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Preparation, Spectral Characterization and Antioxidant Activities of Aminothiophene-Containing Schiff Base and Co(II) and Pd(II) Complexes

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ABSTRACT

This research focused on synthesizing a Schiff base (L) and its Co(II) and Pd(II) complexes under appropriate conditions to contribute to enhancing antioxidant activity. The ligand was synthesized by reacting methyl 2-amino-4-ethyl-5-methylthiophene-3-carboxylate with 5-bromo-2-hydroxybenzaldehyde. Various analytical techniques were employed to characterize the ligand and its resulting complexes, including microanalysis, ¹H and ¹³C NMR spectroscopy, FTIR, electronic spectra, mass spectrometry, magnetic susceptibility, molar conductance, and thermo-gravimetric analysis. Electronic spectra and magnetic moment values were used to confirm the geometric structures of the synthesized compounds. Thermo-gravimetric analyses (TGA-DTA) were conducted to evaluate the thermal stability of the complexes. The Pd(II) complex was found to have a square planar structure, while a tetrahedral structure was suggested for the Co(II) complex. The results from the elemental analysis were consistent with both the ligand and metal complexes, showing good agreement between calculated and experimental values. The *in vitro* antioxidant activities of the newly synthesized compounds were examined using different antioxidant methods. Experiment results exhibited that the Pd(II) complex had better activities than the Schiff base and Co(II) complex. According to ABTS experimental results, Pd(II) complex (IC₅₀:1.25) showed similar activity to the standards. In contrast, CUPRAC results revealed that Pd(II) complex (7.55 µg TE/mL) showed better activity than the standards. The results indicate that Pd(II) complex has strong potential as effective antioxidants, making them valuable for food and medicine applications.

1 | Introduction

Schiff bases and their metal complexes are essential in medical and biological applications. The activity properties of these complexes are greatly influenced by the type of Schiff base and the metal involved. Metal complexes of Schiff bases often demonstrate superior drug efficacy compared to organic complexes. The biologically active azomethine group makes Schiff

bases excellent metal coordinated ligands [1, 2]. This group consists of an electron-deficient carbon and an electron-rich nitrogen, allowing the complex to undergo various electrophilic and nucleophilic reactions at these centers [3, 4]. Schiff bases are fundamental in various fields, including biological, organic, analytical, and inorganic industries, owing to their multiple properties such as chelation, chemical sensing, structure modification, and reactivity [5–8].

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Coordination complexes, a significant class of inorganic compounds, are vital in medicinal chemistry due to their numerous attributes such as dentality, flexibility, and reactivity. Recently, there has been increasing interest in transition metal coordination compounds. These compounds are integral to a variety of biological processes and are present in electron-rich molecules like proteins, nucleic acids, enzymes, and carbohydrates. Research in bio-inorganic chemistry highlights the significance of transition metal compounds that utilize Schiff bases [9–12].

The efficacy of Schiff bases has been observed to increase after complexation with transition metal ions due to factors like the behavior of bound groups, solubility, cell permeability, and enzymatic effects [13]. Thus, studying transition metal complexes with Schiff base ligands is of interest in medicine, as the pharmaceutical industry's widespread use of bioactive compounds has greatly stimulated the utilization of transition metal complexes by confirming the binding of both organic ligands and inorganic metal ions [14, 15]. In addition, the structural, electronic and steric properties of the synthesized complexes can be tailored, making Schiff bases excellent ligands for the preparation of a wide range of coordination compounds. This flexibility in ligand design enables the customization of the properties and functions of the resulting compounds, broadening their potential uses across different areas of chemistry and materials science [16–18].

Recently, many researchers have been investigating the synthesis and properties of metal complexes with Schiff bases to evaluate their potential applications in both pharmaceutical and industrial fields. Transition metal ions are crucial for enzyme activity in the human body, and Schiff base ligands interact with these ions, thereby enhancing their medicinal properties. The increased biological activity of these complexes is attributed to the partial sharing of the metal ion's positive charge with the donor group, which reduces the metal's polarity through the chelation process. Current research has focused on synthesizing and applying Schiff base ligand complexes with transition metals (Co(II, III), Ni(II), Cu(II), Pd(II), Pt(II), Ru(II, III), V(II), Cd(II), Zn(II), Mn(II, III) e.g.) [19–22].

Consequently, these Schiff base metal complexes are extensively studied in the medical industry for their significant biological applications, including anticancer, antifungal, antiradical, antioxidant [23], antibacterial, antiviral, antituberculosis, anti-inflammatory, anti-HIV, antimalarial, and antiparasitic properties [10, 24–26].

Studies have indicated that excessive accumulation of free radicals can lead to oxidative damage, potentially causing various diseases [27, 28]. Consequently, research focused on synthesizing exogenous antioxidants, which play a crucial role in antioxidant defense, is growing rapidly. The synthesis of Schiff bases and metal complexes, which represent a significant position among exogenous antioxidants, continues to contribute new compounds to the scientific literature [29, 30].

Aminothiophene derivatives are used as building blocks and synthetic intermediates in organic synthesis. Today, aminothiophene analogues (e.g., 2-*N*-substituted thiophenes) are of great interest, especially due to their biological and

pharmacological properties. Known biological effects of aminothiophene's include anticancer, antiviral, antimicrobial and antitubercular activities, allosteric ligands for adenosine A₁ receptors, antioxidants and various favorable effects on neurological diseases. Many thiophene- or aminothiophene-containing drugs and drug candidates showing promising pharmacological effects in early preclinical studies have once again confirmed the advantageous potential of the aminothiophene skeletal structure; therefore, aminothiophene derivatives remain an active area of research and clinical development [31–33].

Schiff base ligands and their metal complexes play an important role in the synthesis and development of new drugs, considering their promising biological potential. In this study, methyl 2-amino-4-ethyl-5-methylthiophene-3-carboxylate was synthesized using the Gewald method [34]. This compound was then reacted with 5-bromo-2-hydroxybenzaldehyde to form the Schiff base, methyl 2-(5-bromo-2-hydroxybenzylideneamino)-4-ethyl-5-methylthiophene-3-carboxylate (L). Transition metal complexes were subsequently synthesized using CoCl₂·6H₂O and PdCl₂(CH₃CN)₂. Various spectroscopic and analytical techniques were used to characterize the synthesized compounds and confirm their structure formation, including microanalysis (CHNS), ¹H NMR, ¹³C NMR, FT-IR, UV-visible, thermo-gravimetric analysis, mass spectrometry, magnetic susceptibility and molar conductivity measurements. The antioxidant activities of these complexes were also evaluated using several methods, such as DPPH, ABTS, CUPRAC, and FRAP.

2 | Experimental

2.1 | Materials and Physical Measurements

The synthesis procedures were conducted in an inert argon environment using anhydrous solvents. High-purity solvents and reagents were utilized, including 5-bromo-2-hydroxybenzaldehyde, glacial acetic acid, CoCl₂·6H₂O, PdCl₂(CH₃CN)₂, ethanol, toluene, DMSO, DMF, chloroform, acetone, methanol, hexane, water, and diethyl ether, which were used as commercially supplied. Thin layer chromatography (TLC) was employed to verify the purity of newly synthesized compounds.

Microanalysis was obtained on a CHNS-932 LECO instrument, and a mass spectrum was carried out using an Agilent 1100 MSD spectrophotometer. The FT-IR spectra were performed as KBr pellets within the 4000–400 cm⁻¹ range using a Perkin Elmer Spectrum 65 FT-IR spectrometer. Electronic spectra were obtained in the 200–800 nm range on a Shimadzu UV-1800 spectrophotometer. ¹H and ¹³C NMR spectra were performed in DMSO-*d*₆ solution using Bruker's high-performance Avance III NMR spectrometer 400 MHz, and thermo-gravimetric analysis were measured with a Shimadzu DAT/TG-50 model thermal analyzer with platinum crucibles with a heating rate of 10°C/min under nitrogen atmosphere. The magnetic susceptibility of the complexes was measured using Guoy's method with a vibrating sample magnetometer and Hg[Co(SCN)₄] as the calibrant at room temperature. Molar conductivity of the complexes was measured with Jenway 4010 conductivity meter at 25°C.

2.2 | Synthesis

2.2.1 | Synthesis of Methyl 2-(5-Bromo-2-Hydroxybenzylideneamino)-4-Ethyl-5-Methylthiophene-3-Carboxylate (L)

A solution of 0.80 g (4.01 mmol) of amine compound in 40 mL of ethanol was placed in a 100 mL round-bottom flask and stirred. To this, 0.81 g (4.01 mmol) of 5-bromo-2-hydroxybenzaldehyde in 20 mL of ethanol was gradually added. Additionally, 5–6 drops of glacial acetic acid were introduced to the mixture. The reaction was heated under reflux, and progress was monitored using TLC. After cooling the reaction mixture, colored precipitates were obtained, filtered, washed with diethyl ether and purified by recrystallization from a chloroform/methanol solution. The synthetic pathway for the preparation of the Schiff base ligand (L) is illustrated in Scheme 1.

Yield: 85% (0.68 g); Color: Dirty yellow; m.p. 210°C–212°C. Anal. Calc. for $C_{16}H_{16}BrNO_3S$ (382.27 g/mol): C, 50.22; H, 4.18; N, 3.66; S, 8.37%. Found: C, 50.44; H, 4.20; N, 3.70; S, 8.38%. FT-IR (KBr, ν , cm^{-1}): 3438, 3316 (OH)br, 3080 (-CH, Ar.), 2966, 2907 (-CH, Alip.), 1617 (C=O), 1596 (CH=N), 1597, 1546 (C=C, Ar.), 1184 (C-O), 733 (C-S-C). 1H -NMR (400 MHz, DMSO- d_6 , δ , ppm): 10.27 (s, H, OH), 8.86 (s, H, CH=N), 7.99 (s, H, -CH, Ar.), 7.64–7.62 (d, 2H, -CH, Ar.), 7.03–7.00 (d, 2H, -CH, Ar.), 3.59 (s, 3H, -OCH₃), 2.57 (q, 2H, -CH₂), 1.46 (s, 3H, CH₃), 1.20 (t, 3H, -CH₃). ^{13}C -NMR (100 MHz, DMSO- d_6 , δ , ppm): 160.01 (C=O), 158.05 (CH=N), 141.81–106.54 (thionyl and benzen rings), 52.69 (OCH₃), 37.25 (-CH₂CH₃), 34.16 (CH₂CH₃), 29.82 (CHCH₃). UV-Vis. λ_{max} , nm (ϵ in parenthesis, $M^{-1} cm^{-1}$): 196 (1393), 204 (150), 219 (246), 245 (115), 287 (50), 337 (65), 348 (69), 399 (112). MS [ESI⁺]: m/z 383.27 (Calc.), 383.30 (Found) [M + H]⁺.

2.2.2 | Synthesis and Characterization of Co(II) and Pd(II) Complexes

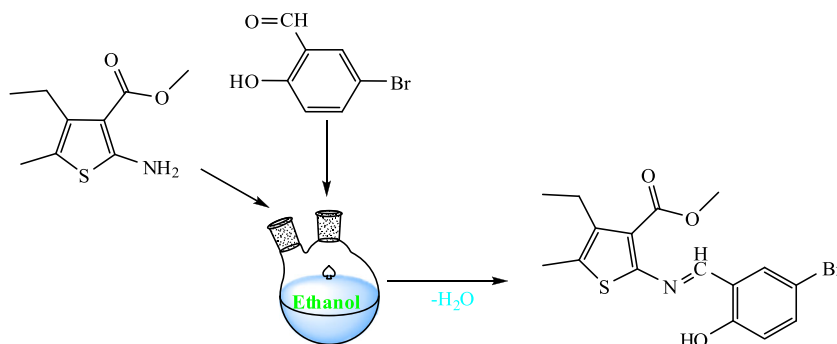
A solution of $CoCl_2 \cdot 6H_2O$ (0.37 g, 1.57 mmol) in 15 mL of ethanol was added slowly, with constant stirring, to a 25 mL ethanol solution containing the Schiff base (0.60 g, 1.57 mmol). The resulting mixture underwent reflux heating and stirring for an appropriate duration, then was left undisturbed overnight. The resulting-colored precipitates were collected, washed

several times with diethyl ether, filtered, recrystallized from a chloroform/methanol mixture and dried, and then used for spectral analysis.

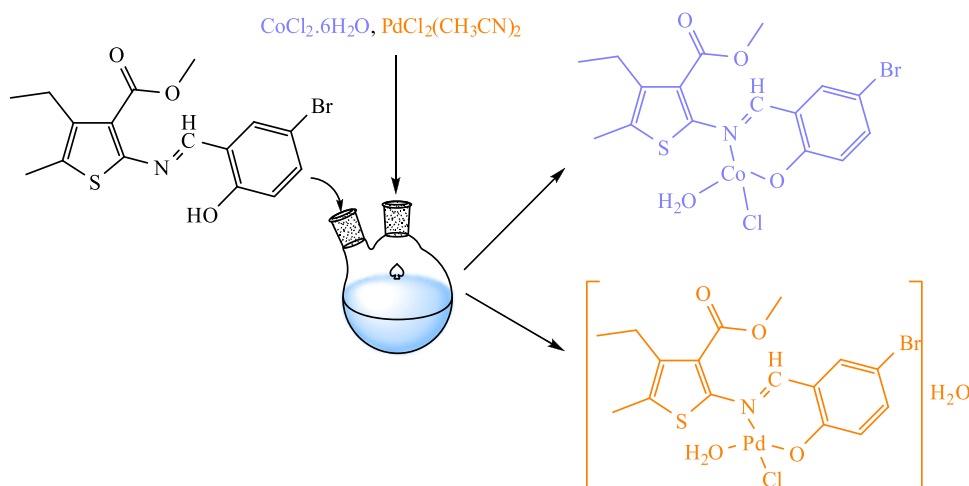
[LCoCl \cdot H₂O]: Yield: 84% (0.31 g); Color: Light brown; m.p. 254°C–256°C. Anal. Calc. for $C_{16}H_{17}NO_4SBrCoCl$ (493.50 g/mol.): C, 38.93; H, 3.44; N, 2.83; S, 6.48%. Found: C, 38.97; H, 3.46; N, 2.86; S, 6.53%. FT-IR (KBr, ν , cm^{-1}): 3553 (OH/H₂O), 3032 (-CH, Ar.), 2972, 2903 (-CH, Alip.), 1617 (C=O), 1602 (CH=N), 1592, 1560 (C=C, Ar.), 1175 (C-O), 828 (H₂O), 734 (C-S-C), 573, 550 (M-O), 503, 457 (M-N). UV-Vis. λ_{max} , nm (ϵ in parenthesis, $M^{-1} cm^{-1}$): 210 (1928), 218 (1840), 265 (1740), 287 (878), 340 (906), 359 (1053), 398 (1508), 550 (137), 1025 (128). MS [ESI⁺]: m/z 491.50 (Calc.), 491.66 (Found) [M-2H]⁻². μ_{eff} (B.M.): 4.50. Molar conduct. (10^{-3} M, DMF): $13 \Omega^{-1} cm^2 mol^{-1}$.

Schiff base (0.60 g, 1.57 mmol) and $PdCl_2(CH_3CN)_2$ (0.40 g, 1.57 mmol) were mixed in equal proportions and dissolved in 40 mL of dry toluene in inert atmosphere. The solution was refluxed for 8 h, and the reaction was monitored by TLC. When the reaction was confirmed to be complete, the colored mixture was removed from the solvent under vacuum. The resulting-colored precipitates were collected, washed several times with diethyl ether, filtered, recrystallized from a chloroform/methanol mixture and dried, and then used for spectral analysis. The synthesis of the ligand and its Co(II) and Pd(II) metal complexes is illustrated in Scheme 2.

[LPdCl \cdot H₂O] \cdot H₂O: Yield: 78% (0.31 g); Color: Brown; m.p. 271°C–273°C. Anal. Calc. for $C_{16}H_{19}NO_5SPdBrCl$ (559.01 g/mol): C, 34.37; H, 3.39; N, 2.50; S, 5.73%. Found: C, 34.44; H, 3.40; N, 2.55; S, 5.75%. FT-IR (KBr, ν , cm^{-1}): 3445 (OH/H₂O)br, 3058 (-CH, Ar.), 2979 (-CH, Alip.), 1621 (C=O), 1602 (CH=N), 1585, 1541, 1508 (C=C, Ar.), 1164 (C-O), 837 (H₂O), 735 (C-S-C), 560, 539 (M-O), 489, 459 (M-N). 1H -NMR (400 MHz, DMSO- d_6 , δ , ppm): 8.90 (s, H, CH=N), 8.13–6.90 (m, 3H, -CH, Ar.), 4.13 (s, 3H, -OCH₃), 2.79 (q, 2H, -CH₂), 1.42 (t, 3H, CH₃), 1.69 (s, 3H, -CH₃). ^{13}C -NMR (100 MHz, DMSO- d_6 , δ , ppm): 162.89 (C=O), 159.32 (CH=N), 140.01–105.25 (thionyl and benzene ring), 55.57 (OCH₃), 36.46 (-CH₂CH₃), 27.45 (CH₂CH₃), 21.57 (CHCH₃). UV-Vis. λ_{max} , nm (ϵ in parenthesis, $M^{-1} cm^{-1}$): 211 (2451), 220 (2034), 228 (2070), 241 (2158), 298 (1319), 301 (1315), 420 (480), 480 (563). MS [ESI⁺]: m/z 561.01 (Calc.), 561.60 (Found) [M + 2H]⁺². μ_{eff} (B.M.): Dia. Molar conduct. (10^{-3} M, DMF): $17 \Omega^{-1} cm^2 mol^{-1}$.



SCHEME 1 | Synthesis of Schiff base ligand (L).



SCHEME 2 | Synthesis scheme of metal complexes.

2.3 | Reducing Power of Fe^{+3} Ions

The Oyaizu method [35] was employed to assess the capacity of Fe^{+3} ions to be reduced to Fe^{+2} ions. Samples of varying concentrations (25, 50, and 100 $\mu\text{g}/\text{mL}$) were placed in tubes and brought to a total volume of 200 μL using distilled water. Subsequently, 500 μL each of buffer solution (pH: 6.6) and $\text{K}_3\text{Fe}(\text{CN})_6$ were added to each tube, followed by a 20-min incubation period at 50°C. The reaction was then halted by introducing 500 μL of trichloroacetic acid (TCA). After centrifugation, 500 μL of the resulting supernatant was extracted and combined with an equal volume of distilled water and 100 μL of FeCl_3 . The absorbance values were then measured at 700 nm using a micro-volume spectrophotometer.

2.4 | Reducing Power of Cu^{+2} Ions

The CUPRAC method was employed to assess the reducing abilities of Cu^{+2} ions. Various concentrations of compounds and standards were placed in test tubes and diluted to 1 mL with distilled water. Equal volumes (0.25 mL) of CuCl_2 , ethanolic neocuprin, and acetate buffer were then added to each tube. Following a 30-min incubation period, the samples' absorbance was measured at 450 nm [36].

2.5 | ABTS Radical Scavenging Assay

The ABTS solution was diluted using phosphate buffer until its absorbance reached 0.750 ± 0.025 at 734 nm. The samples were then brought to a total volume of 200 mL with water, followed by the addition of 1 mL of ABTS^{+} solution. The mixtures were incubated for 30 min, after which the absorbance of the extracts was measured at 734 nm [37].

2.6 | DPPH Radical Scavenging Assay

The samples (25, 50 and 100 $\mu\text{g}/\mu\text{L}$) were combined with ethanol in test tubes to reach a total volume of 600 μL . Subsequently, 200 μL of DPPH solution was introduced, and the

mixture was left to react for approximately 30 min to ensure completion. A spectrophotometer was utilized to measure the absorbance values at 517 nm [38].

3 | Result and Discussion

The synthesis of Co(II) and Pd(II) complexes was achieved by combining the ligand with the appropriate metal salt in equal proportions, resulting in yields of 84% and 78% respectively. The proposed structures of all synthesized compounds were confirmed by spectral and analytical data. The low molar conductivity values of the metal complexes indicate their non-electrolytic nature [39, 40].

The FT-IR spectrum of the synthesized Schiff base showed no aldehyde group ($-\text{CHO}$) signals near $2700\text{--}2600\text{ cm}^{-1}$, which are typically present in the starting material. Additionally, the amino group ($-\text{NH}_2$) stretching bands were absent around 3200 cm^{-1} , supporting the successful completion of the Schiff base reaction. The FT-IR spectrum of the Schiff base displayed a distinct azomethine stretching band at 1596 cm^{-1} . In the FT-IR spectra of the complexes, this band shifted to higher wavenumbers, indicating that the imine group was coordinated with the metal ions [41]. In the ligand's FT-IR spectrum, the phenolic OH band observed at 3438 and 3316 cm^{-1} vanished, while a C-O stretching absorption band appeared at 1184 cm^{-1} . This band shifted to 1175 and 1164 cm^{-1} in the Co(II) and Pd(II) complexes spectra, respectively [42], confirming successful C-O group coordination with metal ions [43]. The involvement of phenolic oxygen in coordination was further verified through ^1H NMR spectroscopy. The proposed coordination sites were further supported by the appearance of new bands in the $573\text{--}539$ and $503\text{--}457\text{ cm}^{-1}$ regions, corresponding to M-O and M-N stretching vibrations. The C=O and C-S-C bond frequencies at 1617 and 733 cm^{-1} remained largely unchanged in the metal complexes, suggesting that these groups did not participate in metal ion coordination [44, 45]. Concurrently, stretching vibrations at $3553\text{--}3455\text{ cm}^{-1}$ and 837 and 828 cm^{-1} suggested the presence of H_2O molecules in the complex structures [46]. The distinct rocking frequencies at 837 cm^{-1} and 828 cm^{-1} further suggest the coordination of water molecules, which was corroborated by thermal studies.

The Schiff base and Pd(II) complex NMR spectra (^1H and ^{13}C) aligned well with their proposed structures. DMSO- d_6 was used to obtain the ^1H NMR spectra of the ligand and its Pd(II) complex (Figures S1–S4). In the ^1H -NMR spectrum of the ligand, the proton signals for the azomethine ($-\text{CH}=\text{N}$) group appeared at δ 8.86 ppm, while the proton signals for the phenolic ($-\text{OH}$) group were observed at 10.27 ppm [47]. In the ^1H -NMR spectrum of the Pd(II) complex, the azomethine signals shifted slightly to 8.90 ppm, and the phenolic ($-\text{OH}$) proton signals were absent. This indicates that the metal binding occurs through the azomethine nitrogen and the phenolic oxygen. Aromatic protons were found between 7.99 and 7.00 ppm for the ligand and 8.13–6.90 ppm for the complex, as anticipated. In ^{13}C NMR spectra, azomethine carbon ($\text{CH}=\text{N}$) in the ligand appeared as singlets at 158.05 ppm, shifting to 159.32 ppm in the Pd(II) complex spectra. This shift supports azomethine nitrogen coordination to the metal center [48]. The C-OH signals in the Schiff base ligand appeared between 141.81 and 106.54 ppm and experienced a shift upon complex formation, indicating successful complexation through deprotonation of the phenol group. Due to their paramagnetic properties, ^1H and ^{13}C NMR spectra of Co(II) complex were not obtained.

Electronic absorption spectra of the compounds were performed in 10^{-5}M DMF. The ligand's UV-Vis spectra showed intense peaks at 196–287 nm, ascribed to $\pi \rightarrow \pi^*$ transitions from benzene and thiophene ring aromatic structures. Absorption bands at 337–399 nm were assigned to $n \rightarrow \pi^*$ transitions of azomethine and phenolic oxygen structures. Upon coordination with metal ions, both the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions for the ligand were shifted to higher wavelengths, confirming the formation of the Schiff base metal complexes. In the Co(II) complex, peaks between 210 and 287 nm signify $\pi \rightarrow \pi^*$ transitions, while those between 340 and 398 nm indicate $n \rightarrow \pi^*$ transitions. The 550 nm peak in the cobalt complex results from d-d transitions. Electronic spectra of Pd(II) complex show $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transition peaks at 211–298 nm and 301–420 nm, respectively. A peak at 480 nm suggests MLCT (metal-to-ligand charge transfer transitions), strongly supporting a square planar geometry around the central metal ion [49].

The thermal decompositions of the metal complexes, along with the decomposition stages and the percentage of mass loss at each step, are illustrated in the TGA-DTA thermograms (Figures S14 and S15). Table 1 presents the thermal analysis outcomes for the complexes. Examination of the $[\text{LCoCl}\cdot\text{H}_2\text{O}]$ complex's thermal analysis spectrum reveals a three-stage decomposition process. The initial stage showed no mass loss between 50°C and 200°C , indicating the absence of hydration water. The second stage demonstrated the separation of one mole of coordination water between 230°C and 300°C . The third and fourth stages exhibited the separation of the substance's organic components, with decomposition continuing beyond 725°C . The $[\text{LPdCl}]\text{H}_2\text{O}$ complex's thermal analysis spectrum displayed a two-stage decomposition. The first stage involved the separation of one mole of hydrate water, resulting in a 3.22% mass loss (3.60% found) between 50°C and 200°C . Following a stable period up to 300°C , the second stage between 300°C and 700°C showed the separation of the $\text{C}_6\text{H}_{13}\text{O}_3\text{BrCl}$ group from the structure, with decomposition persisting after 700°C .

Analysis of the ligand complexes' magnetic susceptibility values revealed diamagnetic properties for the Pd(II) complex and paramagnetic properties for the Co(II) complex. The Co(II) complex's magnetic moment was measured at 4.50 B.M., approximating the calculated 3.87 B.M. value for d^7 metal ions with three unpaired electrons. This value indicates sp^3 hybridization and tetrahedral geometry for the Co(II) complex [50]. The Pd(II) complex exhibited square planar geometry [51].

Mass spectra results for the ligand's complex compounds showed molecular ion peaks aligning with the empirical formula. Other peaks represented decomposition products separated from the molecular ion. The complexes' molecular weights corresponded to the molecular ion masses, confirming the complexes' structure and verifying successful syntheses.

Four distinct in vitro methods were employed to assess the extracts' antioxidant properties. Butylated hydroxyanisole (BHA) and ascorbic acid (AA) served as comparative standards. Experimental findings indicated that the ligand and metal complexes generally demonstrated lower activity than the standard antioxidants (BHA and AA). Nevertheless, the compounds exhibited significant activities, which typically increased with higher sample concentrations (Table 2). The compounds demonstrated comparable activity to standard antioxidants, particularly in the CUPRAC and ABTS tests.

While iron is crucial for organisms, its excessive accumulation can lead to various adverse effects. The fenton reaction can transform excess Fe^{+2} ions into hydroxyl radicals, which are highly dangerous free radicals [52]. Consequently, researchers frequently employ the FRAP method in antioxidant studies. The results indicated that BHA (5.46) and AA (4.70) had the highest reducing capacities, with the Pd(II) complex (0.66) exhibiting stronger reducing properties than other compounds (Table 2). The CUPRAC results revealed that the extracts were similar to each other, approximating standard antioxidants and displaying enhanced reducing properties (Table 2). The ABTS $^+$ test results mirrored these findings (Table 2). Notably, metal complexes demonstrated stronger radical scavenging abilities compared to ligands and showed activities similar to BHA and AA antioxidants. In the DPPH test, the AA molecule exhibited the highest activity. The ranking of DPPH radical scavenging activities for Schiff base (L), metal complexes, and standard antioxidants, based on IC50 values, was as follows: AA > BHA > Pd(II) complex > L > Co(II) complex (Table 2).

The antioxidant activity of Schiff base and its metal complexes can be explained by the redox properties of the metal complexes, which are influenced by factors such as the size of the chelate ring and the degree of unsaturation within the ring. Pd(II) complexes exhibit higher antioxidant activity compared to other synthesized compounds due to the strong reduction potential of Pd^{+2} and its ability to donate protons, making it an effective free radical scavenger. In contrast, Co(II) complexes show moderate antioxidant activity, possibly due to steric hindrance within their geometric structure, which prevents DPPH radicals from approaching the active centers of the complex. Overall, these findings suggest that Pd(II) complex could be considered for therapeutic applications in treating diseases associated with oxidative stress [53].

TABLE 1 | Thermo-gravimetric analysis results of the complexes.

Complexes	Stage	Temp. range (°C)	Mass loss (%)		Assignment
			Calc./Found		
[LCoCl·H ₂ O]	2	230–300	3.60	3.60	H ₂ O
	3	300–425	16.70	15.75	Cl and CH ₃ O ₂
	4	425–725	11.34	11.70	C ₄ H ₈
	Residue	725–			C ₁₁ H ₄ NBrS + CoO
[LPdCl·H ₂ O]H ₂ O	1	50–200	3.22	3.60	H ₂ O
	2	300–700	44.40	45.00	C ₆ H ₁₃ O ₃ BrCl
	Residue	700–			C ₁₀ H ₄ NS + PdO

TABLE 2 | In vitro antioxidant test results of ligand, metal complexes and standard antioxidants.

Samples	Fe ⁺³ reducing power (µg TE/mL)	Cu ⁺² reducing power (µg TE/mL)	ABTS activity (IC50)	DPPH activity (IC50)
L	0.49 ± 0.14	7.22 ± 0.02	1.28 ± 0.01	5.71 ± 0.06
Co(II) complex	0.45 ± 0.06	7.23 ± 0.02	1.25 ± 0.05	10.63 ± 0.01
Pd(II) complex	0.66 ± 0.12	7.55 ± 0.04	1.25 ± 0.05	4.21 ± 0.01
BHA	5.46 ± 0.03	7.32 ± 0.11	1.23 ± 0.02	1.62 ± 0.01
AA	4.70 ± 0.01	6.95 ± 0.03	1.22 ± 0.07	1.30 ± 0.03

The scientific literature contains numerous studies documenting the antioxidant properties of Schiff base ligands and their metal complexes. Buldurun et al. [54, 55] reported that the Pd(II) complex demonstrated superior antioxidant activity compared to the ligand alone. Nevertheless, both the metal complex and the ligand exhibited comparable effectiveness to standard antioxidants (BHA, AA and α -tocopherol). A separate investigation employing FRAP and DPPH methods revealed that the Pd(II) complex possessed more potent antioxidant characteristics than Fe(II) and Ni(II) complexes [21]. Research conducted by Turan et al. indicated that the ABTS and DPPH radical scavenging activities of Schiff base-Pd(II) complexes yielded results approximating those of standard oxidants [56]. Similar results have been reported in the literature for metal complexes and ligands. Antioxidant properties vary depending on the presence of hydroxyl, imine and aromatic rings. In addition, the central metal ion plays a decisive role in forming antioxidant capacity [57–59]. Overall assessment of this study's findings demonstrates consistency with previous research outcomes.

4 | Conclusion

The successful synthesis of a bidentate ligand and its complexes with Co(II) and Pd(II) was verified through various analytical techniques, including microanalysis, FT-IR, NMR, and UV-Vis. spectroscopy, mass spectrometry, thermal analysis, and magnetic susceptibility measurements. Spectral data indicated that the Schiff base ligand acted as bidentate chelates, coordinating through the phenolic oxygen and azomethine nitrogen atoms. The Pd(II) complex exhibited a square planar geometry, based on magnetic susceptibility results, while the Co(II) complex displayed a tetrahedral geometry. Antioxidant activities of compounds by different methods were determined by comparing

them with standard antioxidants. The experimental results showed that the compounds generally had lower activities than standard antioxidants. However, the Pd(II) complex showed typically better activities than the other synthesized compounds. In the next phase of this study, the structure–activity relationships of these bio-inorganic compounds can be explored, to advance their application in vivo as part of the drug design process.

Author Contributions

Naki Çolak: project administration, supervision, investigation, writing – original draft, methodology, resources. **Ahmet Savcı:** investigation, writing – original draft, visualization, methodology, validation, supervision. **Nevin Turan:** investigation, writing – original draft, visualization, validation, supervision, data curation. **Kenan Buldurun:** investigation, writing – review and editing, validation, visualization, supervision, data curation.

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Data Availability Statement

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.