 37 ºC for 1 h. After incubation, they were checked by the agarose electrophoresis method.

RESULTS AND DISCUSSION

The $[M(SB)_2(NO_2)_2]$ molecular formula of the synthesized metal(II) complexes was derived based on the results of elemental analysis and metal estimation with the stoichiometry nature of metal ions and ligand used in the synthesis. The physical and conductivity values of the metal(II) complexes are given in Table-1.

Molar conductance and magnetic moment: The conductivity values of novel Schiff base and its metal(II) complexes were found at 6.4, 1.2 and 4.2 and Ω^{-1} cm^2 mol^{-1}, respectively, from the results the Schiff base shows slightly higher values are non-electrolyte nature of the complexes [12] (Table-1).

Mass spectra: The Schiff base exhibit the *m/z* value at 267 is in good agreement with its molecular weight and confirmed the proposed formula of the Schiff base ($C_{16}H_{17}N_3O$) (Fig. 1). A series of peaks at 106, 130 and 145 corresponds to the various fragments *viz.* C_4N_3O, $C_7H_2N_2O$ and $C_8H_5N_2O$. The peak intensity gives an idea of the stability of the fragments [13].

Cyclic voltammetry: The electrochemical property of Ni(II) complex shows cathodic peak E_{pc} at –0.9175 V, with an associated anodic peak E_{pa} at 1.6340 V and the potential separation is 02.551 increases with scan rate, the current ratio I_{pa}/I_{pc} at 2.3700 V (Fig. 2). The fact confirmed by the quasi-reversible one-electron redox process with nickel(II)/nickel(I) couple [14].

TABLE-1
ANALYTICAL DATA OF THE SYNTHESIZED SCHIFF BASE AND METAL COMPLEXES

Compounds	m.w. (g/mol)	m.p. (°C)	Colour	Elemental analysis (%): Found (calcd.)				Molar conductance (Ohm^{-1} cm^2 mol^{-1})
				C	H	N	O	
Schiff base	267.33	170	White	68.30 (68.24)	6.98 (7.01)	14.80 (14.73)	15.84 (15.74)	6.4
$[Ni(SB)_2(NO_2)_2]$	58.69	139	Green (chartreuse & lime)	56.02 (56.18)	4.96 (4.84)	16.34 (16.32)	14.00 (14.05)	1.2
$[Cu(SB)_2(NO_2)_2]$	63.54	210	Green (basil)	55.63 (55.48)	4.92 (4.80)	16.22 (16.24)	13.90 (13.86)	4.2

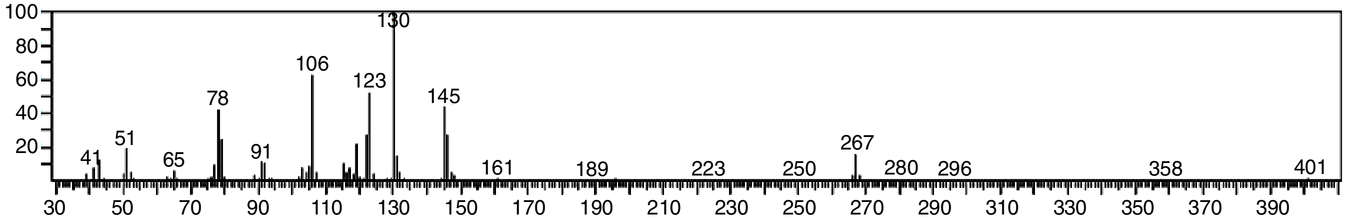

Fig. 1. Mass spectrum of the synthesized Schiff base (E-*N*-(4-isopropylbenzylidine)isonicotinohydrazide)

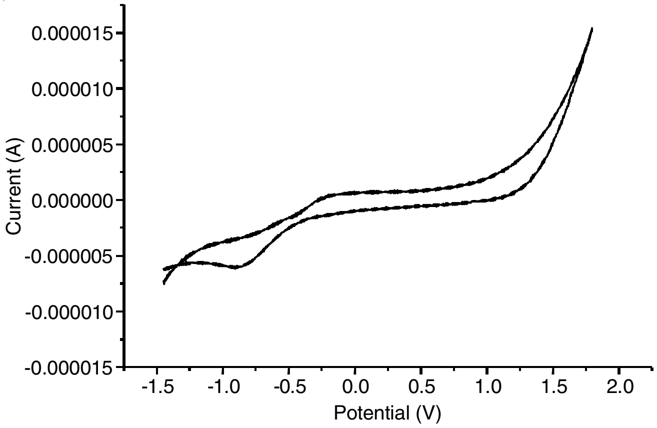

Fig. 2. Electrochemical property of nickel(II) complex

Infrared studies: The characteristic absorption bands in the IR spectra of the Schiff base and its metal(II) complexes are summarized in Table-2. The IR spectra of metal(II) complexes show significant changes compared to the ligand. The IR spectrum of the Schiff base gives a sharp peak at 1606 cm^{-1} corresponding to the ν(C=N) group of imine. After effec-tive formation of complexes with Schiff base, these are shifted to low frequencies at 1599 cm^{-1} and 1602 cm^{-1}, respectively in both complexes confirmed by the metal chelates through nitrogen atom azomethine group [15]. The strong intense peak at 1665 cm^{-1} of the spectra indicates the carbonyl group, which is shifted to a higher region 1693-1677 cm^{-1} confirming the coordination by an oxygen atom in the carbonyl group. Aromatic ν(C-H) and ν(C-C) stretching bands occurred at 2849 cm^{-1} and 2950 cm^{-1}, these bands are shifted to higher/lower frequency regions at 2869 cm^{-1} and 2932 cm^{-1} in Ni(II) complex and 2870 cm^{-1} and 2932 cm^{-1} in Cu(II) complex. The ν(N-H) vibration of ligand was observed at 3198 cm^{-1} was shifted to lower frequencies at 3198 cm^{-1} and 3180 cm^{-1} upon complex formation revealing the coordination of amine nitrogen atom [16]. New bands at 547-542 cm^{-1} and 461-460 cm^{-1} attributed to the existenceof ν(M-O) and ν(M-N) vibrations. The appearance of these vibra-tions, which are not present in the free ligand indicates the involvement of nitrogen and oxygen atoms in chelation.

EPR studies: The electron paramagnetic resonance spectra of Cu(II) complex shows the well-resolved spin Hamiltonian parameters which are calculated as $g_{\|} = 2.2254$, $g_{\perp} = 2.0755$, and $A_{\|} = 169.79 \times 10^{-4}$ cm^{-1}. The values $g_{\|} > g_{\perp} > 2.0023$, which show the unpaired electrons are present in $d_{x^2-y^2}$ orbital [17]. The anisotropic factor G was calculated using $G = (g_{\|} - 2.0023)/(g_{\perp} - 2.0023)$, which is a measure of the exchange interaction between copper(II) centers [18]. The trend G > 4 in which the exchange interaction is negligible and G < 4 indicates considerable exchange coupling is present in the complex [19]. The synthesized copper(II) complex showed a G value of 3.0474, which is < 4 suggests that the complex has regular tetragonal geometry with the exchange coupling is present. The covalence parameter α^2 was predicted using the formula:

$$\alpha^2 = (A_{\|} / 0.036) + (g_{\|} - 2.0023) + \frac{3}{7}(g_{\perp} - 2.0023) + 0.04$$

in the present complex, the α^2 is 0.47, which is almost equal to 0.5 and < 1 indicating the covalent nature of the complex [20].

Thermal stability: Thermal stability of $[Cu(SB)_2(NO_2)_2]$ complex shows a three-step decomposition process in the range of 192-580 ºC, respectively; the weight loss of 37.56% at 277.9 ºC was observed, which corresponds to the removal of one Schiff base moiety from the coordination sphere. The second decomposition was observed at 295-397 ºC shows a mass loss of 14.67% (368.2 ºC) and can be assigned to the loss of one nitrite ion from the sphere of the complex. The last stage at 397-580 ºC shows the degradation of the Schiff base having a mass loss of 23.76% (472.1 ºC). After 580 ºC, some unreacted residue had been observed with a weight loss of 18.48% which correspond to the degradation of metal oxide (Fig. 3) [21].

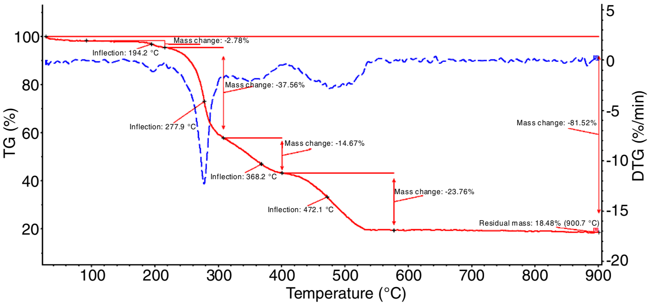

Fig. 3. TG-DTG curves of Cu(II) complex

The DTG curve of $[Ni(SB)_2(NO_2)_2]$ exhibit the two-step decomposition process in the range of 332-390 ºC and 390-580 ºC, respectively. The first stage decomposition starts from 332 ºC and continued to 390 ºC and in between at 381 ºC, a mass change of 28.58% was observed, which corresponds to the loss of nitrite group. Thermal degradation at 400 ºC there is a mass loss of 38% due to the decomposition of the ligand. After 580 ºC, the decomposition of nickel(II) complex can be observed with 14.83% of final residue (Fig. 4).

***in vitro* antioxidant activity:** The *in vitro* antioxidant activity of the synthesized metal(II) complex indicates the lower the IC_{50} value, the higher the antioxidant activity of samples. The IC_{50} value for Schiff base is 267 µg/mL, which is lower than the value of 386.1 µg/mL in Cu(II) complex. The observed

TABLE-2
FT-IR SPECTRAL DATA (cm^{-1}) OF SCHIFF BASE AND ITS METAL COMPLEXES

Compound	Ar(C-H)	Ar(C-C)	ν(C=N)	ν(C=O)	ν(N-N)	ν(N-H)	ν(M-N)	ν(M-O) keto group	ν(M-O)
Schiff base	2849	2950	1606	1665	1155	3198	-	-	-
$[Ni(SB)_2(NO_2)_2]$	2869	2932	1599	1677	1153	3195	461	542	352
$[Cu(SB)_2(NO_2)_2]$	2870	2932	1602	1693	1150	3180	460	547	359

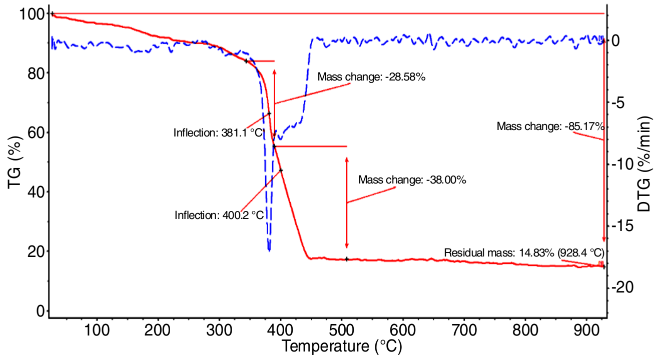

Fig. 4. TG-DTG curve of Ni(II) complex

IC_{50} value showed that the Schiff base exhibited the highest antioxidant activity. The antioxidant activity of Schiff base and its metal(II) complexes was also compared with ascorbic acid standard (Table-3). The increasing antioxidant activity of the complexes is due to the complexation of the system, increasing its unpaired electrons which is stabilized the ring and therefore the complexes scavenge free radicals [22].

DNA cleavage studies: The agarose electrophoresis experiment of plasmid DNA predicted it is clear that the complex Ni(II) (Lane 5 & 6) does not exhibit significant DNA cleavage activity in the presence of H_2O_2 when compared to the control plasmid DNA. However, Cu(II) complex at both concentrations (Lane 7 & 8), slow-moving Form-II (open circular form) increases when compared to the control DNA (Lane1). Similarly, DNA + H_2O_2 (Lane 2), DNA + H_2O_2 + Schiff base (Lane 3 & 4), does not show any significant cleavage of plasmid DNA when compared to the control (Fig. 5). This difference may be ascribed to the different binding affinities of complexes to DNA [23,24].

Conclusion

New mixed Schiff base ligand and its metal(II) complexes have been synthesized and characterized. It can bind to the metal ions in bidentate fashion through the nitrogen atom of imine group and the oxygen atom of keto group and also the nitrito-O coordination of nitrite. The synthesized metal (Ni^{2+} & Cu^{2+}) complexes exhibit a good affinity towards antioxidant activity. The DNA cleavage metal(II) complexes were confirmed by the cleavage studies of them.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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TABLE-3
ANTIOXIDANT ACTIVITY OF SCHIFF BASE AND Cu(II) COMPLEX

Compound	Concentration (µg/mL) average absorbance				Control	Inhibition (%)				IC_{50} value
	10	100	500	1000		10	100	500	1000	
Schiff base	1.196	0.881	0.546	0.319	1.689	29	47.8	67.6	81.1	267
$[Cu(SB)_2(NO_2)_2]$	1.258	0.958	0.648	0.467		25.5	43.2	61.6	72.3	386.1

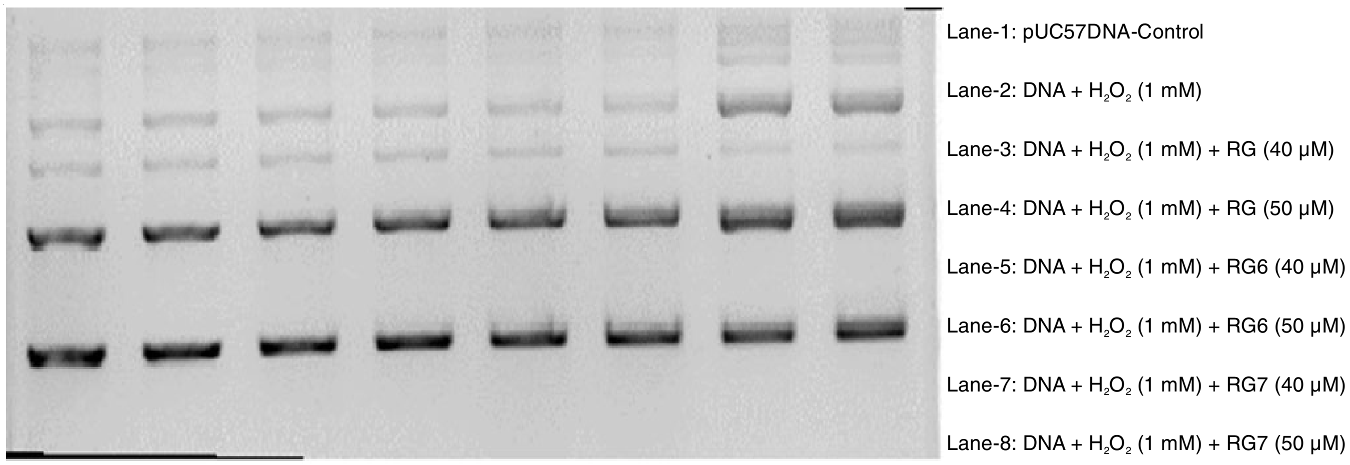

Fig. 5. DNA cleavage activity of Schiff base and its metal complexes

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