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Abstract: An iron(III) complex, [Fe(L¹)Cl].H₂O, was synthesized by template condensation reaction of 1,1,1-Trifluoroacetylacetone-S-methylthiosemicarbazone hydrogen iodide (L) and 2,3-dihydroxybenzaldehyde in the presence of iron(III) ions. The complex was characterized by IR, ESI MS and X-ray diffraction techniques. Free radical scavenging (FRS) ability and antioxidant capacity of the S-methylthiosemicarbazone and the iron(III) complex were evaluated through DPPH and CUPRAC methods, respectively. The complex exerted better than the S-methylthiosemicarbazone in both TEAC and FRS% values. In addition, iron(III) complex was found to be 3.1 times more antioxidant than the reference ascorbic acid according to the CUPRAC method.

Keywords: Thiosemicarbazone; iron complex; X-ray analysis; antioxidant activity.


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INTRODUCTION

Iron plays a vital role in adjusting many redox processes essential for cell homeostasis and is also substantial for cellular respiration, oxygen carry, ATP production, heme and DNA synthesis (1,2). Research on iron-based complexes to discover chemotherapeutic ingredients is often preferred in medical chemistry (3). On the other side; thiosemicarbazones are a rising class of compounds that display marked and selective antitumor activity and can overcome resistance to standard chemotherapy (4–6). The metal complexes of thiosemicarbazones show variable binding properties and structural diversity, as well as promising anticancer activities (7–10). Especially, metal complexes of S-alkylthiosemicarbazones exert significant cytotoxic activity against various cancer cells (11–13). For instance, an iron(III) complex with an N₂O₂ donor S-methylthiosemicarbazone showed a significant cytotoxicity on HeLa and HT-29 cells (14). Another iron(III)-S-methylthiosemicarbazone complex with the same donor atom set was cytotoxic in K562 cells at very low concentrations (15).

Free radicals are forms of atoms or molecules with unpaired electrons, which are unsteady and very reactive against chemical reactions (16). Under physiological conditions, specific organelles of the cell produce reactive oxygen species (ROS) as by-products of metabolism, normal respiration, and autoxidation of xenobiotics or as an outcome of stress associated with certain diseases (17,18). Therefore, it is necessary to investigate new antioxidants that can be effective in protecting organisms. Metal complexes are an alternative to the use of well-known antioxidants as they offer benefits such as variation in coordination geometry and number, and oxidation states that facilitate and support the redox processes involved in antioxidant effect (19).

Thiosemicarbazones and their metal complexes often exhibit useful antioxidant activity in vitro (20). In recent years, antioxidant properties of some nickel(II), manganese(III) oxovanadium(IV),
iron(III) and zinc(II) complexes with S-alkylthiosemicarbazones have been reported (21-25). In a study, oxovanadium(IV), nickel(II) and iron(III) complexes of 3-hydroxysalicylaldehyde-S-methylthiosemicarbazone have been tested according to the CUPRAC method and observed that the iron(III) complex displayed a higher TEAC value than the other complexes (26). Another study showed that iron(III) complexes of S-alkylthiosemicarbazones have usable levels of inhibition against reactive oxygen species, $H_2O_2$, $O_2^{-}$ and $\cdot OH$ (27).

To discover new iron(III) derivatives of S-alkylthiosemicarbazones with potent antioxidant activity, S-methylthiosemicarbazone (L) and its iron(III) complex $[Fe(L)\cdot Cl].H_2O$ were synthesized. Since it is generally assumed that the antioxidant activity is related to the number of hydroxyl groups in the phenyl ring (16), it was aimed to increase the antioxidant activity by adding a hydroxyl group to the complex structure. The structural characterization of the complex was performed using elemental analysis, IR, ESI MS and X-ray diffraction techniques. The antioxidant potential of the S-methylthiosemicarbazone and iron(III) complex was screened in the scavenging activity of DPPH• and cupric ions (Cu$^{2+}$) reducing power (CUPRAC) in vitro.

Figure 1: The synthesis of the iron(III) complex.

**EXPERIMENTAL SECTION**

**Physical Measurements**

Thermo Finnigan Flash EA 1112, Agilent Carry 630 FTIR, Varian UNITY INOVA 500 MHz NMR and Thermo Finnigan LCQ Advantage Max LC/MS were used for elemental, IR, NMR and ESI MS analysis, respectively. Magnetic moment measurement was performed using the Gouy technique with Sherwood Scientific’s MK I model device at room temperature.

The crystallographic data for the suitable crystal of $[Fe(L)\cdot Cl].H_2O$ was collected at room temperature with a D8-QUEST diffractometer equipped with graphite-monochromatic Mo-Kα radiation. Following procedures was used for analysis: solved by direct methods; SHELXS-2013 (28); refined by full-matrix least-squares methods; SHELXL-2013 (29); data collection: Bruker APEX2 (30); molecular graphics: MERCURY (31); solution: WinGX (32). The crystallographic data of the complex are listed in Table 1 and selected bond distances and angles, in Table 2.

**Synthesis**

Starting material, 1,1,1-Trifluoro-acetylaceton-S-methylthiosemicarbazone hydrogen iodide (L), was prepared in accordance with a standard procedure reported earlier (33,34). Briefly, 1,1,1-Trifluoroacetylacetone and S-methylthiosemicarbazide hydrogen iodide were reacted in ethanol. The resulting cream colored compound was filtered and dried in vacuo. Elemental analysis and spectroscopic data for the thiosemicarbazone was confirmed its preparation.

Yield: 2.42 g, 65.0%; m.p. (°C): 149; Calc. for C$_7$H$_{11}$N$_3$OSF$_3$I (M=369.14), %: C, 22.78; H, 3.00; N,
The iron(III) complex was synthesized using the literature methods (14,33). 1,1,1-Trifluoroacetylacetone-S-methylthiosemicarbazone hydrogen iodide (0.37 g, 1 mmol) and 2,3-dihydroxybenzaldehyde (0.14 g, 1 mmol) were dissolved in ethanol (10 mL) and the solution was added to a solution of FeCl₃·6H₂O (0.27 g, 1 mmol) in ethanol (5 mL). Et₃N (0.1 mL) was added and the mixture was left to stand at room temperature for 6-8 hours. The solid was filtered off and recrystallized from a mixture of ethanol-dichloromethane (1:3).

Yield: 0.16 g, 35%. M.p. 275 °C. \( \mu_{\text{eff}} (\mu_B) \): 5.89. Anal. Calc. for C₁₆H₂₁ClF₆FeN₂O₁₂S (468.64 g·mol⁻¹): C, 35.88; H, 3.01; N, 8.97; S, 6.84. Found: C, 35.53; H, 2.79; N, 8.71; S, 6.48%. IR: ν(OH) 3477-3442; ν(C=N) 1602; ν(N=C) 1578; ν(N=C) 1530.

\[ \text{m/z ESI MS (relative abundance)}: [M-H}_{\text{O-Cl}}^{+} \text{ClF}_3 \text{FeN}_{\text{2}} \text{O}_{\text{12}} \text{S}^{+} \rightleftharpoons \text{C}_{\text{16}} \text{H}_{\text{21}} \text{ClF}_{\text{6}} \text{FeN}_{\text{2}} \text{O}_{\text{12}} \text{S}^{+} \text{Cl}^{-} = 415.1 \text{ (30.58%)}, 416.1 \text{ (6.51%)}, 417.2 \text{ (2.43%)}, \] 

\[ ([M-H-O-Cl]+\text{CH}_3\text{OH})_\text{100%} \text{ (100%)}], 447.6 \text{ (16.30%)}, 448.5 \text{ (5.99%)}, ([M-H-O-Cl]+\text{CH}_3\text{OH}+\text{SCH}_3)_\text{99.76%} = 492.9 \text{ (46.85%)}, 493.9 \text{ (9.76%)}, 494.8 \text{ (5.93%)}, ([M-H-O]+\text{Na}+\text{CH}_3\text{OH}]_\text{27.52%} = 488.9 \text{ (5.56%)}, 490.9 \text{ (2.71%)}. \]

Antioxidant Tests

1 mL of 1.10⁻⁴ M each compound was added to 2 mL of the DPPH (2,2-diphenyl-1-picrylhydrazyl) solution (4 mg/100 mL) and the final volume was completed to 4 mL using methanol. Reference solution was obtained by adding 2 mL of methanol to 2 mL of DPPH. The mixture was shaken and incubated. After 30 min absorbances were measured at 515 nm (35). The percentage of scavenging activity was calculated from the following equation: Radical scavenging activity(%) = \( (A_{\text{control}} - A_{\text{sample}})/A_{\text{control}} \) × 100.

CUPRAC (Cupric ion reducing antioxidant capacity) method was applied for determining cupric ions reducing potentials of the compounds (36). Briefly, 1 mL of each CuCl₂ (10 mM), neocuproine (NC, 7.5 mM) and NH₄H₂CH₂COO (1 M) solutions were added to the samples at 4.88-24.4 \( \mu \)g concentrations. Each of the volumes was adjusted to 4.1 mL with distilled water and incubated for 30 min. The increasing absorbance at 450 nm indicates the cupric reducing potential of the compounds. Trolox equivalent antioxidant capacities as TEAC values were calculated as the ratio of the molar absorption of each compound to that of trolox \( \left( E_{\text{trolox}}: \right. \) 1.58x10⁻⁴ L mol⁻¹ cm⁻¹).
**Figure 2:** The molecular structure of [Fe(L1)Cl].H2O, showing the atom numbering scheme.

**X-ray Analysis**

The molecular structure of [Fe(L1)Cl].H2O is shown in Figure 2 with an atom numbering scheme. The asymmetric unit of complex [Fe(L1)Cl].H2O contains one Fe(III) ion, one L1 ligand, one coordinated chlorine atom and one non-coordinated water molecule. The Fe(III) ion is coordinated by two oxygen [Fe1-O1= 1.893(2) Å and Fe1-O3= 1.935(3) Å] and two nitrogen atoms [Fe1-N1= 2.083(3) Å and Fe1-N3= 2.075(3) Å] from the thiosemicarbazidato structure and chlorine atom [Fe1-Cl1= 2.2114(13) Å]. The tau value, \[ \tau = (\beta-\alpha)/60, \alpha \text{ and } \beta \text{ being the two largest angles around the central atom} \], can be usefully used to estimate the degree of distortion from a square pyramidal to trigonal bipyramid structures. For an ideal square pyramidal geometry, the \( \tau \) value is equal to zero, while it becomes one for a perfect trigonal bipyramidal geometry (39). The value of \( \tau \) for the iron(III) ion is 0.09, indicating a distorted square pyramid. The molecules of [Fe(L1)Cl].H2O are connected by O-H⋯O and O-H⋯F hydrogen bonds (Table 3). The O4 atom of water molecule at (x, y, z) acts as a hydrogen-bond donor, via H4A atom, to O3i and F3ii atoms, forming a R1i(5) ring. Similarly, the O4 atom at (x, y, z) acts as a hydrogen-bond donor, via H4B atom, to O1i and O2i atoms, forming a R1ii(5) ring. The combination of hydrogen bonds produces edge-fused R1ii(5), R1ii(6) and R1ii(8) rings (Figure 3).

**Table 1:** Crystal data and structure refinement parameters for [Fe(L1)Cl].H2O.

<table>
<thead>
<tr>
<th></th>
<th>CCDC: 2132527</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Empirical formula</strong></td>
<td>C14H14ClF3FeN3O4S</td>
</tr>
<tr>
<td><strong>Formula weight</strong></td>
<td>468.64</td>
</tr>
<tr>
<td><strong>Space group</strong></td>
<td>P-1</td>
</tr>
<tr>
<td><strong>Crystal system</strong></td>
<td>Triclinic</td>
</tr>
<tr>
<td><strong>a (Å)</strong></td>
<td>7.928 (3)</td>
</tr>
<tr>
<td><strong>b (Å)</strong></td>
<td>8.744 (3)</td>
</tr>
<tr>
<td><strong>c (Å)</strong></td>
<td>14.269 (5)</td>
</tr>
<tr>
<td><strong>α (°)</strong></td>
<td>97.039 (11)</td>
</tr>
<tr>
<td><strong>β (°)</strong></td>
<td>94.454 (11)</td>
</tr>
<tr>
<td><strong>γ (°)</strong></td>
<td>109.047 (9)</td>
</tr>
<tr>
<td><strong>Dc (g cm(^{-3}))</strong></td>
<td>1.691</td>
</tr>
<tr>
<td><strong>V (Å(^3))</strong></td>
<td>920.6 (6)</td>
</tr>
<tr>
<td><strong>µ (mm(^{-1}))</strong></td>
<td>1.13</td>
</tr>
<tr>
<td><strong>Z</strong></td>
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</tr>
<tr>
<td><strong>Independent refls.</strong></td>
<td>4578</td>
</tr>
<tr>
<td><strong>Measured refls.</strong></td>
<td>35138</td>
</tr>
<tr>
<td><strong>R</strong></td>
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</tr>
<tr>
<td><strong>R_{int}</strong></td>
<td>0.047</td>
</tr>
<tr>
<td><strong>R1/WR2</strong></td>
<td>0.055/0.143</td>
</tr>
<tr>
<td><strong>T_{max}/T_{min}</strong></td>
<td>1.03/-0.43</td>
</tr>
</tbody>
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Table 2: Selected bond distances (Å) and angles (°) for [Fe(L¹)Cl].H₂O.

<table>
<thead>
<tr>
<th>Bond</th>
<th>Distance (Å)</th>
</tr>
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<tbody>
<tr>
<td>Fe1-O1</td>
<td>1.893(2)</td>
</tr>
<tr>
<td>Fe1-N3</td>
<td>2.075(3)</td>
</tr>
<tr>
<td>Fe1-Cl1</td>
<td>2.2114(13)</td>
</tr>
<tr>
<td>O1-Fe1-O3</td>
<td>92.96(11)</td>
</tr>
<tr>
<td>O3-Fe1-N3</td>
<td>87.52(12)</td>
</tr>
<tr>
<td>O3-Fe1-N1</td>
<td>143.46(12)</td>
</tr>
<tr>
<td>O1-Fe1-Cl1</td>
<td>106.66(9)</td>
</tr>
<tr>
<td>N3-Fe1-Cl1</td>
<td>102.24(9)</td>
</tr>
</tbody>
</table>

Figure 3: Crystal structure of [Fe(L¹)Cl].H₂O, showing the formation of R₁²(5), R₂²(6) and R₄⁴(8) rings.

Table 3: Hydrogen-bond parameters for [Fe(L¹)Cl].H₂O (Å, °).

<table>
<thead>
<tr>
<th>D-H···A</th>
<th>D-H</th>
<th>H···A</th>
<th>D···A</th>
<th>D-H···A</th>
</tr>
</thead>
<tbody>
<tr>
<td>O2—H2···O4¹</td>
<td>0.82</td>
<td>1.94</td>
<td>2.740 (4)</td>
<td>164</td>
</tr>
<tr>
<td>O4—H4A···F3ii</td>
<td>0.79  (7)</td>
<td>2.52  (7)</td>
<td>3.292 (6)</td>
<td>166</td>
</tr>
<tr>
<td>O4—H4A···O3³</td>
<td>0.79  (7)</td>
<td>2.57  (7)</td>
<td>3.136 (4)</td>
<td>130</td>
</tr>
<tr>
<td>O4—H4B···O1¹</td>
<td>0.85  (7)</td>
<td>2.28  (7)</td>
<td>2.978 (4)</td>
<td>140</td>
</tr>
<tr>
<td>O4—H4B···O2¹</td>
<td>0.85  (7)</td>
<td>2.25  (7)</td>
<td>3.009 (5)</td>
<td>149</td>
</tr>
</tbody>
</table>

Symmetry codes: (i) x−1, y−1, z; (ii) −x+1, −y+1, −z+1.

Antioxidant Properties
The DPPH radical is stable and commonly used for specifying the capability of an antioxidant agent (40). The result reveals that free radical (DPPH) scavenging activity follows the order: ascorbic acid > [Fe(L¹)Cl].H₂O > L (Table 4). According to this order, the iron complex is a more active radical scavenger than the free thiosemicarbazone (L). This might be due to the phenolic hydroxyl group in the complex structure. Based on the literature data, it was confirmed that the radical scavenging activities of the compounds were quite controlled by the number of phenolic hydroxyl groups (41,42). Among synthetic antioxidants, TBHQ with two hydroxyl groups has been shown to be a more potent antioxidant than BHA and BHT with one hydroxyl group (16). In a study, the DPPH assay showed that the ruthenium thiosemicarbazone complex is a more potent scavenger than the nickel thiosemicarbazone complex because it has two free hydroxyl groups (43). It was also reported that zinc(II) complexes containing four free hydroxyl groups showed remarkable scavenging activity (44).
The CUPRAC method is based on the ability of a compound to reduce a $[\text{Cu(II)-Nc}]$ complex to a $[\text{Cu(I)-Nc}]$ complex. The TEAC coefficients are given in Table 5. According to the results, the antioxidant capacity of both iron(III) complex and free thiosemicarbazone was superior to standard ascorbic acid. Especially, the TEAC value of the iron(III) complex was found to be 3.1 times higher than the value of ascorbic acid. Similar to the antiradical activity, the antioxidant capacity obtained for the iron(III) complex was also higher than for the free thiosemicarbazone.

The literature, the antioxidant activity of the metal complexes was found to be higher (45,46) or lower (47,48) than the corresponding free ligands. The CUPRAC assay demonstrated that Zn(II), Co(II), Cu(II) and Ni(II) complexes of a chicoric acid ligand increased antioxidant properties compared to their free ligands (49). In another study, the antioxidant activity of the Zn(II) complex of chlorogenic acid ligand was tested and it was found that coordination with the metal nucleus increases the ability to reduce the ligand by about 1.5 times (50).

**Table 4**: The free radical scavenging (FRS) activity as inhibition ratio %.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>FRS activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>L</td>
<td>39.65±2.43</td>
</tr>
<tr>
<td>$[\text{Fe(L')}\text{Cl}]\text{H}_2\text{O}$</td>
<td>66.77±1.44</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>98.75±1.45</td>
</tr>
</tbody>
</table>

CONCLUSION

The new iron(III) complex with S-methylthiosemicarbazone was synthesized and structurally confirmed. The crystal analysis showed that the complex had 6,5,6-membered chelate rings formed by coordination of the two oxygen and nitrogen atoms on the thiosemicarbazidato structure to the iron(III). The fifth coordination was completed with a chlorine atom, and coordination geometry around iron(III) could be defined as a distorted square pyramid.

The cupric ions ($\text{Cu}^{2+}$) reducing power (CUPRAC) and DPPH• scavenging activity of the synthesized S-methylthiosemicarbazone and iron(III) complex were studied. TEAC values showed that the iron(III) complex had a higher antioxidant capacity than the free thiosemicarbazone and ascorbic acid, and its DPPH radical scavenging ability was higher than that of the free thiosemicarbazone. The superior antioxidant properties of the iron(III) complex might be due to the presence of the hydroxyl group in the phenyl ring. Consequently, the complex with potent antioxidant activity is a promising component for research related to oxidative stress-induced diseases and likely cancers.

**CONFLICT OF INTEREST**

The author declares no conflict of interest.

**ACKNOWLEDGMENTS**

I dedicate this paper to Dr. Bahri ÜLKÜSEVEN, the mentor of my research career. Also, I gratefully acknowledge Dr. Onur ŞAHİN for X-ray diffraction studies and Scientific and Technological Research Application and Research Center, Sinop University, Turkey, for the use of the Bruker D8 QUEST diffractometer.

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SUPPLEMENTARY INFORMATION

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Figure S1: ESI MS of L in MeOH.
Figure S2: ESI MS of [Fe(L₁)Cl].H₂O in MeOH.

Figure S3: IR spectrum of L.
**Figure S4:** IR spectrum of [Fe(L¹)Cl].H₂O.

**Figure S5:** ¹H NMR spectrum of L in DMSO-d₆.