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Copper(II) and nickel(II) complexes of benzyloxybenzaldehyde-4-phenyl-3-thiosemicarbazone: Synthesis, characterization and biological activity

B. Prathima [a], Y. Subba Rao [a], S. Adinarayana Reddy [b], Y.P. Reddy [c], A. Varada Reddy [a,*]

[a] Analytical Division, Dept. of Chemistry, S.V. University, Tirupati 517502, India
[b] Dept. of Material Science and Nanotechnology, Y.V. University, Kadapa 516001, India
[c] Dept. of Physics, Sri Padhmavati Mahila Visva Vidyalayam (Women's University), Tirupati 517502, India

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ABSTRACT

Benzyloxybenzaldehyde-4-phenyl-3-thiosemicarbazone ligand (L) has been synthesized from benzyloxybenzaldehyde and 4-phenyl-3-thiosemicarbazide. Complexes of this ligand with chlorides of Cu(II) and Ni(II) have been prepared. The structure of the ligand (L) is proposed based on elemental analysis, IR and ^{1}H NMR spectra. Its complexes with Cu(II) and Ni(II) ions are characterized from the studies of electronic as well as EPR spectra. On the basis of electronic and EPR studies, rhombically distorted octahedral structure has been proposed for Cu(II) complex while the Ni(II) complex has been found to acquire an octahedral structure. The ligand and their metal complexes have been tested *in vitro* for their biological effects. Their antibacterial activities against Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*) and Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) have been investigated. The prepared metal complexes exhibit higher antibacterial activities than the parent ligand. The *in vitro* antioxidant activity of free ligand and its metal(II) complexes have also been investigated and the results however reveal that the ligand exhibits greater antioxidant activity than its complexes.

1. Introduction

The chemistry of thiosemicarbazones has received considerable attention in view of their variable bonding modes, promising biological implications, structural diversity and ion-sensing ability [1–3]. Thiosemicarbazones are now well established as an important class of sulphur donor ligands particularly for transition metal ions [4–6]. This is due to remarkable biological activities observed for these compounds, which have since been shown to be related to their metal complexing ability. These compounds present a great variety of biological activities ranging from antiviral [7] to anticancer [8], antitumour [9,10], antibacterial [11–13], anti-inflammatory and antiamoebic [14–16] activities. The inhibitory action of these compounds is attributed to their chelating properties [17]. The activity of these compounds is strongly dependent upon the nature of the hetero atomic ring and the position of attachment of thiosemicarbazone group to the ring as well as the form of thiosemicarbazone moiety [18]. These compounds are studied extensively due to their flexibility, their selectivity and sensitivity towards the central metal atom, their structural similarities with natural biological substances and the presence of imine group(–N=CH–) which imparts the biological activity [19]. Collins et al. have reported the correlation between structure and anti-mycobacterial activity in a series of 2-acetylpyridine thiosemicarbazones [20]. In many cases, due coordination to different transition metal ions that can be found in biological systems, it is possible to obtain complexes that are more efficient drugs than the corresponding free ligands [21–24].

In the present work, as a continuation of the research program related to the coordination chemistry and biological activity of thiosemicarbazones in our laboratory [25], the synthesis and spectroscopic characterization of Cu(II) and Ni(II) complexes of the ligand, benzyloxybenzaldehyde-4-phenyl-3-thiosemicarbazone are reported. The *in vitro* antibacterial and antioxidant activities of these compounds are evaluated.

2. Experimental

2.1. Materials

All the chemicals used are of analytical grade. Organic chemicals such as thiobarbituric acid (TBA), trichloroaceticacid (TCA), α-tocopherol, butylated hydroxytoluene (BHT), 1,1-diphenyl-2-picryl hydrazyl (DPPH), 4-phenyl-3-thiosemicarbazide and dimethyl formamide (DMF) are procured from Sigma–Aldrich and all metal salts are procured from E-Merck chemical companies.

* Corresponding author. Tel.: +91 877 2249666x303; fax: +91 877 2249611.
E-mail address: ammireddyv@yahoo.co.in (A. Varada Reddy).

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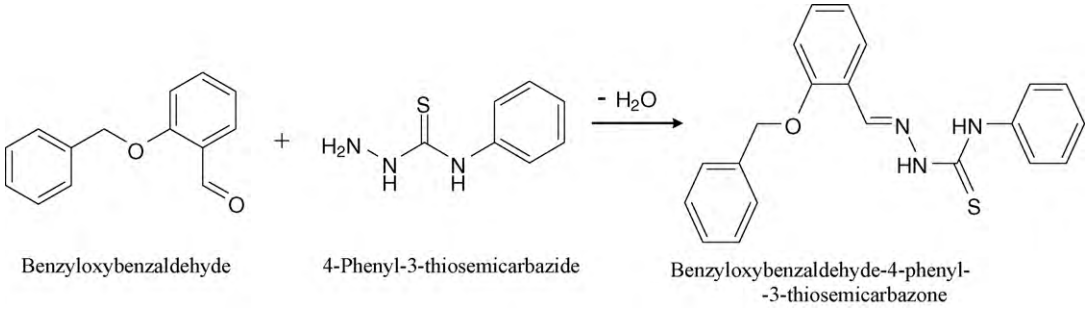
2.2. General procedures

The IR spectra of the compounds are recorded on a Nicolet FT-IR 560 Magna spectrometer using KBr(neat). The Bruker 300 MHz NMR spectrometer is used to obtain the ^{1}H NMR spectrum of the ligand. A mass spectrum was recorded in a Quattro LC-Micro mass. Elemental analysis is obtained from vario-micro qub elementar analyzer. The electronic spectra of the complexes are recorded on a PerkinElmer UV/VIS Lambda 950. EPR spectra are recorded on an EPR spectrometer (JEOL FE-1X) operating in the X-band frequencies with a modulation frequency of 100 kHz. 100 mg of each compound is taken in a quartz tube for EPR measurements. The magnetic field is scanned from 2200 to 4200 G, with a scan speed of 250 G min^{-1}. Absorbance is measured using systronics UV–VIS spectrometer-117. Centrifugation is done using REMI centrifuge. A digital pH meter (model L1-10 Elico, India) is used for measuring pH.

2.3. Synthesis of benzyloxybenzaldehyde-4-phenyl-3-thiosemicarbazone

Hot ethanolic solution containing 2.12 g of benzyloxybenzaldehyde is mixed with hot ethanolic solution containing 1.67 g of 4-phenyl-3-thiosemicarbazide. The mixture obtained is refluxed for an hour, then stirred for 3.5 h at 60–70 °C and kept at room temperature for a day. The resulting intense yellow colored precipitate is filtered, washed with ethanol and dried. The scheme is shown below.



2.4. Synthesis of metal complexes

A hot ethanolic solution (25 ml) of free ligand (0.002 mol) and a hot ethanolic solution (25 ml) of the corresponding metal salt (0.001 mol) are refluxed for 3–4 h at 50 °C and on cooling the contents, the colored complexes are precipitated out. They are filtered, washed with 50% ethanol and dried. The purity of the complex is checked by TLC.

2.5. Antibacterial screening

In vitro antibacterial screening is performed by the agar disc diffusion method [26,27]. The bacterial species used in the screening are Gram-negative bacteria such as *Klebsiella pneumoniae* and *Escherichia coli* and Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*. Stock cultures of the test bacterial species are maintained on nutrient agar media (Hi-media Laboratories, Mumbai) by sub culturing in petri dishes. The media are prepared by adding the components as per manufacturer's instructions and they are sterilized in the autoclave at 121 °C and atmospheric pressure for 15 min. Each medium is cooled to 45–60 °C and 20 ml of it is poured into a Petri dish and allowed to solidify. After solidification, Petri plates with media are spread with 1.0 ml of bacterial suspension prepared in sterile distilled water. The wells are bored with cork borer and the agar plugs are removed. To each agar well, 100 ml of the compound reconstituted in DMF in concentration of 1.0 mg/ml is added. DMF is used as a negative control and antibiotics such as ampicillin and tetracycline are used as positive control standards. All the plates are incubated at 37 °C for 24 h and they are observed for the growth inhibition zones. The presence of clear zones around the wells indicated that the compound is active. The diameter of zone of inhibition is calculated in millimeters. The well diameter is deducted from the zone diameter to get the actual zone of inhibition diameter and the values are tabulated.

2.6. DPPH scavenging activity

The principle for the reduction in DPPH free radicals is that the antioxidant reacts with stable free radical DPPH and converts it to 1,1-diphenyl-2-picrylhydrazine. The ability to scavenge the stable free radical DPPH is measured by decrease in the absorbance at 517 nm. Solutions of BBPTS and its Cu(II) and Ni(II) complexes at 100 μM concentrations are added to 100 μM DPPH and kept in ethanol tubes. The tubes are kept at ambient temperature for 20 min and absorbances are measured at 517 nm. α-Tocopherol is used as a positive control [28]. These measurements are run in triplicate. The percentage of scavenging activity is calculated as follows:

$$\text{Scavenging activity}\,(\%) = \left[\frac{A_{\text{DPPH}} - A_{\text{TEST}}}{A_{\text{DPPH}}}\right] \times 100$$

where A_{DPPH} is the absorbance of DPPH without test sample (control) and A_{TEST} is the absorbance of DPPH in the presence of test sample.

2.7. Inhibition of lipid peroxidation in rat brain homogenate

2.7.1. Preparation of rat brain homogenate

Albino Wistar rats (180–200 g) are used for the study. Prior to decapitation and removal of the brain, the animals are anesthetized with ether and perfused transcardially with ice-cold normal saline to prevent contamination of brain tissue with blood. Tissue was weighed and its homogenate (10%, w/v) was prepared in 0.15 M KCl and centrifuged at 800 rpm for 10 min. The supernatant is used immediately for the study [29].

2.7.2. Iron(III) induced lipid peroxidation

The incubation mixture contains in a final volume of 1.5 ml brain homogenate (0.5 ml of 10%, w/v), KCl (0.15 M) and ethanol (10 μl) or test compound dissolved in ethanol. Peroxidation is initiated by adding ferric chloride (100 μM) to give the final concentration stated. After incubation for 20 min at 37 °C, reactions were stopped by adding 2 ml of ice-cold 0.25 M HCl containing 15% trichloroaceticacid(TCA), 0.38%. thiobarbituric acid (TBA) and 0.05% butylated hydroxy toluene (BHT). The samples are heated at 80 °C for 15 min, cooled and centrifuged at 1000 rpm for 10 min. The absorbances

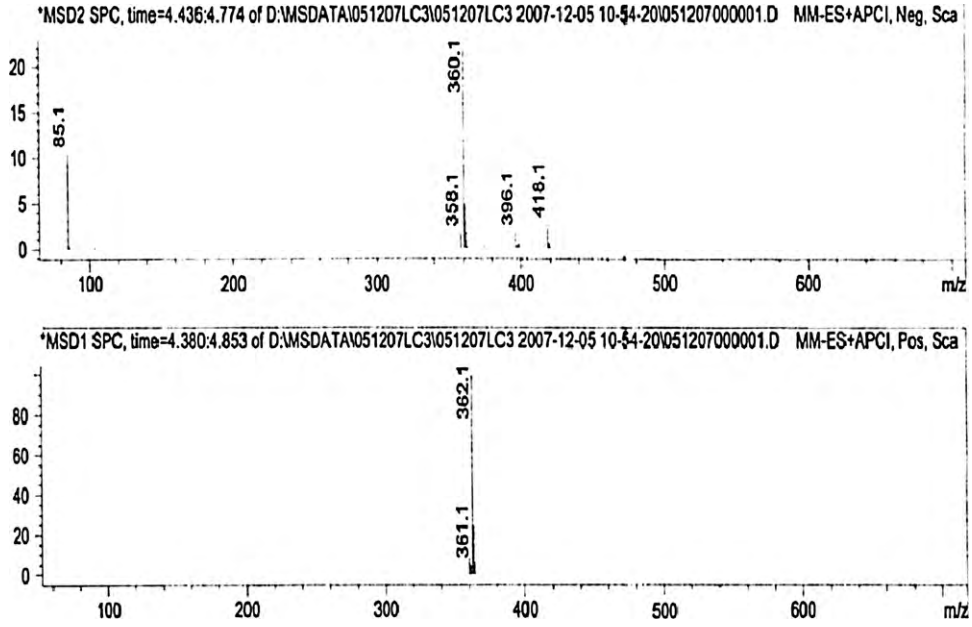


Fig. 1. Mass spectrum of the benzyloxybenzaldehyde-4-phenyl-3-thiosemicarbazone.

of the supernatant solutions are measured at 532 nm. Percentage inhibition of thiobarbituricacid reactive substances (TBARS) formed by test compounds are calculated by comparing with the control. Iron(III) solutions are prepared fresh in distilled water and other solutions in 0.15 M KCl. Since most buffers trap hydroxyl radical or interfere with iron conversion, the reactions are carried out in unbuffered 0.15 M KCl solution [30,31].

The inhibition percentages of the selected ligand and its metal complexes are evaluated using lipid peroxidation method. The following formula is used in calculating inhibition percentages.

$$\text{Inhibition percentage} = \left[\frac{A_{CONT} - A_{TEST}}{A_{CONT}}\right] \times 100$$

where A_{CONT} is the absorbance of the control reaction and A_{TEST} is the absorbance in the presence of the test sample.

3. Results and discussion

3.1. Characterization of benzyloxybenzaldehyde-4-phenyl-3-thiosemicarbazone

Benzyloxybenzaldehyde-4-phenyl-3-thiosemicarbazone is analyzed by IR and ^{1}H NMR spectroscopy. The IR spectrum of ligand exhibits absorption bands around 1543.18 cm^{-1} (C=N), 1253.18 cm^{-1}(C=S) and 3307.85 cm^{-1}(–NH). The ^{1}H NMR (300 MHz, δ (ppm), $CDCl_3$) investigations provide the following information 8.0–7.15 (m, 14H, Ar–H), 11.66 (s, NH), 5.13 (s, N=CH), 3.12 (s, OCH_2). Elemental analysis (Found C 69.78; H 5.30; N 11.63; S 8.87; Calcd: C 69.32; H 5.043; N 11.35; S 8.725%). The mass spectrum of ligand (Fig. 1) shows a molecular ion (M^+) peak at m/z value is 362.1, corresponding to the species $[C_{21}H_{19}N_3OS]^+$, which confirms the proposed formula. Benzyloxybenzaldehyde-4-phenyl-3-thiosemicarbazone is a yellow crystalline solid with a melting point of 150–152 °C.

3.2. Characterization of metal complexes

The complexes are synthesized by reacting benzyloxybenzaldehyde-4-phenyl-3-thiosemicarbazone (L) with the metal ions in 2:1 molar ratio in ethanolic medium. The complexes Cu(II) and Ni(II) are stable in air and insoluble in most of the common organic solvents. Thus the complexes may be formulated as $[M(L)_2X_2]$, where M = Cu(II) or Ni(II).

3.3. Electron paramagnetic spectrum of the Cu(II) complex

The EPR spectrum of the Cu(II) complex recorded at room temperature with polycrystalline sample is shown in Fig. 2. The spectrum of Cu(II) complex at room temperature reveals three sets of resonances at low, mid and high fields corresponding to g_1, g_2 and g_3 respectively. From the peak positions, the g values evaluated are $g_1 = 2.285$, $g_2 = 2.167$ and $g_3 = 2.066$. Hyperfine structure is not resolved. The calculated g values provide valuable information on the electronic ground state of the ion. For g values $g_1 > g_2 > g_3$, if the quantity of R $\{R = (g_2 - g_3)/(g_1 - g_2)\}$ is greater than unity, the ground state is $^2A_1(d_{z^2})$ and R is less than unity, the ground state is

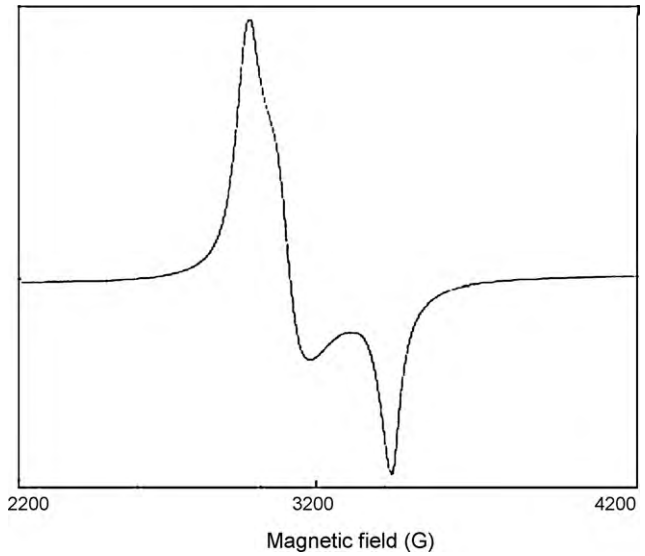


Fig. 2. Powder X-band EPR spectrum of $[Cu(L)_2Cl_2]$ at room temperature (ν = 9.205 GHz).

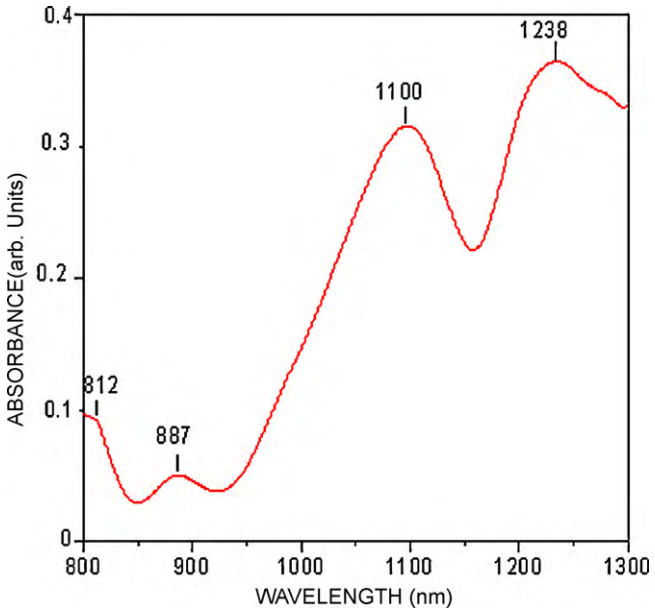


Fig. 3. Solid state electronic spectrum (nm) of $[Cu(L)_2Cl_2]$ complex.

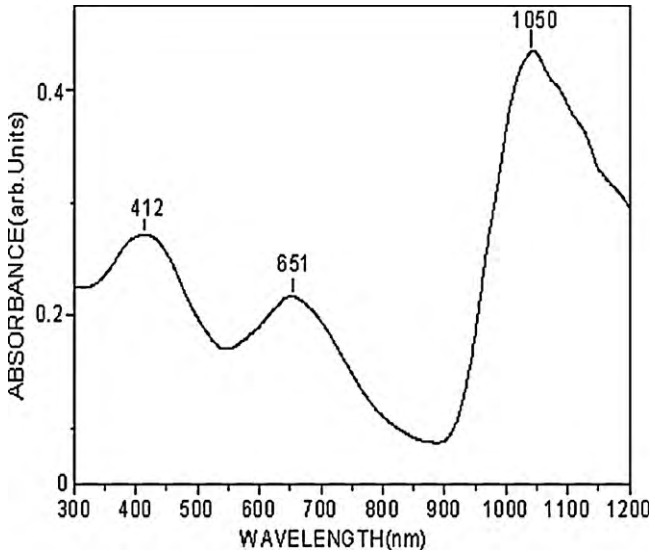


Fig. 4. Solid state electronic spectrum (nm) of $[Ni(L)_2Cl_2]$ complex.

$^2A_1(d_{x^2-y^2})$. The data compare well with those of the other reported rhombic octahedral complexes.

3.4. Electronic spectrum of the Cu(II) complex

The electronic spectrum (optical absorption) of Cu(II) complex displays four bands at 1238 nm, 1100 nm, 887 nm and 812 nm (Fig. 3) which indicates characteristic of rhombic symmetry with the general ordering of the energy levels as $^2A_1(d_{x^2-y^2}) < {}^2A_1(d_{z^2}) < {}^2A_2(d_{xy}) < {}^2B_1(d_{xz}) < {}^2B_2(d_{yz})$. Accordingly the absorption bands observed at room temperature are assigned as follows: 1238 nm (8075 cm^{-1}) to $^2A_1(d_{x^2-y^2}) \rightarrow {}^2A_1(d_{z^2})$, 1100 nm (9088 cm^{-1}) to $^2A_1(d_{x^2-y^2}) \rightarrow {}^2A_2(d_{xy})$, 887 nm (11,271 cm^{-1}) to $^2A_1(d_{x^2-y^2}) \rightarrow {}^2B_1(d_{xz})$ and 812 nm (12,312 cm^{-1}) to $^2A_1(d_{x^2-y^2}) \rightarrow {}^2B_2(d_{yz})$ respectively. These observations are in tune with those reported earlier [32] and the bands are accordingly ascribed to Cu(II) complex in octahedral coordination with rhombic (C_{2v}) distortion.

3.5. Electronic spectrum of the Ni(II) complex

The electronic spectrum of Ni(II) complex displays three absorption bands (Fig. 4) at 412 nm (24,265 cm^{-1}), 651 nm (15,357 cm^{-1}) and 1050 nm (9521 cm^{-1}). All these bands are characteristic of Ni(II) complex in octahedral symmetry. The broad bands at 24,265 cm^{-1} (ν_1), 15,357 cm^{-1} (ν_2) and 9521 cm^{-1} (ν_3) are assigned to the spin allowed transitions from the ground state $^3A_{2g}(F) \rightarrow {}^3T_{1g}(P)$, $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ and $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ states respectively. The positions of the bands indicate that the complex has six coordinated octahedral geometry [33]. The approximate values of D_q = 955 cm^{-1} and B = 720 cm^{-1} give good fit of the experimental and calculated values of band heads. The observed and the calculated band partitions and their assignments are depicted in Table 1.

3.6. Antibacterial activity

The antibacterial screening data show that the ligand (L) does not exhibit antibacterial activity. Ni(II) complex does not inhibit the growth of test organisms. Our results are in agreement with the earlier findings that 6-coordinate paramagnetic Ni(II) complex with two ligands do not inhibit the growth of test organisms[34]. From Table 2, it is clear that Cu(II) metal chelate exhibits effective antibacterial activities. The increased activity of the metal chelate can be explained on the basis of chelation theory. It is known that chelation tends to make the ligand act as more powerful and potent bactericidal agent, killing more of the bacteria than the ligand. It is observed that in a complex, the positive charge of the metal is partially shared with the donor atoms present in the ligands and there may be Π-electron delocalization over the whole chelation. This increases the lipophilic character of the metal chelate and favors its permeation through the lipoid layer of the bacterial membranes. The other factors like solubility; conductivity and bond length between the metal and ligand also increase the activity.

The synthesized ligand and its metal complexes have been screened for reduction in DPPH free radicals and inhibition of iron(III) induced lipid peroxidation at 100 μm concentration. The free ligand shows good activity in DPPH scavenging (42%) and ferric ion induced lipid peroxidation (60%) as seen in the case of standard antioxidant α-tocopherol, but Cu(II) and Ni(II) complexes have not shown any activity against DPPH scavenging and ferric ion induced lipid peroxidation. Relevant data is given in Table 3.

Table 1
Band head data and assignment for Ni(II) in ligand at room temperature.

Transitions from $^3A_{2g}(F)$	Band positions	
	Observed wave number (cm^{-1})	Calculated wave numbers (cm^{-1})
$^3T_{1g}(P)$	24,265	24,310
$^3T_{1g}(F)$	15,357	15,140
$^3T_{2g}(F)$	9521	9550

Table 2
Antibacterial screening data of the ligand and its Ni(II) and Cu(II) complexes (diameter of zone of inhibition in mm).

Compound	*K. pnuemoniae*	*E. coli*	*B. subtilis*	*S. aureus*
Ligand (L)	–	–	–	–
$[Ni(L)_2Cl_2]$	–	–	–	–
$[Cu(L)_2Cl_2]$	28	19	20	24
Ampicillin	43	40	43	42
Tetracycline	32	33	30	32

Table 3
Effect of ligand and its metal complexes on scavenging of DPPH and Fe^{3+} induced lipid peroxidation at 100 μM concentration.

Compound	DPPH scavenging (%)	Fe^{3+} induced lipid peroxidation
Ligand(L)	42	60
$[Ni(L)_2Cl_2]$	–	–
$[Cu(L)_2Cl_2]$	–	–
α-Tocopherol	53	65

4. Conclusions

In this paper coordination chemistry of ligand, obtained from the reaction of benzyloxybenzaldehyde and 4-phenyl-3-thiosemicarbazide is described. Cu(II) and Ni(II) complexes have been synthesized using the ligand and characterized on the basis of analytical and spectral data. The EPR and electronic spectral studies suggested that Cu(II) complex has rhombically distorted octahedron with $^2A_{1g}(d_{x^2-y^2})$ as the ground state, where as N(II) complex exhibits octahedral geometry. The antibacterial activity of the ligand enhanced upon complexation with metal ions particularly for Cu(II), against four bacteria (*B. subtilis*, *S. aureus*, *E. coli* and *K. pneumonia*), but Ni(II) complex did not show antibacterial activity. The synthesized ligand and its metal complexes were screened for reduction of DPPH and inhibition of iron(III) induced lipid per oxidation at 100 μM concentration. Among them, free ligand shows good activity in DPPH scavenging (42%) and ferric ion induced lipid per oxidation (60%) as seen in the case of standard antioxidant α-tocopherol, but Cu(II) and Ni(II) complexes have not show activity against DPPH scavenging and ferric ion induced lipid peroxidation.

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