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Co(II) and Ni(II) Complexes with Schiff Base Ligands: Synthesis, Characterization, and Biological Activity[1]

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Abstract—The ligands, 1-acetylferrocenehydrazinecarboxamide (HL^1) and 1-acetylferrocenehydrazinecarbothioamide (HL^2), and their Ni(II) and Co(II) complexes were synthesized. The properties of the synthesized compounds were determined by the elemental and spectroscopic analyses. Ni(II) and Co(II) acetates interact with the ligands at the molar ratios 1 : 1 and 1 : 2 to give coloured products. The complexes have octahedral geometry. The ligands are coordinated to Co(II) and Ni(II) centers via the azomethine nitrogen and thiolic sulfur /enolic oxygen atom. The ligands and their Co(II) and Ni(II) complexes were screened for antibacterial and antifungal activities. The Co(II) and Ni(II) complexes show enhanced inhibitory activity as compared to their parent ligands. The DNA cleavage activity of the Co(II) and Ni(II) complexes was determined by gel electrophoresis. It was shown that the complexes have better cleavage activity than the ligands. The antioxidant activity of the complexes was also evaluated and used to examine their scavenging ability on hydrogen peroxide.

Keywords: Ni(II) and Co(II) complexes, 1-acetylferrocene, spectroscopic characterization, DNA cleavage activity, antioxidant activity

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INTRODUCTION

Schiff bases are an important class of ligands in the coordination chemistry. The interest in these compounds is due to their wide abundance in biological systems. Imines are used in various scientific fields. Schiff bases and their metal complexes are increasingly being used as catalysts in a variety of biological systems, polymers, and dyes. Furthermore, they can act as enzyme preparations [1]. They are also applied in organic synthesis, chemical catalysis, medicine, pharmaceutics, chemical analysis, and new technologies [2]. Imine complexes have diversity of biological properties, including antitumor, antiviral, antifungal, and antibacterial activity [3]. They are used as preparations to overcome drug resistance in cancer and are often tested as antimalarials. Also, they could be used for enzyme immobilization [4, 5].

Schiff base ligands such as thiosemicarbazones and semicarbazone are used in pharmacology as antibacterial, anticancer, and antifungal agents. Also, they are of significant interest for biochemistry, biomedicine, and environmental protection owing to their ability for chemical recognition of anions and metals. Thiosemicarbazones and semicarbazone belong to important class of mixed nitrogen–sulphur/oxygen donor ligands. Commonly, they coordinate with a metal atom via the sulphur/oxygen and azomethine nitrogen atoms to form chelates. Thiosemicarbazones are known as analytical reagents [6–8]. Metal chelates of the reagents inhibit tumor growth and increase biological activity of some drugs.

Ferrocenyl Schiff base ligands and their metal complexes have attracted attention of researchers due to a reversible redox couple [9] and two rotatory coplanar cyclopentadienyl rings. Different ferrocenyl Schiff bases are under clinical trials as potential compounds with antitumour, antibacterial, and antiviral activity. Ferrocene-containing metal-chelate complexes can be regarded as multinuclear molecules possessing both organometallic and coordination compounds properties. The use of ferrocenyl derivatives as bioactive molecules has been growing rapidly and several promising applications have been developed. This is because ferrocene is stable and nontoxic compound with interesting redox properties. It can be

[1] The text was submitted by the authors in English.

Table 1. Analytical data and physical properties of the ligands and their complexes synthesized by conventional heating

Compound	Color	mp, °C	Elemental analysis data												Molar mass found / calculated
			found, %						calculated, %						
			C	H	N	S	Co	Ni	C	H	N	S	Co	Ni	
L^1H	Brown	186	54.37	5.14	14.63	–	–	–	54.76	5.30	14.74	–	–	–	285.01 / 285.12
L^2H	Light brown	175	51.75	4.98	13.91	10.42	–	–	51.84	5.02	13.95	10.65	–	–	301.02 / 301.19
$Co(L^1)(OAc)\cdot 3H_2O$	Coffee	210	39.38	5.00	9.11	–	2.72	–	39.50	5.08	9.21	–	12.92	–	456.02 / 456.14
$Co(L^1)_2\cdot 2H_2O$	Dark yellow	200	46.94	4.68	12.59	–	8.73	–	47.09	4.86	12.67	–	8.89	–	663.10 / 663.19
$Ni(L^1)(OAc)\cdot 3H_2O$	Yellow	220	38.89	4.89	9.06	–	–	12.52	39.52	5.09	9.22	–	–	12.87	455.34 / 455.90
$Ni(L^1)_2\cdot 2H_2O$	Yellow	205	46.79	4.39	12.36	–	–	8.55	47.10	4.87	12.68	–	–	8.85	662.01 / 662.95
$Co(L^2)(OAc)\cdot 3H_2O$	Dark brown	198	38.01	4.67	8.76	6.49	12.09	–	38.15	4.91	8.90	6.79	12.48	–	471.89 / 472.20
$Co(L^2)_2\cdot 2H_2O$	Black	160	44.48	4.24	12.40	9.08	8.29	–	44.91	4.64	12.65	9.22	8.48	–	664.01 / 664.07
$Ni(L^2)(OAc)\cdot 3H_2O$	Coffee	260	38.02	4.71	8.64	6.54	–	11.89	38.17	4.91	8.90	6.79	–	12.44	471.51 / 471.96
$Ni(L^2)_2\cdot 2H_2O$	Brown	209	44.35	4.23	11.89	9.0	–	8.32	44.93	4.64	12.09	9.23	–	8.44	694.78 / 695.08

easily derivatized and functionalized or oxidized to ferrocenium salts. Many ferrocenyl compounds display interesting cytotoxic, antitumor, antimalarial, antifungal, and DNA cleavage activities [10-14]. Therefore, the application of ferrocene in medicine has been extensively studied. According to many studies, ferrocene derivatives possess highly promising activity against several diseases. In this study, the Co(II) and Ni(II) complexes with the ligands, 1-acetylferrocenehydrazinecarboxamide (HL^1) and 1-acetylferrocenehydrazinecarbothioamide (HL^2), were synthesized and their biological and spectral properties were determined.

RESULTS AND DISCUSSION

The elemental and spectral analyses revealed ligands (HL^1 and HL^2) and their complexes of the type $Co(L^1)(OAc)\cdot 3H_2O$, $Co(L^1)_2\cdot 2H_2O$, $Ni(L^1)(OAc)\cdot 3H_2O$, $Ni(L^1)_2\cdot 2H_2O$, $Co(L^2)(OAc)\cdot 3H_2O$, $Co(L^2)_2\cdot 2H_2O$, $Ni(L^2)(OAc)\cdot 3H_2O$, and $Ni(L^2)_2\cdot 2H_2O$. The solid complexes were prepared by mixing a hot ethanol solution of HL^1 and HL^2 with an aqueous ethanol solution of Co(II) and Ni(II) acetates to cause immediate precipitation of metal complexes (Table 1). The solid complexes were filtered off and thoroughly washed with warm water and aqueous ethanol to remove unreacted metal acetates or ligands.

Spectroscopic characterization. *IR spectra.* Results of the IR spectral analysis of the ligands and their metal complexes are given in Table 2. After the complexation, strong band at 1615–1620 cm^{-1}, which is due to the azomethine group ν(C=N), is shifted to lower frequency by 15–20 cm^{-1} owing to coordination of azomethine nitrogen to the metal ion. Compared to spectra of the ligands $HL^1$and HL^2, the spectra of the complexes have no broad band of the –(NH) vibrations in the region 3242–3248 cm^{-1}, which confirms deprotonation of this group on coordination with a metal atom. In spectra of the complexes, bands of the >C= S and >C=O bonds at 1040 and 1685 cm^{-1},

Table 2. IR vibrational frequences (cm^{-1}) for the ligands and their corresponding complexes

Compound	ν(NH)	ν(C=N)	ν($OCOCH_3$)	ν(M–N)	ν(M–O)	ν(M–S)	ν(H_2O/OH)
$L^1H(N^{\cap}OH)$	3242	1615	–	–	–	–	–
$L^2H(N^{\cap}SH)$	3248	1620	–	–	–	–	–
$Co(L^1)(OAc)\cdot 3H_2O$	–	1597	1728	487	512	–	3356
$Co(L^1)_2\cdot 2H_2O$	–	1598	–	490	520	–	3350
$Ni(L^1)(OAc)\cdot 3H_2O$	–	1592	1720	502	526	–	3348
$Ni(L^1)_2\cdot 2H_2O$	–	1596	–	508	532	–	3300
$Co(L^2)(OAc)\cdot 3H_2O$	–	1598	1726	488	–	352	3352
$Co(L^2)_2\cdot 2H_2O$	–	1595	–	489	–	351	3350
$Ni(L^2)(OAc)\cdot 3H_2O$	–	1592	1722	502	–	348	3346
$Ni(L^2)_2\cdot 2H_2O$	–	1593	–	506	–	350	3300

Table 3. Chemical shift (δ, ppm) in ^1H NMR and ^{13}C NMR spectra of the ligands

Compound	^1H NMR			^{13}C NMR			
	–NH(s)	$–NH_2$(s)	$–CH_3$(s)	>C=O/S	>C=N	–CH	ferrocenyl carbons
$L^1H(N^{\cap}OH)$	8.96	2.21	1.85	162.68	183.61	11.15	69.89,71.16,72.95,77.44
$L^2H(N^{\cap}SH)$	9.20	2.35	1.78	158.46	163.30	11.59	69.91,71.16, 73.12,77.45

respectively, are shifted towards lower frequency, indicative of keto-enol tautomerism and subsequent coordination of the complexes through the enolic oxygen / thiolic sulphur atom and a broad band of the coordinated water molecules is present in the region 3300–3356 cm^{-1}. Intense band within 1720–1728 cm^{-1} is assigned to the ν($OOCCH_3$) vibration in the 1 : 1 metal complexes [19]. Bands at (cm^{-1}) 510±10, 480±10, 350±10, 525±10, 500±10, and 340±10 are assigned to ν(Co–O), ν(Co–N) , ν(Co–S), ν(Ni–O), ν(Ni–N), and ν(Ni–S) vibrations, respectively.

^1H NMR spectra. ^1H NMR spectra of the ligands (DMSO-d_6 solutions) were recorded using TMS as the internal standard. The chemical shift values (δ, ppm) are given in Table 3. The ^1H NMR spectra of the ligands have signal of the –NH proton at δ = 8.96–9.20 ppm. Other protons, CH_3 and NH_2, resonate at δ = 1.80–1.85 and 2.20–2.35 ppm, respectively. A sharp singlet at δ = 6.22–6.62 ppm is due to the $–C_5H_5$ group.

Electron absorption spectra. The stereochemistry of metal ions in the complexes was determined from the position and number of the *d–d* transition peaks in the electron absorption spectra (see Table 4). The spectra of the Schiff base and its Co(II) and Ni(II) complexes (ethanol solutions) were recorded at room temperature. Our results are in good agreement with the literature data on the high spin six-coordinated octahedral complexes, according which the high spin octahedral Co(II) complex is characterized by three electron transitions: $^4T_{1g}(F)\rightarrow {}^4T_{2g}(P)$ (ν_1), $^4T_{1g}(F)\rightarrow {}^4A_{2g}(F)$ (ν_2), and $^4T_{1g}(F)\rightarrow {}^4T_{1g}(F)$ (ν_3). The spectra of the Co(II) complexes contain three absorption bands in the regions 9275–9490, 19 425, 20 420, 21 490, 22 001 cm^{-1} and, as for high spin octahedral complexes, the transition energy ratio of second to first is 2.0–2.1 [20].

The ligand field parameters (*Dq*, *B*, β, and β%) are calculated for the Co(II) complexes. The Racah parameter (*B*) is found to be 870–935 cm^{-1} (< 971 cm^{-1}), which shows that the ligand and the metal overlap with their orbitals. The nephelauxetic ratio (β) for the 1 : 1 and 1 : 2 cobalt complexes is less than unity, which shows that metal bonding to the ligand has a partially covalent character. The electron spectra of the Ni(II) complexes have three absorption bands of the $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ (ν_1), $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ (ν_2), and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$

Table 4. Electron transition frequences and ligand field parameters of the metal complexes

Compound	Frequency, cm^{-1}			*Dq*, cm^{-1}	*B*, cm^{-1}	ν_2/ν_1	β	β, %
	ν_1	ν_2	ν_3					
$Co(L^1)(OAc)\cdot 3H_2O$	9278	19425	21490	1014.6	871.9	2.09	0.898	10.2
$Co(L^1)_2\cdot 2H_2O$	9341	19616	21527	1026.5	874.0	2.09	0.900	10.0
$Ni(L^1)(OAc)\cdot 3H_2O$	9925	15384	22739	992.5	556.5	1.55	0.534	46.6
$Ni(L^1)_2\cdot 2H_2O$	9985	15478	22814	998.5	555.8	1.55	0.533	46.7
$Co(L^2)(OAc)\cdot 3H_2O$	9489	19896	21826	1040.6	883.6	2.09	0.910	9.00
$Co(L^2)_2\cdot 2H_2O$	9476	20417	22001	1094.0	932.6	2.12	0.960	4.00
$Ni(L^2)(OAc)\cdot 3H_2O$	9931	15095	24843	993.1	676.3	1.52	0.649	35.1
$Ni(L^2)_2\cdot 2H_2O$	9947	15318	24851	994.7	688.5	1.54	0.661	33.9

(ν_3) transitions in the regions 9925–9985, 15090–15480, and 22740–24855 cm^{-1}, respectively [20], which confirms octahedral coordination of the Ni(II) ion. The ligand field parameters (*Dq*, *B*, β, and β%) show that the contribution of covalent bond into the metal-ligand bonding is significant.

Bioassay. *Biological aspect.* The antimicrobial activity of the ligands and their Co(II) and Ni(II) complexes against pathogenic fungi and bacteria is demonstrated in Figs. 1, 2. As seen, the Schiff bases possess biological activity and the antifungal and antimicrobial activity of their metal complexes against

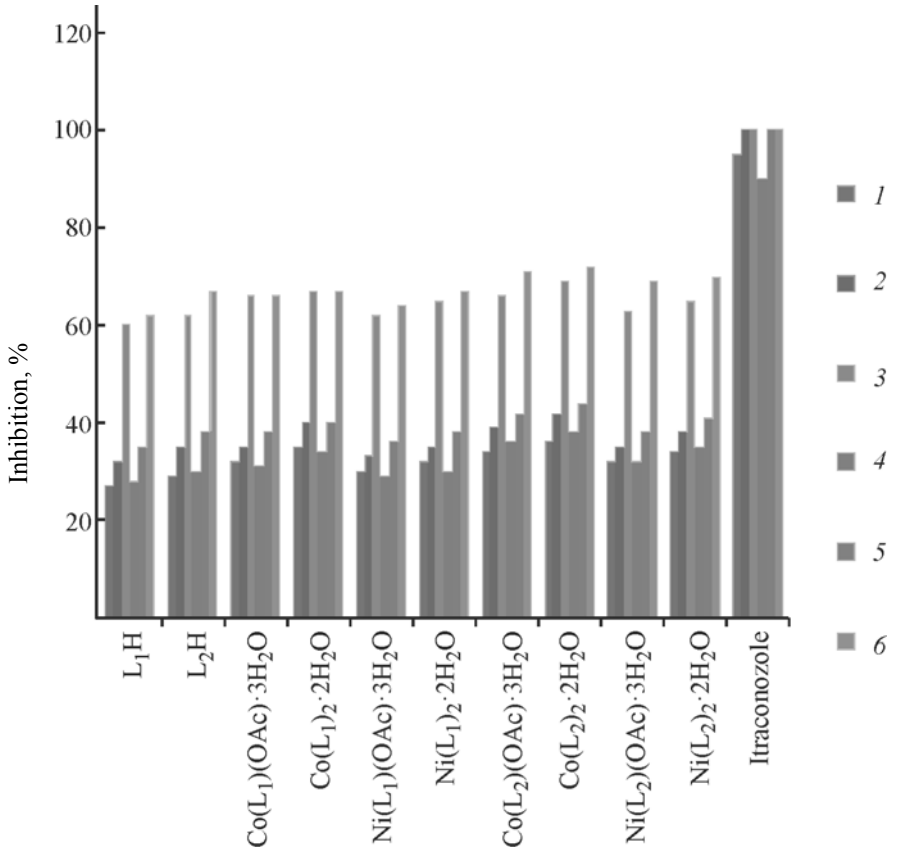

Fig. 1. Data on the antifungal screening of the ligands and their Co(II) and Ni(II) complexes after 96 h of inhibition (%). The concentration is given in ppm. (*1*) *Fusarium semitectum* 50 ppm, (*2*) *Fusarium semitectum* 100 ppm, (*3*) *Fusarium semitectum* 200 ppm, (*4*) *Aspergilus flavus* 50 ppm, (*5*) *Aspergilus flavus* 100 ppm, and (*6*) *Aspergilus flavus* 200 ppm.

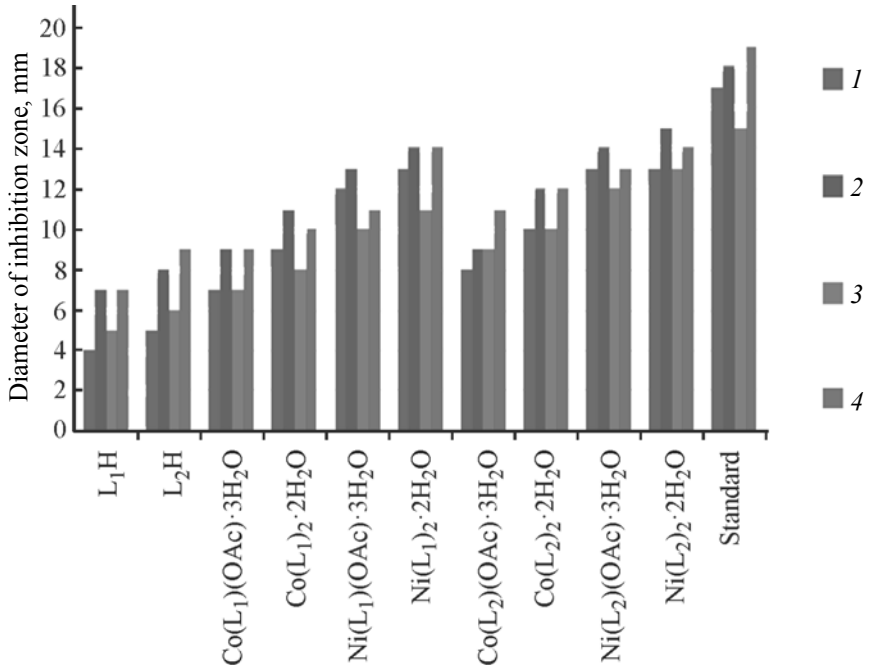

Fig. 2. Data on the antibacterial screening of the ligands and their Co(II) and Ni(II) complexes: (*1*) *Pseudomonas aerugenosa* 500 ppm, (*2*) *Pseudomonas aerugenosa* 1000 ppm, (*3*) *Streptococcus mutans* 500 ppm, and (*4*) *Streptococcus mutans* 1000 ppm.

microbial strains is significantly higher compared to the ligands. The fungicidal and bacterial activities increase with increasing concentration. Thus, it is evident that the concentration plays a vital role in enhancing the degree of inhibition. The enhanced activity of the synthesized complexes, as compared to the activity of the ligands, may be explained in terms of the Overtone's concept and Tweedy's chelation theory [21, 22]. According to the Overtone's concept, a lipid membrane surrounding the cell facilitates the passage of substances that dissolve in lipids. Therefore, the liposolubility is an important factor to control antimicrobial activity. On the chelation, the polarity of the metal ion is reduced to a larger extent owing to overlapping of the ligand orbitals and a partial sharing of the positive charge of the metal ion with the charge of the donor groups.

Moreover, delocalization of π-electrons over the entire chelate ring is higher and lipophilicity of the complexes is enhanced. Owing to increased lipophilicity, the complexes can easily penetrate into a lipid membrane and block the metal binding sites in enzymes of microorganisms. These complexes also disturb cellular respiration and thus block proteins synthesis, which makes further growth of the organism impossible. In general, metal complexes are more active than the ligands as they may serve as principal cytotoxic species. Thus, the complexes, as antimicrobial agent, can find further application in pharmaceutical industry after testing their toxicity to human beings.

DNA cleavage activity . The DNA cleavage activity of the ligands and their Co(II) and Ni(II) complexes against *Pseudomonas aerugenosa* (ATCC 27853) was evaluated by agarose gel electrophoresis. Gel electrophoresis is based on the migration of DNA under the influence of an electric potential. In the control experiments with the untreated DNA, the gel electrophoresis clearly showed no cleavage (Fig. 3, lane *2*), whereas the DNA cleavage activity of all metal complexes and the ligands is remarkable.

The migration of the ligands and their cobalt and nickel complexes differs from that of the control DNA of *Pseudomonas aerugenosa* (Fig. 3, lanes *2* and *3*–*12*). The cleavage activity was confirmed by band tailing in the DNA spectrum. In the presence of the complexes, a double stranded or super-coiled DNA is converted into a single stranded or an open circular DNA. The oxidative cleavage mainly occurs through the sugar phosphate breakdown. In this study, the Co(II) and Ni(II) complexes with thiosemicarbazone exhibited the highest activity towards DNA cleavage and all metal complexes exhibited better activity than free ligands.

Antioxidant activity. Free radicals including hydroxyl, superoxide anion, and hydrogen peroxide species play an important role in biochemical processes of the immune system, cell differentiation, and signal transduction [23]. Generally, free radicals in the body are maintained at constant levels by the antioxidant defence mechanism. Small molecule antioxidants, such as ascorbic acid (vitamin C), uric acid, and glutathione, are also significant cellular antioxidants [24]. However, many situations, such as an unhealthy physical conditions, ageing, or a stressful environment perturb the free radical balance, which leads to several diseases, including inflammation, neurodegenerative disorders, cardiovascular diseases, and cancer [25].

The ability of free radicals to scavenge hydrogen peroxide was studied on the ligands and metal complexes using ascorbic acid as standard compound. Results of the antioxidant activity show that the compound $Ni(L^2)_2 \cdot 2H_2O$ exhibited the highest free radical scavenging activity. It is shown that the antioxidant activity of the Ni(II) Schiff base complexes is better than of Co(II) complexes. The marked antioxidant activity of the metal complexes in comparison with a free ascorbic acid may be due to the metal coordination in the complex.

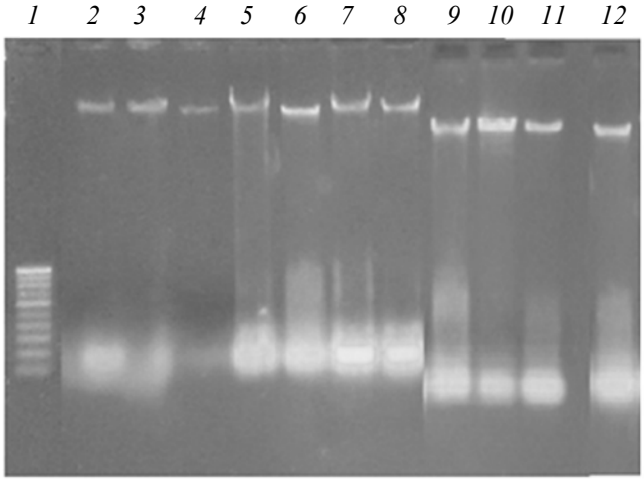

Fig. 3. Gel electrophoresis diagram demonstrating the DNA cleavage activity of the synthesized compounds. Lanes: (*1*) standard molecular weight marker, (*2*) control DNA of *P. Aeruginosa,* (*3, 4*) *P. Aeruginosa* DNA treated with the ligands L^1H and L^2H, respectively, and (*5–12*) *P. Aeruginosa* DNA treated, respectively, with the complexes $[Co(L^1)(OAc)\cdot 3H_2O$, $Co(L^1)_2\cdot 2H_2O$, $Ni(L^1)(OAc)\cdot 3H_2O$, $Ni(L^1)_2\cdot 2H_2O$, $Co(L^2)(OAc)\cdot 3H_2O$, $Co(L^2)_2\cdot 2H_2O$, and $Ni(L^2)(OAc)\cdot 3H_2ONi(L^2)_2\cdot 2H_2O]$.

EXPERIMENTAL

The Co(II) and Ni(II) compounds, $Co(OAc)_2\cdot 4H_2O$ and $Ni(OAc)_2\cdot 4H_2O$, as well as 1-acetylferrocene, semicarbazide hydrochloride, and thiosemicarbazide were purchased from Alfa Aesar. Chemicals and solvents (all of analytical grade) were dried and purified by standard methods. The molecular weight was determined by the Rast's camphor method, chlorine, by the Volhard's titration [15], nitrogen, by the Kjeldahl's method [16], and sulphur, by the Messenger's method [17]. IR spectra of the ligands and their complexes (KBr pellets) were recorded on a Nicolet Magna FTIR-550 spectrophotometer. ^1H NMR

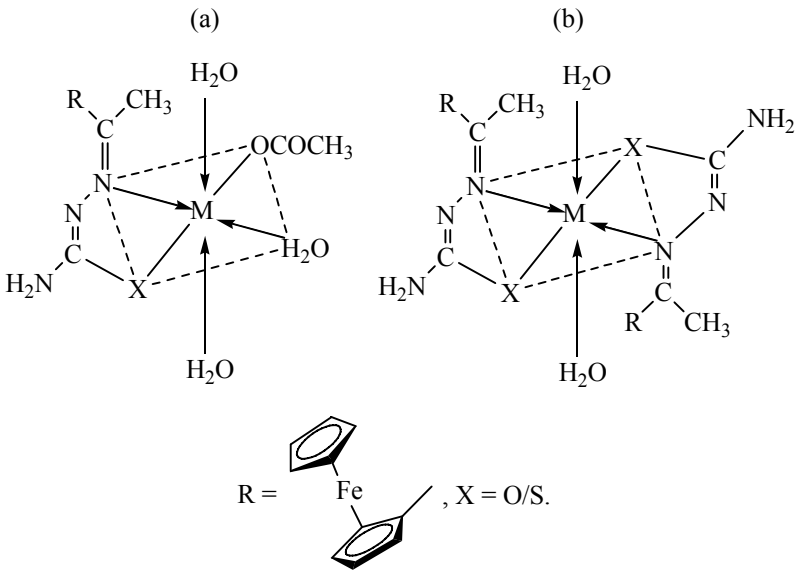

Fig. 4. The structure of the complex (a) 1 : 1 and (b) 1 : 2.

Table 5. Analysis of the hydrogen peroxide scavenging activity of the ligands and their metal complexes

Activity	Sample	OD at 230 nm	Activity 1, %	OD at 230 nm	Activity 2, %	The average activity, %
Hydrogen peroxide scavenging activity	L^1	0.555	55.33	0.512	64.180	59.76
	L^2	0.415	57.08	0.418	71.100	64.09
	$Co(L^1)(OAc)\cdot 3H_2O$	0.425	63.34	0.214	64.567	63.95
	$Co(L^1)_2\cdot 2H_2O$	0.446	65.83	0.297	64.937	65.38
	$Ni(L^1)(OAc)\cdot 3H_2O$	0.431	79.98	0.413	78.254	78.39
	$Ni(L^1)_2\cdot 2H_2O$	0.442	81.29	0.443	79.267	80.25
	$Co(L^2)(OAc)\cdot 3H_2O$	0.482	67.15	0.356	66.982	67.06
	$Co(L^2)_2\cdot 2H_2O$	0.502	68.24	0.506	68.742	68.49
	$Ni(L^2)(OAc)\cdot 3H_2O$	0.449	82.15	0.461	80.415	81.28
	$Ni(L^2)_2\cdot 2H_2O$	0.512	84.30	0.528	83.254	83.27
	Control	0.759	64.56	0.776	66.647	65.60

and ^{13}C NMR spectra (DMSO-d_6 solutions) were recorded at the Therachem Research Organization, India **(**internal standard was TMS).

Ligand preparation. To prepare ligands (HL^1) and (HL^2), 2 g of 1-acetylferrocene, 0.97 g of semicarbazidehydrochloride (or 0.79 g of thiosemicarbazide, respectively) were dissolved in ethanol (100 mL) in the presence of sodium acetate (0.72 g) at the molar ratio 1 : 1 with following reflux for 7–8 h. The residue formed was separated, filtered off, recrystallized from ethanol, and finally dried in vacuum over a fused calcium chloride. The structure of the ligands is represented in Fig. 4.

Preparation of the metal complexes. *Metal complexes of HL^{1-2} (1 : 1).* An aqueous ethanol solution of Co(II) acetate (0.26 g, 1.03 mmol) and Ni(II) acetate (0.26 g, 1.03 mmol) was added to a hot ethanol solution of the ligand HL^{1-2} (0.35 g, 1.03 mmol) to cause immediate precipitation. The solid complexes were filtered off and thoroughly washed with warm water and aqueous ethanol to remove unreacted metal acetates or ligands.

Metal complexes of HL^{1-2} (1 : 2). An aqueous ethanol solution of Co(II) (0.12 g, 0.50 mmol) and Ni(II) (0.12 g, 0.50 mmol) acetates was added to a hot ethanol solution of the ligand (HL^{1-2}) (0.35 g, 1.03 mmol) to cause immediate precipitation. The solid complexes were filtered off and thoroughly washed with warm water and aqueous ethanol to remove unreacted metal acetates or ligands.

The purity was checked by thin-layer chromatography using silica gel-G as stationary phase. The physical properties of the compounds and results of their elemental analysis are collected in Table 5.

Biological evaluation. *Antifungal activity.* All the newly synthesized ligands [HL^{1-2}] and their metal complexes were screened *in* v*itro* for their antifungal activity against two pathogenic fungi, *Fusarium semitectum* and *Aspergilus flavus*, by the agar plate method using Sabouraud Dextrose Agar as selective medium. The medium has the following composition: dextrose 40 g, mycological peptone 10 g, agar 15 gm, and distilled water 1000 mL; pH was adjusted to 5.6–5.9 at 28±2°C. Then, a required amount of the test sample (ethanol solution) was introduced into it to obtain a concentration of 50, 100, and 200 ppm.

The medium was then poured into petri plates and the spores of the fungi were inserted into the medium through an inoculum needle. The petri plates were wrapped in polythene bags containing a few drops of alcohol and placed in an incubator at 25±4°C. The activity, i.e., the linear growth of the fungi, was determined after 96 h of incubation by measuring the diameter of the fungal colony. The percentage inhibition was calculated as:

$$\text{Inhibition} = \frac{C - T}{C} \times 100\ \%,$$

where C and T are the diameters of fungal colony in control and test plates, respectively, after 96 h.

The antifungal activity of the compounds was compared with that of the standard compound (itraconazole).

Antibacterial activity. The antibacterial activity of the ligands and their Co(II) and Ni(II) complexes against gram positive (*Pseudomonas aeruginosa*) and gram negative (*Streptococcus mutans*) bacteria was evaluated. Test organisms were grown on a Mueller-Hinton agar. The agar medium contained starch (0.15 g), beef infusion (30 g), casein hydrolysate (1.75 g), agar (1.7 g), and distilled water (1000 mL); its pH was adjusted to neutral at 37±2°C. The desired compounds were separately dissolved in methanol in concentrations 500 and 1000 ppm and soaked in Whatman no. 1 filter paper discs of 5 mm diameter. The discs were dried and placed on the previously seeded petri plates and incubated at 35°C for 24 h. The diameter of the inhibition zone around each disc was measured accurately in mm and compared with that of the standard antibiotic (Streptomycin), whose antibacterial activity was also determined by the same procedure.

Antioxidant activity. *Hydrogen peroxide scavenging ability.* The ability of the cobalt and nickel complexes to scavenge hydrogen peroxide was determined by the method [18] developed by Ruch et al. in 1989. A 40 mM solution of hydrogen peroxide was prepared in phosphate buffer (pH = 7.4). The concentration of hydrogen peroxide was determined spectrophotometrically (8500 II Biochrom spectrophotometer, Switzerland). The cobalt and nickel complexes (50 μg/mL solutions in distilled water) were added to a 0.6 mL of hydrogen peroxide solution. Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing a phosphate buffer without hydrogen peroxide (Table 5). The percentage scavenging activity of hydrogen peroxide by control and compounds was calculated from the following equation:

$$[H_2O_2] = [(A_0 - A_1)]/A_0 \times 100\%,$$

where A_0 is the absorbance of the control and A_1, the absorbance in the presence of the sample.

DNA cleavage analysis. *Preparation of culture media.* The DNA binding and cleavage experiments were performed at room temperature**.** The DNA cleavage activity of the Co(II) and Ni(II) complexes was evaluated by agarose gel electrophoresis. *Pseudomonas aeruginosa* plasmid was cultured, isolated, and the DNA was used for the experiment. For the cultivation we used a nutrient broth medium containing peptone (5 g), beef extract (3 g), and NaCl (5 g), and distilled water (1000 mL); pH was adjusted to 7.0, autoclaving was done at 121°C at the pressure 15 psi. The medium was inoculated with the *Pseudomonas aeruginosa* (ATCC 27853).

Isolation of DNA. The fresh bacterial culture (1000μL) was centrifuged (6 000 rpm, 10 min) to obtain a pellet, which was then dissolved in 250 μL of a cell lysis buffer. To this, 250 μL of saturated phenol, chloroform, and isoamyl alcohol were added at the ratio 25 : 24 : 1 and the mixture was incubated at –20°C for 1 h. Then, it was centrifuged at 10 000 rpm for 10 min and the upper aqueous layer was collected. To this supernatant, two volumes of chilled absolute alcohol and 50 μL of sodium acetate were added. The DNA precipitate was separated by centrifugation. The pellet was dried, collected, dissolved in a TE buffer (10 mM Tris, pH = 8.0, 1 mM EDTA), and stored in cold conditions.

Agarose gel electrophoresis. Agarose gel electrophoresis is the easiest and commonest way of separateing and analyzing DNA. Test samples (1 mg/mL) were prepared in DMF. The samples (25 μg) were added to the isolated DNA of *Pseudomonas aeruginosa*. The samples were incubated at 37°C for 2 h. During electrophoresis, the gel was submersed in an electrophoresis chamber containing a TAE buffer solution (4.84 g Tris base, pH 8.0, 0.5 m EDTA/L) and a DNA sample (20 μL) mixed with a bromophenol blue dye at the ratio 1 : 1 was carefully loaded into the wells of the agarose gel. The voltage 50 V was supplied for about 30 min. The gel was removed and stained with 10.0 g/mL of ethidium bromide for 10–15 min. After the electrophoresis, the gel was photographed under UV transilluminator and documented. The results obtained were compared with those for standard DNA marker.

CONCLUSIONS

The Schiff bases synthesized here act as bidentate ligands and are coordinated to metal ion through the nitrogen and sulphur/oxygen atoms. The ligand binding to the metal ion is confirmed by elemental and spectroscopic analyses. The ligand field parameters (*Dq*, *B*, β, and β%) correspond to the metal-ligand bonds with a significant covalency. The metal complexes have significantly enhanced antibacterial and antifungal activity against microbial strains in comparison with free ligands. Thus, they may find

application as a new antimicrobial agent after further study of their biological properties. The study of the antioxidant activity showed that the Ni(II) Schiff base complexes are better antioxidants than Co(II) complexes. According to the study on the DNA cleavage activity, the Co(II) and Ni(II) complexes of thiosemicarbazone have the highest activity towards DNA cleavage and all metal complexes exhibit better activity compared with the ligands.

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REFERENCES

1. Kalaivani, S., Priya, N.P., and Arunachalam, S., *Int. J. Appl. Biol. Pharm.,* 2012, vol. 3, pp. 219–223.
2. Upadhyay, K.K., Kumar, A., Upadhyay, S., and Mishra, P.C., *Mol. Struct.,* 2008, vol. 73, pp. 5–16**.** DOI: 10.1016/j.molstruc.2007.02.031.
3. Radecka-Paryzek, W., Pospieszna-Markiewicz, I., and Kubicki, M., *Inorg. Chim. Acta,* 2007, vol. 360, pp. 488–496. DOI: 10.1016/j.ica.2006.07.071.
4. Boghaei, D.M., Askarizadeh, E., and Bezaatpour, A., *Spectrochim. Acta A,* 2008, vol. 69, pp. 624–628. DOI: 10.1016/j.saa.2007.05.013.
5. Prashanthi, Y., Kiranmai, K., and Subhashini, N.J.P., *Spectrochim. Acta A,* 2008, vol. 70, pp. 30–35. DOI: 10.1016/j.saa.2007.07.028.
6. Riopedre, G.G., Garca, M.I.F., Noya, A.M.G., Fernandez, M.A.V., Bermejo, M.R., and Maneiro, M., *Phys. Chem.,* 2011, vol. 13, pp. 18069–18077. DOI: 10.1039/C1CP21154D.
7. Reddy, D.N. and Reddy, K.V., *Momona Ethiop. J. Sci.*, 2012, vol. 4, p. 70.
8. Karthikeyan, J., Parameshwara, P., and Shetty, A.N., *Environ. Monit. Assess.*, 2011, vol. 173, p. 569. DOI: 10.1007/s10661-010-1405-8.
9. Zhang, W., Chen, M., and Diao, G., *Electrochim. Acta*, 2011, vol. 56, pp. 5129–5136. DOI: 10.1016/j.electacta.2011.03.062.
10. Gallagher, J.F., Kenny, P.T.M., and Sheehy, M.J., *Inorg. Chem. Commun.*, 1999, vol. 2, p. 200. DOI: 10.1016/S1387-7003(99)00048-9.
11. Suer, R.L., Basta, R., Arif, A.M., Geiger, W.E., and Ernst, R.D., *Organometallics,* 2003, vol. 22, p. 1487. DOI: 10.1021/om020779g.
12. Ohs, A.C., Rheingold, A.L., Shaw, M.J., and Nataro, C., *Organometallics,* 2004, vol. 23, p. 4655. DOI: 10.1021/om049735t.
13. Tomapatanaget, B., Tuntulani, T., Chailapakul, O., Tomapatanaget, B., Tuntulani, T., and Chailapakul, O., *Org. Lett.,* 2003, vol. 5, p. 1539. DOI: 10.1021/ol034355+.
14. Biyala, M.K., Sharma, K., Fahmi, N., and Singh, R.V., *Phosphorus. Sulfur,* 2007, vol. 182, pp. 2955–2965. DOI: 10.1080/10426500701544540.
15. Vogel, A.I., *A Textbook of Organic Quantitative Analysis,* London: Longman, 2004, 5th ed. DOI: 10.1016/0160-9327(90)90087-8.
16. Vogel, A.I., *A Text Book of Quantitative Inorganic Analysis,* Longman**:** London, 1961. DOI: 10.1002/ange.19620741645.
17. Makode, J.T. and Aswar, A.S., *Indian J. Chem.*, 2004, vol. 43A, p. 2120.
18. Ruch, R.J., Cheng, S.J., and Klaunig, J.E., *Carcinogenesis,* 1989, vol. 10, pp. 1003–1008. DOI: 10.1093/carcin/10.6.1003.
19. Singh, K., Kumar, Y., Puri, P., Sharma, C., and Aneja, K.R., *Med. Chem. Res.*, 2012, vol. 187, pp. 1498–1507. DOI: 10.1080/10426507.2012.692128.
20. Cotton, F.A., Williknson, G., Murillo, C.A., and Bochman, M., *Advanced Inorganic Chemistry*, Wiley: New York, 2003, 6 ed.
21. Raman, N., Kulandaisamy, A., and Jayasubramanian, K., *Polish J. Chem.,* 2002, vol. 76, pp. 1085–1094.
22. Tweedy, B.G., *Phytopathology,* 1964, vol. 55, p. 910. DOI: 10.3186/jjphytopath.29.55.
23. Droge, W., *Physiol. Rev.*, 2002, vol. 82, pp. 47–95. DOI: 10.1152/physrev.00018.2001.
24. Marciniak, G. and Petty, M.A., *Drugs Futur.,* 1996, vol. 21, pp. 1037–1046. DOI: 10.1358/dof.1996.021.10.385490.
25. Hail, N. Jr., Cortes, M., Drake, E.N., and Spallholz, J.E., *Free Radic. Bio. Med*., 2008, vol. 45, pp. 97–110. DOI: 10.1016/j.freeradbiomed.2008.04.004.