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Article

Fe(III) and Cu(II) Complexes of Chlorogenic Acid: Spectroscopic, Thermal, Anti-/Pro-Oxidant, and Cytotoxic Studies

Monika Kalinowska ^{1,*} , Kamila Gryko ¹ , Ewelina Gołębiewska ¹ , Grzegorz Świdorski ¹ , Hanna Lewandowska ² , Marek Pruszyński ^{2,3} , Małgorzata Zawadzka ¹, Maciej Kozłowski ¹, Justyna Sienkiewicz-Gromiuk ⁴  and Włodzimierz Lewandowski ¹

¹ Department of Chemistry, Biology and Biotechnology, Institute of Environmental Engineering and Energetics, Faculty of Civil Engineering and Environmental Sciences, Białystok University of Technology, Wiejska 45E Street, 15-351 Białystok, Poland

² Institute of Nuclear Chemistry and Technology, 16 Dorodna Street, 03-195 Warsaw, Poland

³ NOMATEN Centre of Excellence, National Centre of Nuclear Research, 7 Andrzeja Soltana Street, 05-400 Otwock, Poland

⁴ Department of General and Coordination Chemistry and Crystallography, Institute of Chemical Sciences, Faculty of Chemistry, Maria Curie-Skłodowska University, Maria Curie-Skłodowska Sq. 2, 20-031 Lublin, Poland

* Correspondence: m.kalinowska@pb.edu.pl



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Abstract: Complexes of chlorogenic acid (5-CQA) with copper(II) and iron(III) were synthesized in a solid state and examined by means of FT-IR, thermogravimetric, and elemental analyses. The molar stoichiometric ratios of metal:ligand for the solid forms of the complexes were established as Cu(II):L = 1:2 and Fe(III):L = 2:3 (L: 5-CQA), with the possible coordination through the carboxylate group and the hydroxyl group from the catechol moiety. In an aqueous solution at pH = 7.4, the composition of the complexes was Cu(II):L = 1:1, and Fe(III):L = 1:1 and 1:2. The Cu(II) and Fe(III) complexes with 5-CQA showed lower antioxidant properties, as estimated by the spectrophotometric methods with DPPH[•], ABTS^{•+}, and HO[•] radicals, than the ligand alone, whereas in the lipid peroxidation inhibition assay, the metal complexes revealed a higher antioxidant activity than 5-CQA. Cu(II) 5-CQA showed the highest pro-oxidant activity in the Trolox oxidation assays compared to the other studied compounds. The lipophilic parameters of the compounds were estimated using the HPLC method. 5-CQA and its complexes with Fe(III) and Cu(II) were not toxic to HaCaT cells in a tested concentration range of 0.15–1000 nM after a 24 h incubation time.

Keywords: phenolic compounds; metal complexes; chlorogenic acid; caffeoylquinic acid; antioxidant activity

1. Introduction

In recent years, the interest in antioxidant compounds of natural origin has been constantly growing. A noteworthy group of chemical compounds in this area are plant phenolic acids [1]. Chlorogenic acids have recently turned out to be some of the more available and active phenolic compounds because of their antioxidant, anti-inflammatory, and anticancer properties [2]. Chlorogenic acids (caffeoylquinic acids, CQAs) are esters of one or more molecules of cinnamic acid (or its derivatives, including caffeic, ferulic and *p*-coumaric acids) and quinic acid, which belong to the hydroxycinnamic acid group [1]. Among all the isomers found in plants, 3-caffeoylquinic acid (3-CQA), 4-caffeoylquinic acid (4-CQA), and 5-caffeoylquinic acid (5-CQA, commonly called chlorogenic acid) stand out as the main ones (the structures of CQAs have been shown in previous publications) [3–5].

1.1. Physicochemical Properties

The compounds that belong to the family of chlorogenic acids exhibit very different physicochemical properties, depending on the identity, number, and position of the acyl residues esterified with quinic acid, as well as the functional groups present on the aromatic group of the acyl residues [3]. CQAs are soluble in water, but the polarity of CQAs consequently decreases with the degree of esterification, i.e., monoCQAs > diCQAs > triCQAs > tetraCQAs. The less-polar CQAs are soluble in lower alcohols or alcohol–water mixtures. They are insoluble in benzene, chloroform, and petroleum ether. The polar nature of CQA makes it relatively insoluble in the lipid matrix. Furthermore, its polyphenolic structure leads to its instability and poor penetration across the lipophilic membrane barrier, limiting its absolute bioavailability in the human organism [6]. CQAs are highly susceptible to the effects of temperature. As temperature increases, they undergo intramolecular isomerization, transesterification, and degradation more easily. CQAs are photosensitive and undergo trans–cis isomerization upon exposure to ultraviolet or visible light [3].

It has been demonstrated that 5-CQA not only isomerizes to 3-O-caffeoylquinic acid and 4-O-caffeoylquinic acid, but also undergoes other transformations such as esterification and reactions with water, i.e., hydrolysis and/or the addition of a water molecule to the double bond. These processes occur not only in CQA solutions, but also during their isolation from plant materials and can lead to extracts with a lower content of biologically active phenolic compounds [3].

1.2. Biological Properties

Chlorogenic acids exhibit a wide range of health-promoting properties (Figure 1), which makes them an attractive food additive or drug. Chlorogenic acids exhibit antioxidant [4,7–10], anti-inflammatory [2,4,7], antibacterial [2,4,7,8,11,12], antiviral [7,13], anticancer [8,9,14,15], neuroprotective [3,4,16], and antidiabetic [14,17] effects, as well as positive effects against gastrointestinal diseases [2,3,18,19]. In addition, they can increase the number of white blood cells, lower blood pressure, and stimulate the central nervous system [16].

In vitro and in vivo data indicate that 5-CQA has antioxidant activity and can alleviate oxidative stress in various disease models [2]. According to their structure, phenols can eliminate radicals directly, through a peroxidase reaction or by forming chelates with metal ions, thus preventing Fenton-type reactions [20]. Despite their significant antioxidant properties, CQAs are also characterized by pro-oxidant properties, which depend on their concentration, the occurrence of transition metal ions, and environmental conditions (presence of oxygen molecules, high pH value) [7,19–22]. Moreover, the mechanism of the pro-oxidant action of CQAs can be based on the ability to produce reactive oxygen species (ROS), i.e., hydrogen peroxide H_2O_2 , superoxide radicals $ROO^{\bullet-}$, and hydroxyl radicals $HO^{\bullet-}$ formed in the Fenton reaction during the reduction of Fe(III) to Fe(II) [23]. The excessiveness of ROS in an organism can contribute to irreversible damage to the proteins, lipids, and nucleic acids present in the cells [24].

In addition, CQAs exhibit broad-spectrum activity against Gram-positive and Gram-negative bacteria as well as fungi and yeasts [5]. 5-CQA shows activity against *Stenotrophomonas maltophilia*, *Candida albicans* (MIC = 80 μ g/mL) [5,12], and *Staphylococcus aureus* (MIC = 500 μ g/mL) [5,13,25].



Figure 1. Biological properties of chlorogenic acids (CQAs) [2,4,5,11–19,25–27].

1.3. Bioavailability and Metabolism

The pharmacokinetics of CQAs have been studied. In an experimental model involving humans and animals, CQAs and their metabolites were noted in the blood. One third of ingested CQAs in beverages and food are absorbed in the small intestine, which can be measured by high-performance liquid chromatography as 5-CQA, 4-CQA, and 3-CQA present in the plasma. The remaining two-thirds enters the large intestine, where the phenolic acid is further metabolized by the gastrointestinal microflora and then absorbed [26].

Chlorogenic acids (acyl-quinic acids), as with other polyphenols, show low bioavailability due to several factors: interactions with the food matrix, and metabolic processes in the liver (phase I and II metabolism), intestines, and microflora. On the other hand, the biological activity of phenolic compounds may be mediated by their metabolites, which are produced *in vivo*, and recent studies have confirmed that these molecules may have antioxidant and anti-inflammatory properties. For example, unabsorbed in the small intestine, dietary 5-CQAs are hydrolyzed into caffeic and quinic acid and both are then metabolized by the colonic microflora to a series of lower-molecular-weight metabolites such as ferulic acid, isoferulic acid, *p*-coumaric acid, and gallic acid, which are mainly absorbed in the colon. The remaining metabolites enter the bloodstream and are absorbed or further metabolized (e.g., to vanillic or protocatechuic acid) in the liver [28,29].

Various ways to increase the bioavailability of CQAs have been investigated, including metal complexation, nanoformulation, and the synthesis of sulfonate derivatives. Zhang et al. studied the interactions of chlorogenic acid with whey proteins. CGA bound to three whey proteins, β -Lg, α -La, and BSA, mainly through hydrophobic force. The study was designed to provide information for further studies of milk proteins with polyphenols and the fabrication of whey protein-based carriers to improve the bioavailability of polyphenols [1].

1.4. Sources of Chlorogenic Acid

Chlorogenic acid is one of the most widely occurring polyphenols found in plants, resulting in its presence in the diet. It is present in many food products, e.g., apples, stone fruits, berries, cruciferous vegetables, celery, and potatoes [30–37] (Tables S1 and S2). However, the content of chlorogenic acid depends not only on the part of the plant, but also on its maturity and the storage conditions. Additionally, its high content can be found in processed beverages, most often in green and black tea, juices, wines, yerba mate, and coffee [4]. One of the richest dietary sources of CQA are coffee beans. The content of chlorogenic acid in green coffee beans is highly determined by their type; it may range from 6 to 12% of the coffee bean dry mass [4]. The content of this compound for many fruits and vegetables depends on their variety, the cultivation method, exposure to stresses, and storage conditions, which is very evident for apples (12–31 mg/100 mL of apple juice) [23]. However, irrespective of variety, 5-CQA is the major constituent [3].

1.5. Chlorogenic Acid–Metal Complexes

Phenolic compounds play a significant role in binding toxic metals in the environment [15]. CQAs have an important function in plant tolerance to toxic metals, as well as in preventing and reducing biotic and abiotic oxidative stress. The mechanism involves: (a) the secretion of chelating agents, e.g., by roots, to prevent metal uptake or (b) the production of chelating agents that bind metals in the cell wall, symplast, or vacuole [38].

The ability of CQAs to form complexes with selected metal ions and the effect of complexation on changing their properties (including antioxidant) have been studied in recent years (Figure 2). Several publications have described the formation of complexes of CQAs with various metal ions in aqueous solutions, such as Al(III) [39], Fe(III) [40,41], Mg(II) [13], Cu(II) [42,43], or Na(I) [11]. Moreover, the ability to form CQA complexes with Pb(II) and Cu(II) and the lack of this ability for Cd(II) and Zn(II) have been demonstrated [7]. The molecular complexation of this compound with β -cyclodextrin, caffeine, and proteins has been reported to design more advanced and controlled carriers for drugs and food ingredients [15].

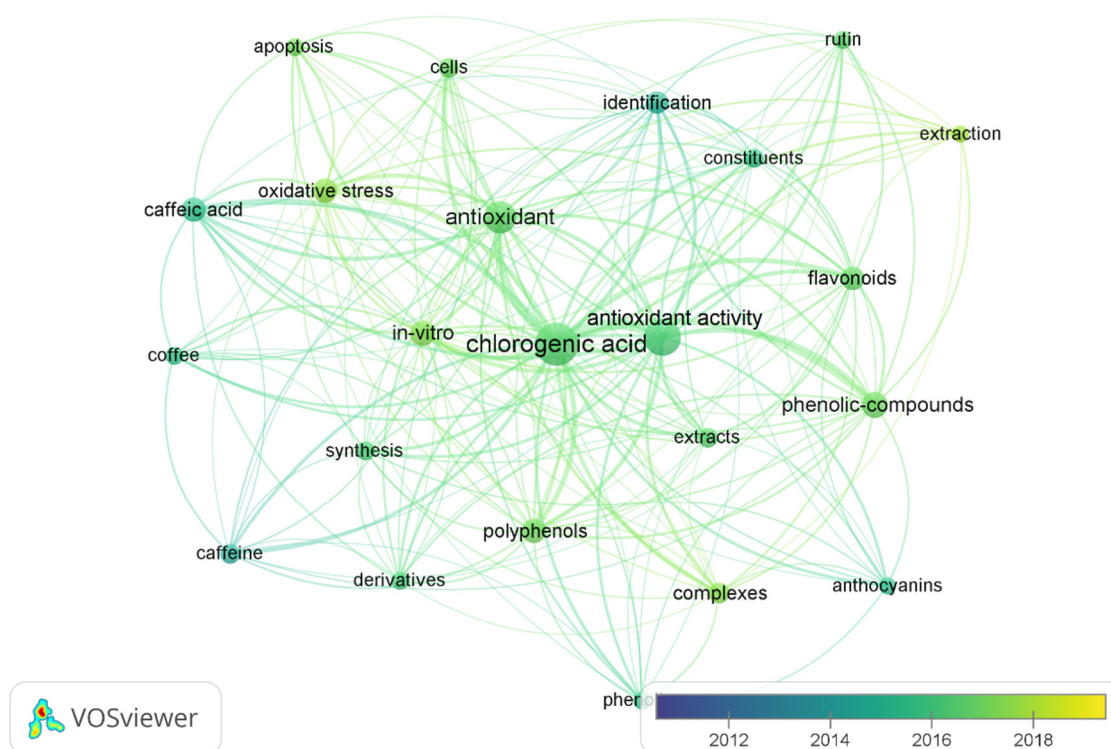


Figure 2. Co-occurrence of selected keywords in articles in 2010–2022 (search terms: “chlorogenic acid”, “antioxidant”), created with VOSviewer [44].

Complexation with metal ions can alter the antioxidant potential of chlorogenic acid. Alkali metal salts [20] and Fe(II) [40], Cu(II) [43], Ce(IV), Mg(II) [13], and Zn(II) [7] complexes of 5-CQAs showed higher antioxidant activity than the ligand itself. Kalinowska et al. [7] showed that Zn(II) 5-CQA is a better scavenger of ABTS^{•+} cationic and DPPH[•] radicals and a better reductor of Fe(III) and Cu(II) ions compared to 5-CQA alone or even natural (L-ascorbic acid, $EC_{50} = 10.32 \pm 0.98 \mu\text{M}$) and synthetic (butylated hydroxyanisole BHA, butylated hydroxytoluene BHT) antioxidants [7]. Chlorogenic acid can interact with Fe(III) to form complexes that interact with ferritin via hydrogen bonds, which promotes the rate of oxidative ion deposition and ion release from ferritin and reduces Fe(III)- and Fe(II)-induced ferritin polymerization [27]. It is important to note that antioxidants can also act as pro-oxidants depending on their concentration or the presence of certain metal cations; moreover, the stabilization of phenoxyl radicals by metal cations results in the prolongation of their lifetime [20,43]. It was shown that metals such as Al(III), Zn(II), Cd(II), Mg(II), and Ca(II) can increase the pro-oxidant activity of chlorogenic acid due to their stabilizing effect on the phenoxyl radical [43].

Therefore, in this paper, the Fe(III) and Cu(II) complexes with 5-CQA were synthesized in a solid state and studied by means of FT-IR, thermogravimetric, and elemental analyses. The composition of the complexes in solution was estimated by means of the spectrophotometric method. The antioxidant activity of the complexes was studied by means of DPPH, ABTS, HO, lipid peroxidation inhibition, and pro-oxidant assays. The lipophilicity of the compounds was estimated by means of the HPLC method. The cytotoxic activity of Fe(III) and Cu(II) 5-CQA as well as 5-CQA toward the HaCaT cell line was studied.

2. Materials and Methods

Chlorogenic acid, NaOH, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), potassium persulfate, $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, Trolox, H_2O_2 , horseradish peroxidase, and phosphate buffer

(pH = 7) were purchased from Sigma-Aldrich and used without purification. Methanol and hydrochloric acid were purchased from Chempur (Karlsruhe, Germany).

2.1. Synthesis

The chlorogenates were prepared by mixing the appropriately weighed mass of chlorogenic acid (about 0.1 g weighed to 4 decimal places) with an aqueous solution of NaOH ($C = 0.1$ M) in a stoichiometric molar ratio of 1:1 at room temperature. Then, the aqueous solution of metal chloride ($C = 0.5$ M) was added to the mixture in order to obtain a molar ratio for the ligand:metal cation of 2:1 for the Cu(II) complex and 3:1 for the Fe(III) complex. Brown (Cu 5-CQA) and dark brown (Fe 5-CQA) precipitates occurred immediately. They were filtered from the solution and washed with deionized water. The precipitates were air-dried at room temperature over 48 h. The yield of the reaction was 64% and 76% in the case of Cu 5-CQA and Fe 5-CQA, respectively.

2.2. Thermal Analysis (TG-DCS) in Air Atmosphere and Elemental Study

The thermal behavior of the iron(III) and copper(II) chlorogenates was investigated using thermogravimetry (TG) coupled with differential scanning calorimetry (DSC). The TG-DSC measurements were conducted on a SETSYS 16/18 (Setaram, Caluire, France) thermal analyzer with dynamic air flow at a rate of $0.75 \text{ dm}^3/\text{h}$. The tested samples weighing 8.149 mg and 8.770 mg were placed in alumina crucibles and heated from a temperature of 30°C to 750°C at a constant heating rate of $10^\circ\text{C}/\text{min}$. The elemental analyses for the mass percentages of carbon and hydrogen were performed with Perkin-Elmer 240 equipment (PerkinElmer, Waltham, MA, USA).

2.3. Spectrophotometric Determination of Cu(II) and Fe(III) 5-CQA Composition in Solution Using the Spectrophotometric Method

To determine the metal ion:ligand molar ratio in an aqueous solution of Cu(II) and Fe(III) 5-CQA, the spectrophotometric mole-ratio method was applied. The spectra in the range of 200–550 nm were recorded for solutions with a constant mole number for 5-CQA and a varied amount of Cu(II) or Fe(III) ions. The concentration of 5-CQA was 0.1 mM, while the concentration of FeCl_3 and CuCl_2 changed from 0 to 0.15 mM. All solutions were prepared in tris-HCl buffer (pH = 7.4; $C = 50$ mM).

2.4. IR and UV Studies

The IR spectra of 5-CQA and the Cu(II) 5-CQA and Fe(III) 5-CQA complexes in the solid state were recorded with a Cary 630 FTIR Agilent Technologies spectrometer, using the ATR technique, within the range of $400\text{--}4000 \text{ cm}^{-1}$. The resolution was 1 cm^{-1} . The UV/Vis spectra of the studied compounds at a concentration of 0.01 mM were recorded in the range of 200–550 nm in tris-HCl (pH = 7.4; $C = 50$ mM) using an Agilent Cary 5000 spectrophotometer (Agilent, Santa Clara, CA, USA).

2.5. Antioxidant Properties

2.5.1. DPPH•

The determination of the antiradical activity of the compounds was performed by conducting a direct reaction of the DPPH• radical with the tested compounds in appropriate concentrations, according to the method described in [45]. The absorbance of the samples was measured after 1 h of incubation in the dark at the wavelength $\lambda = 516 \text{ nm}$. The result was the percentage of DPPH• radical inhibition (%I) calculated using the formula:

$$\%I = \frac{A_c - A_t}{A_c} \cdot 100\%$$

where A_c is the absorbance of the control sample and A_t is the absorbance of the test sample.

The radical scavenging capacity was determined by the EC₅₀ parameter, which is the antioxidant concentration needed to reduce the initial radical concentration by 50%. All measurements were taken for five replicates in three independent experiments.

2.5.2. HO•

The hydroxyl radical inhibition activity was measured according to [46]. In the test sample, 0.3 mL of 8 mM FeSO₄, 1 mL of 3 mM salicylic acid in ethanol, and 0.25 mL of 20 mM H₂O₂ were added to 1 mL of a 0.1 mM solution of the tested compounds. In the control sample, deionized water was added instead of H₂O₂, and in the blank sample, deionized water was added instead of the tested compound solution. The samples were vortexed and incubated for 30 min at 37 °C. After the incubation, 0.5 mL of deionized water was added to each sample. The samples were vortexed and the absorbance was measured immediately at 510 nm, with reference to water. The level of hydroxyl radical inhibition was calculated using the formula:

$$\%I = \left(1 - \frac{A_t^{510} - A_c^{510}}{A_b^{510}}\right) \cdot 100\%$$

where A_t^{510} is the absorbance of the test sample, A_c^{510} is the absorbance of the control sample, and A_b^{510} is the absorbance of the blank sample. All measurements were taken for five replicates in three independent experiments.

2.5.3. ABTS•⁺

To obtain the ABTS•⁺ radical solution, ABTS (5.4 mM) and K₂S₂O₈ (1.74 mM) were mixed in a 1:1 volume ratio. The mixture was then incubated in the dark for 12 h. After that, it was diluted with methanol so that, before the assay, it had an absorbance of about 0.8 at a wavelength of 734 nm [47]. A total of 1.5 mL of the diluted radical solution and 1.5 mL of 0.1 and 0.01 mM tested compound solutions were incubated in glass test tubes for 7 min. Then, the absorbance was measured at $\lambda = 734$ nm against methanol. Control samples, containing methanol instead of tested compounds solutions, were prepared in parallel. The percent of inhibition was calculated, using the same formula as for the DPPH• assay. All measurements were taken for five replicates in three independent experiments.

2.5.4. Lipid Peroxidation Inhibition

The lipid peroxidation inhibition capacity was tested in accordance with [48] with some modifications, by preparing linoleic acid emulsions with the addition of antioxidant at the 0.005 M concentration. From this mixture, incubated at 40 °C, 0.1 mL of the sample was taken every 24 h for 5 days. Then, methanol and 30% ammonium rhodate solution were added, and after 3 min, 0.02 M FeCl₂ was added. The absorbance was measured immediately at the wavelength $\lambda = 500$ nm. A control sample containing no antioxidant was performed in parallel. The percent inhibition of linoleic acid peroxidation was calculated, using the formula analogous to the DPPH• inhibition assay. All measurements were taken for five replicates in three independent experiments.

2.5.5. Pro-Oxidant Activity

The pro-oxidant activity was measured in accordance with a method described in [49], on the basis of the compounds' ability of Trolox oxidation. The reaction mixture contained: 0.5 mL of 0.4 mM Trolox, 0.5 mL of 0.2 mM H₂O₂, 0.5 mL of horseradish peroxidase in 0.05 M phosphate buffer, 50 or 25 μ L of 0.1 mM tested compounds, and 0.45 or 0.475 mL of deionized water. The samples were mixed and the absorbance was measured every 10 min at $\lambda = 272$ nm. The pro-oxidant activity assay was performed in triplicate for three independent series. Calculations were made according to the following formula:

$$\%I = \frac{A_c - A_t}{A_c} \cdot 100\%$$

where A_c is the absorbance of the control sample and A_t is the absorbance of the test sample.

All measurements were taken for five replicates in three independent experiments. The absorbance was measured using an Agilent Cary 5000 spectrophotometer (Agilent, Santa Clara, CA, USA).

2.6. Cell Viability Assay

The influence of 5-CQA and its complexes with Fe(III) and Cu(II) on cell viability was determined by the colorimetric MTS metabolic activity assay, as described previously [50]. HaCaT is a human skin keratinocyte cell line widely used in research due to its high capacity for proliferation in vitro. It provides a reproducible model with long viability in cell culture. These cells are an excellent model of skin cells and are often used in our research alongside the Caco2, which is used as a model of the intestinal epithelial barrier. Briefly, the HaCaT human immortalized keratinocyte cells (Thermo Fisher Scientific, Inc., Waltham, MA, USA) were seeded in 96-well plates at a density of 2×10^3 cells/well in a DMEM medium supplemented with 4.5 g/L of glucose, 2 mM L-glutamine, 10% fetal bovine serum, streptomycin (100 µg/mL), and penicillin (100 IU/mL). All reagents for the cell assays were from Biological Industries (Beth Haemek, Israel). After a 24 h incubation, the growth medium was replaced with one containing increasing concentrations of the tested compounds, between 0.15 and 1000 nM, and the cells were again incubated for 24 h at 37 °C. This was followed by the addition of 20 µL of 5-(3-carboxymethoxyphenyl)-2-(4,5-dimethylthiazolyl)-3-(4-sulfophenyl)tetrazolium inner salt (CellTiter-96® AQueous-Non-Radioactive, Promega, Mannheim, Germany). The absorbance in wells was measured at 490 nm using a micro-plate reader (Apollo 11LB913, Berthold, Bad Wildbad, Germany). The cell viability was expressed as a percentage of normalization to cells grown in medium only. All measurements were taken for three replicates in three independent experiments.

2.7. Lipophilicity Assay

The lipophilicity was determined using an RP-HPLC analysis using a Waters Alliance 2695 HPLC separation module (Milford, MA, USA) and a Waters 2996 photodiode array detector (Milford, MA, USA) ($\lambda = 254$ nm). The experimental methodology and the method of calculating the chromatographic lipophilicity parameter, $\log k_w$, is described in [20].

2.8. Statistical Analysis

To determine the statistical significance between the tested compounds, an analysis of variance (ANOVA) followed by Tukey's test was applied. The results from three independent experiments were expressed as the mean \pm standard deviation (SD) of the mean for parametric data. Significance was considered when $p \leq 0.05$. Statistica 13.0 was used.

3. Results and Discussion

3.1. Elemental Study and Thermal Analysis of the Solid-State Samples

Thermal behavior is an important parameter determining material properties. The TG–DTG–DSC thermal profiles of the analyzed complexes are shown in Figure 3, whereas the thermal data are gathered in Table 1. Additionally, the elemental analysis results obtained for the Cu(II) and Fe(III) 5-CQAs complexes in the solid state are presented in Table 2.

The results showed that Cu(II) formed a complex with 5-CQA with a molar ratio of 1:2 (metal:ligand), while for Fe(III) 5-CQA, the molar ratio was 2:3 (metal:ligand). The thermal decomposition of the complexes in question can be divided into two main stages. The first stage was connected only with the endothermic dehydration process, whereas the second one was related to the strong exothermic decomposition of anhydrous complexes. The presence of a weak endothermic peak seen on the DSC curves at 88 and 91 °C for the Cu(II) and Fe(III) complexes, respectively, confirmed the hydrated nature of both analyzed materials. The identified mass loss between 30 °C and 150 °C for Cu 5-CQA (10.37%) was almost the same as the calculated theoretical value (10.46%), which corresponded to the separation of five water molecules. The Fe(III) complex also contained water as

a solvent, but in a different amount than its predecessor. The experimental value of the breakdown of the water molecules in the range of 30 to 140 °C (10.69%) was near the theoretical one (10.95%), which confirmed the presence of eight solvent molecules in the case of Fe(III) 5-CQA. The second key stage of the thermal decomposition was attributed to the exothermic degradation of the organic 5-CQA ligand. The loss of the organic part of the complexes occurred in several steps, as evidenced by the presence of a number of exothermic jumps on the registered DSC curves. The Cu(II) complex showed a slightly higher thermal stability than the Fe(III) compound (the thermal decomposition of the Cu(II) complex started at 150 °C, whereas the Fe(III) complex began to decompose at 140 °C). The final products of the thermal decomposition were the corresponding metal oxides: brick-red Fe₂O₃ and black CuO.

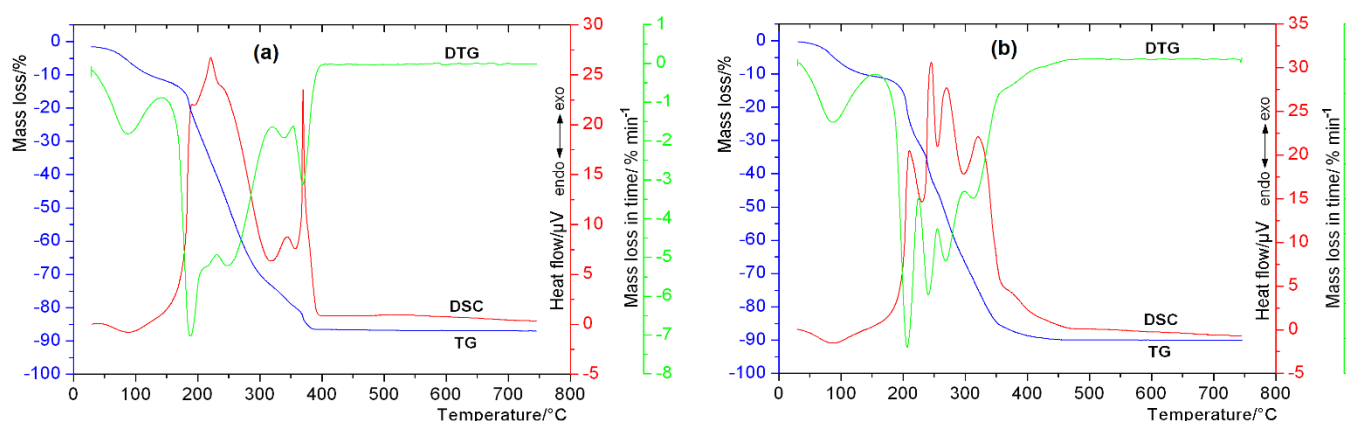


Figure 3. TG–DTG–DSC thermal curves for Fe(III) (a) and Cu(II) (b) chlorogenates.

Table 1. Results of thermal decomposition of Cu(II) and Fe(III) 5-CQAs in air atmosphere.

Complex	T ₁ /°C	T _{endo}	Mass Loss/%		Anhydrous Form	T ₂ /°C	T _{exo}	Residue/%		Residue
			Found	Calc.				Found	Calc.	
Fe ₂ (C ₁₆ H ₁₇ O ₉) ₃ ·8H ₂ O	30–140	91	10.69	10.95	Fe ₂ (C ₁₆ H ₁₇ O ₉) ₃	140–405	191	12.97	12.14	Fe ₂ O ₃
							221			
							344			
							370			
Cu(C ₁₆ H ₁₇ O ₉) ₂ ·5H ₂ O	30–150	88	10.37	10.46	Cu(C ₁₆ H ₁₇ O ₉) ₂	150–465	210	10.06	9.25	CuO
							246			
							270			
							321			

T₁—temperature range of dehydration. T₂—temperature range of degradation of anhydrous complexes to suitable oxides. T_{endo}—peak top of endothermic effect. T_{exo}—peak tops of exothermic effect.

Table 2. Elemental analysis of Cu(II) and Fe(III) complexes with chlorogenic acid.

Compound Formula	C%		H%		M% (Based on TG Profile)	
	Exp	Calc	Exp	Calc	Exp	Calc
Cu(C ₁₆ H ₁₇ O ₉) ₂ ·5H ₂ O	44.08	44.64	5.09	5.12	8.03	7.38
Fe ₂ (C ₁₆ H ₁₇ O ₉) ₃ ·8H ₂ O	43.16	43.78	4.69	5.09	9.07	8.49

3.2. FT-IR Studies of the Solid-State Samples

The FT-IR spectra of the Cu(II) and Fe(III) chlorogenates are shown in Figure 4, and the assignment of the selected bands are gathered in Table S3. The assignment was based on our previous publications [7,10] concerning chlorogenic acid and a zinc(II) complex with chlorogenic acid.

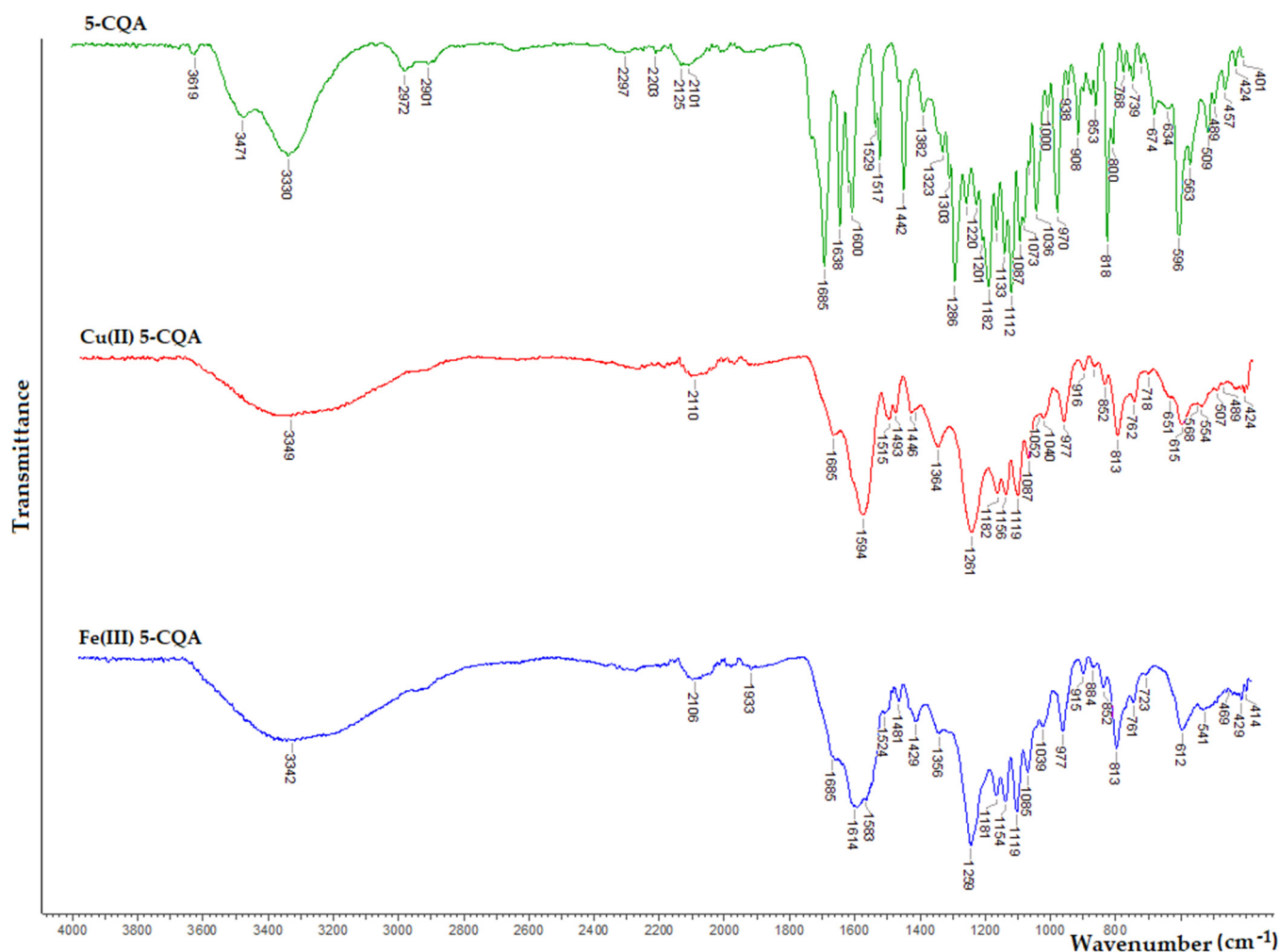


Figure 4. FT-IR spectra of chlorogenic acid and its complexes.

In the FT-IR spectra of the Cu(II) and Fe(III) complexes, the bands related to the vibrations of the carboxylate anion appeared, compared to the absence of these bands in the spectra of the ligand. The asymmetric $\nu_{as}(\text{COO}^-)$ and symmetric $\nu_s(\text{COO}^-)$ stretching vibrations of the carboxylate anion occurred at 1594 and 1364 cm^{-1} in the spectra of Cu(II) 5-CQA and at 1614 and 1583 cm^{-1} and 1364 and 1356 cm^{-1} in the spectra of Fe(III) 5-CQA. In the spectra of the metal complexes, there were no bands derived from the stretching vibrations of the C=O carbonyl group, which was present in the spectrum of the acid at 1725 cm^{-1} . Moreover, in the spectra of the complexes, the bands assigned to the bending vibrations in-plane and out-of-plane of the carboxylate anion occurred at 813 cm^{-1} as well as 615 and 612 cm^{-1} in the spectra of the Cu(II) and Fe(III) complexes, respectively. Moreover, significant changes in the position of the band originating from the vibrations of the catechol group were observed. In the spectrum of the acid, this band is located at 1286 cm^{-1} , while in the spectrum of the Cu(II) and Fe(III) complexes, it is located at 1261 and 1259 cm^{-1} , respectively. The coordination through the carboxylate group probably affected the whole structure of the ligand, including the catechol moiety. On the other hand, these metal ions may form oligomeric structures with chlorogenic acid, similar to the zinc complex [7]. Some of the other bands present in the FT-IR spectra of the 5-CQA complexes were slightly shifted or disappeared compared with the spectra of 5-CQA. This means that the metal ions affected the structure of the quinic and caffeic acid moieties.

3.3. Determination of the Composition of Cu(II) and Fe(III) 5-CQAs in Solution

Figures 5 and 6 show changes in the absorbance of the solutions due to the formation of Fe(III) and Cu(II) complexes with 5-CQA. In the UV/Vis spectrum of 5-CQA, four bands were present at about 218, 232, 298, and 325 nm. These bands arose from the $\pi \rightarrow \pi^*$ transitions within the aromatic ring and the C=C double bond [10]. Due to the complex formation, the bands at 232 and 325 nm disappeared and new bands at ~266 and ~363 nm appeared. As the concentration of metal ions increased, the absorbance of the band at 218 nm increased and the absorbance of the band at 298 nm decreased. The isosbestic point in the absorption spectrum resulted from the formation of an iron(III) complex with chlorogenic acid in the solution. Figure 5 shows selected spectra of the successively prepared solutions. In the spectrum of 5-CQA, four bands were present at 218, 232, 298, and 325 nm. The bands at 298 and 218 nm were derived respectively from the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ electronic transitions within the C=O group, whereas the bands at 232 and 325 were assigned to the $\pi \rightarrow \pi^*$ transitions within the aromatic ring [10]. In the UV/Vis spectra of the Fe(III) and Cu(II) complexes with 5-CQA, the bands at 232 and 325 nm were shifted to ~265 and ~365 nm, which suggested that the metal complexation strongly affected the electronic charge distribution in the catechol moiety. This could have been caused by the participation of the -OH substituents from the aromatic ring in the metal ion coordination.

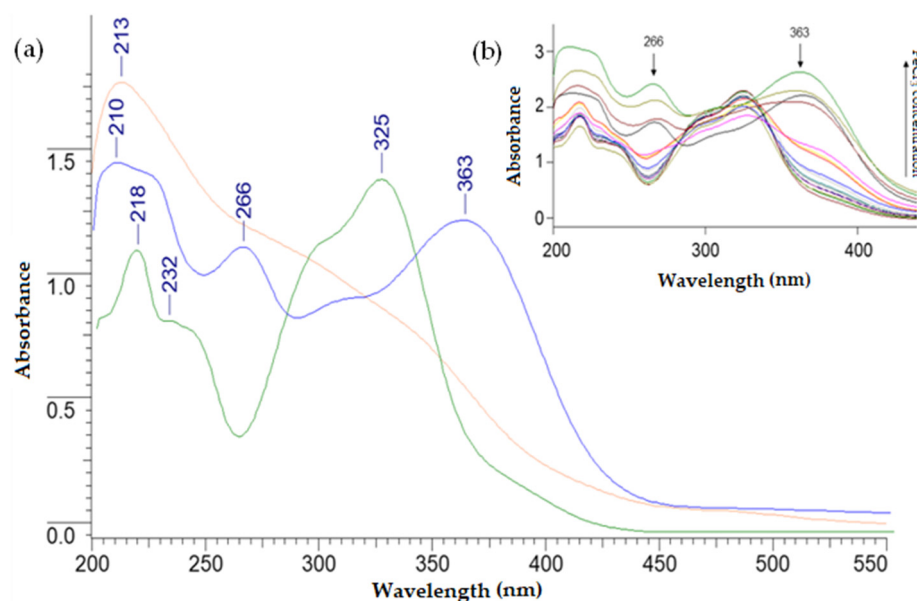


Figure 5. (a) UV/Vis spectra of FeCl₃ in tris-HCl buffer, with C = 10^{−4} M (red line), 5-CQA in tris-HCl buffer, with C = 10^{−4} M (green line), and iron(III) with 5-CQA complex in 1:1 molar ratio in tris-HCl buffer, with C = 10^{−4} M (blue line) recorded in the range of 200–550 nm. (b) The UV/Vis spectra of successive series of prepared solutions; the increasing concentration of FeCl₃ is depicted.

The mole ratio is suitable for examining the composition of chlorogenic acid complexes with iron(III) and copper(II). As a result of the conducted research, the presence of complexes of chlorogenic acid with copper(II) in a molar ratio of 1:1 was found in an aqueous solution (Figure 7a). In the case of the iron(III) complex with chlorogenic acid (Figure 7b), we observed two pitches in the curve, indicating the coexistence of two forms of the complex in solution, in a molar ratio of 1:1 and 1:2 (metal:ligand). According to the literature, chlorogenic acid forms complexes with Cu(II), Mn(II), Zn(II), and Fe(III) and the formula of these compounds was estimated as ML_n, where L is the chlorogenic acid and n = 1, 2, or 3 depending on the pH of the aqueous solution [51]. The authors claimed that in the pH range of 5–6.5, there was an equilibrium between the two forms of the complex (FeL/FeL₂^{3−}), and when pH ≤ 5, the neutral form existed (FeL). The higher complex, FeL₃^{6−}, was formed at a pH of ~7.7. In the case of the Cu(II) complex with

chlorogenic acid, at a pH = 5.7, the main form was CuL^- , whereas at a higher pH, the CuL_2^{4-} complex occurred, which was the major species at pH = 7.3. Other studies revealed that at a nearly neutral pH, Cu(II), Fe(II), and Mn(II) were complexed by 5-CQA with a molar ratio of 1:1 [52]. According to Milic et al., for an aqueous solution at a pH of 7.5, the estimated stoichiometry was 1:1 for Pb(II) 5-CQA and 1:1 and 1:2 for Cu(II) 5-CQA [53]. Taking into account the pH of the solution, it is necessary to consider the participation in the coordination of the metal ion not only of the carboxylate group, but also of the hydroxylic groups of the catechol group [41], as well as the additional possible reduction of iron(III) ions to iron(II) and the formation of chlorogenic acid oxidation products [41]. Studies on the complex of 5-CQA with vanadium(IV) have also confirmed the participation of catechol groups in metal binding [18]. Therefore, the coordination of iron(III) and copper(II) ions through the catechol moiety should also be considered. On the basis of Figures 5 and 6, the stability constants ($\log K$) for the complexes were calculated. For Cu(II) 5-CQA, the stability constant was $\log K = 4.23$, whereas for Fe(III) 5-CQA it was $\log K_1 = 5.20$ (metal:ligand 1:1) and $\log K_2 = 4.56$ (metal:ligand 1:2).

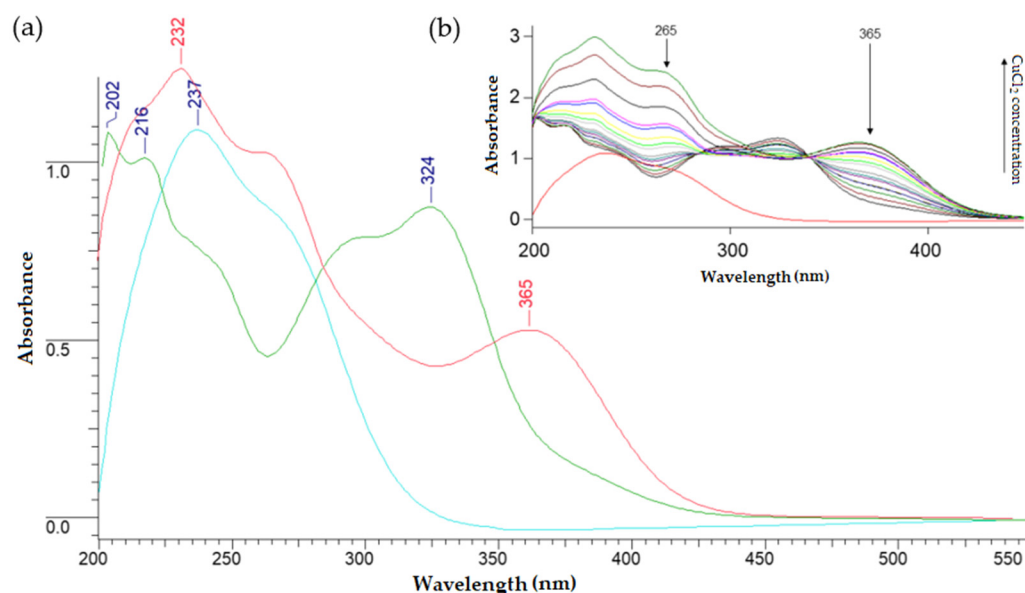


Figure 6. (a) The UV-Vis spectra of CuCl_2 in tris-HCl buffer, with $C = 10^{-4}$ M (blue line), 5-CQA in tris-HCl buffer, with $C = 10^{-4}$ M (green line), and copper(II) with 5-CQA complex in 1:1 molar ratio in tris-HCl buffer, with $C = 10^{-4}$ M (red line), recorded in the range of 200–550 nm. (b) The UV/Vis spectra of successive series of prepared solutions; the increasing concentration of CuCl_2 is depicted (red line— CuCl_2 spectrum in tris-HCl, with $C = 10^{-4}$ M).

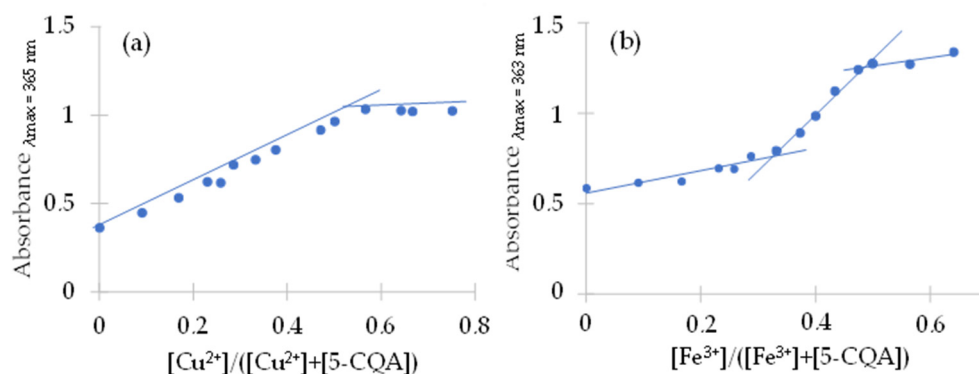


Figure 7. The mole-ratio plot for the estimation of the composition of (a) Cu(II) and (b) Fe(III) chlorogenate complexes.

The difference in the composition of the complexes in solid form and in solution may be explained by a slightly different pH of the reaction environment. In solution, the pH was kept at 7.4 by the presence of the tris-HCl buffer, whereas during the synthesis of the complexes in solid form, the solution of NaOH was added to 5-CQA to deprotonate the acid and facilitate the formation of metal complexes ($\text{pH} \approx 8.2$). The slightly higher pH probably resulted in an increase in the amount of the deprotonated form of 5-CQA with the participation of the $-\text{OH}$ group at the para position of the catechol moiety. This might have caused the formation of the complexes in solid form with different molar stoichiometry (metal:ligand) compared to the complexes in solution. The stability constants for 5-CQA were: pK_a1 (COOH) = 3.35; $\log K_{a2}$ (OH in the para position) = 8.30; and pK_a3 (OH in the meta position) = 12.06 [51].

3.4. DPPH•, HO•, and ABTS•+ Antiradical Activity Assays

The antioxidant properties of chlorogenic acid and its complexes with Fe(III) and Cu(II) ions were measured using a DPPH• assay and expressed as the concentration of antioxidant required to inhibit 50% of the DPPH• radicals (IC_{50}) [45]. The obtained results are presented in Figure 8. The value of the IC_{50} parameter equaled $9.87 \pm 0.05 \mu\text{M}$ for 5-CQA, $11.03 \pm 0.34 \mu\text{M}$ for Cu(II) 5-CQA, and $14.31 \pm 0.08 \mu\text{M}$ for Fe(III) 5-CQA. 5-CQA was found to have the greatest ability to scavenge DPPH• radicals from the studied compounds. Similar IC_{50} values to those obtained in the DPPH• assay for 5-CQA can be found in the literature. For example, in a study by Zheng et al. [54], the IC_{50} for 5-CQA was $6.9 \pm 0.1 \mu\text{M}$ [54]. In other studies, chlorogenic acid inhibited 50% of the DPPH• radicals at a concentration of 7.23 ± 0.76 [7] and $7.39 \pm 0.71 \mu\text{M}$ [20]. It can be concluded that 5-CQA is an effective scavenger of DPPH• radicals.

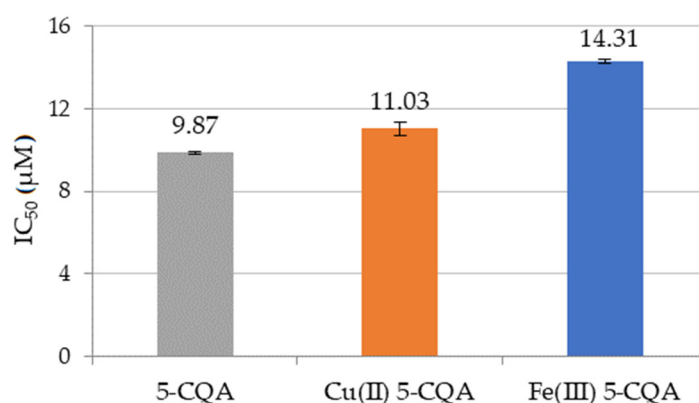


Figure 8. Antioxidant properties of 5-CQA, Cu(II) 5-CQA, and Fe(III) 5-CQA, expressed as the ability to scavenge DPPH• radicals (IC_{50}). Mean values from three independent experiments \pm SDs are shown. The same letter near the means indicates no significant difference (Tukey's test, $p < 0.05$).

The ABTS•+ cation radical assay was carried out for two concentrations of the tested compounds (0.05 and 0.005 mM). The results are shown in Figure 9. The antioxidant activity increased with increasing compound concentration. All the studied compounds showed a significant radical scavenging ability (ranging from 95.67 to 98.76%) at a concentration of 0.05 mM. Larger differences in the activity of the tested compounds were observed at a concentration of 0.005 mM. The methanol solution of 5-CQA showed a higher ABTS•+ cation radical scavenging activity ($60.66 \pm 0.002\%$) than its complexes with Cu(II) and Fe(III) ions ($37.94 \pm 0.03\%$ and $46.71 \pm 0.01\%$, respectively).

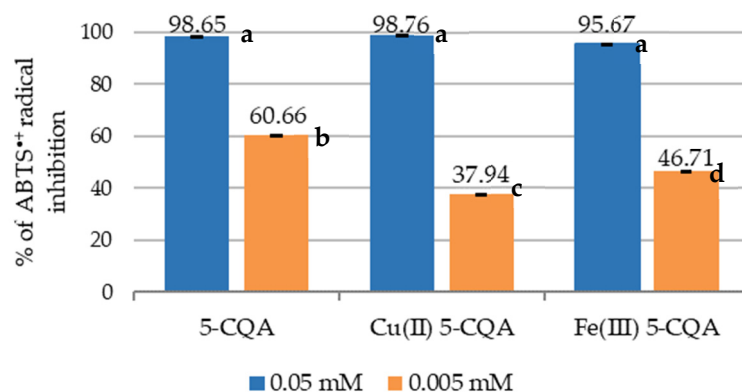


Figure 9. Antioxidant properties of 5-CQA, Cu(II) 5-CQA, and Fe(III) 5-CQA (0.05 and 0.005 mM) expressed as the ability to scavenge ABTS^{•+} cation radicals (%I). Mean values from three independent experiments \pm SDs are shown. The same letter near the means indicates no significant difference (Tukey's test, $p < 0.05$).

The hydroxyl radical (HO[•]) is a highly reactive oxygen species that is produced by the Fenton reaction [46]. The antioxidant activity of chlorogenic acid and the studied chlorogenates was measured as the ability of these compounds to scavenge HO[•] radicals. The obtained results are presented in Figure 10. In this study, 5-CQA and Cu(II) 5-CQA showed a higher percentage of HO[•] radical inhibition ($49.44 \pm 6.05\%$ and $47.19 \pm 3.56\%$, respectively) compared to Fe(III) chlorogenate (%I = $47.19 \pm 3.56\%$). No significant differences were observed between the results obtained for the individual compounds.

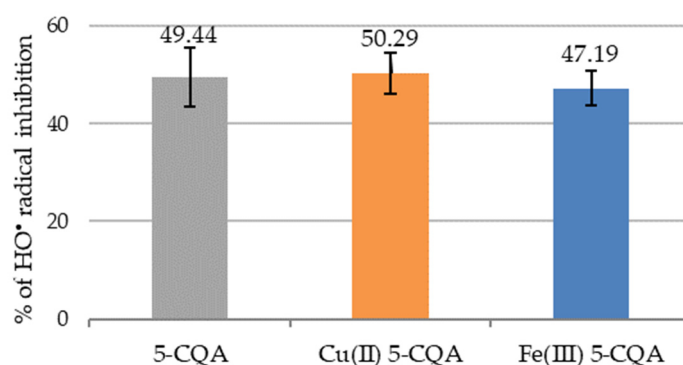


Figure 10. Antioxidant properties of 5-CQA, Cu(II) 5-CQA, and Fe(III) 5-CQA (0.1 mM) expressed as the ability to scavenge HO[•] radicals (%I). Mean values from three independent experiments \pm SDs are shown. There were no significant statistical differences between particular compounds (Tukey's test, $p < 0.05$).

The obtained results indicated that the complexation of chlorogenic acid with Cu(II) and Fe(III) ions did not significantly increase their antioxidant properties when measured in DPPH[•], HO[•], and ABTS^{•+} antiradical activity assays (for a sample concentration of 0.05 mM). The greatest differences in the results were obtained in the ABTS^{•+} cation radical assay for compounds at a concentration of 0.005 mM. In this assay, chlorogenic acid (0.005 mM) showed significantly higher radical-scavenging properties (60.66%) compared to its complexes with Fe(III) or Cu(II) ions (46.71 and 37.94%, respectively) (Figure 9). These three tests were based on mixed HAT (hydrogen atom transfer) or SET (single electron transfer) mechanisms of reaction, which more or less depend on the pH and the type of solvent. In the HAT mechanism, the bond dissociation energy is an important factor influencing the antioxidant effect, while in the SET reaction, such a parameter is the ionization potential [55,56]. In an acidic pH, the antioxidant undergoes protonation, which decreases the ionization potential and its ability to scavenge radicals, whereas in an alkaline

pH, the proton dissociation increases, which facilitates the scavenging of the radicals [20]. The stability constants for 5-CQA were the following: $pK_{a1} = 3.35$, $pK_{a2} = 8.30$, and $pK_{a3} = 12.06$ [50]. Under acidic conditions, the protonated form (AH_3) and the monoanion (AH_2^-) were the main species, whereas in a neutral or basic pH, AH_2^- and the dianion AH^{2-} were the dominant forms of 5-CQA. Above $pH \sim 11.2$, the trianionic form A^{3-} was dominant. Taking into account the experimental conditions in the DPPH, ABTS, and hydroxyl radical assays, the three species AH_3 , AH_2^- , and AH^{2-} should be considered. In the pH range of 4–8, the main form was AH_2^- (at $pH > 7$, the successive deprotonation starts, forming AH^{2-}), which is responsible for the antioxidant properties of 5-CQA in the ABTS and DPPH assays, although some authors have claimed that for the DPPH assay, the pH is irrelevant due to the use of an organic solvent (methanol, in our case) [57]. In the hydroxyl radical assay, the AH form should be predominant, which hinders the formation of complexes with metals. Therefore, there were no distinct differences in the hydroxyl radical assay in the antioxidant activity between 5-CQA and its metal complexes. In both the DPPH and ABTS assays, the antioxidant properties of 5-CQA were higher because Fe(III) and Cu(II) were coordinated through the carboxylate group, and at $pH > 7$, the deprotonation of the $-OH$ of the aromatic ring enabled the additional metal coordination through the catechol moiety. The participation of the catechol moiety in the metal coordination decreased the radical scavenging properties of the metal complexes compared to the ligand alone.

There are some other reports in the literature concerning the antiradical activity of metal complexes with chlorogenic acid. In a study by Kalinowska et al. [7], a CQA/Zn(II) complex inhibited the DPPH \bullet and ABTS \bullet^+ radical scavenging activity more than chlorogenic acid alone. The values of the IC_{50} parameter in the DPPH \bullet assay were 5.45 and 7.23 μM for the CQA/Zn(II) and CQA, respectively. In the ABTS \bullet^+ assay, at a compound concentration of 25 μM , CQA/Zn(II) inhibited ABTS \bullet^+ cation radicals by 97.65%, while CQA inhibited the radicals by 89.53% [7]. Other work by Kalinowska et al. [20] showed that Li, Na, K, Rb, and Cs ions increased the antioxidant activity of the chlorogenic acid measured in DPPH \bullet and FRAP assays compared to that of the ligand alone, but with one exception. CQA/Li in a concentration of 5 μM showed slowly lower activity (106.92 μM Fe^{2+}) than CQA in the same concentration (114.22 μM Fe^{2+}) [20].

Moreover, in the literature there are many other examples of the complexation of phenolic compounds with the transition metals Fe(III) and Cu(II). Rutin, taxifolin, (-)-epicatechin, and luteolin complexes with Fe(III) and Cu(II) ions were synthesized in the work of Kostyuk et al. [58]. In their research, it was found that the obtained flavonoid complexes (flavonoid:metal ion ratio of 1:1) showed a significantly higher scavenging power than the free ligands. For example, in their tests, rutin alone inhibited the superoxide-driven reduction of NBT by 50% at a concentration of 9.0 μM , while the IC_{50} parameters for its complexes with Cu(II) and Fe(III) ions were 0.5 μM and 2.5 μM , respectively [58]. A study conducted by Dowling et al. [59] showed that Cu(II) genistein and biochinin A complexes (flavonoid:metal ion ratio of 2:1) exhibited greater antioxidant activity against DPPH \bullet radicals than free isoflavones, while the chelation of the same ligands with Fe(III) ions increased their pro-oxidant activity against ligands [59]. It can be concluded that the chelation of phenols does not always increase antioxidant ligand properties.

3.5. Inhibition of Linoleic Acid Peroxidation Assay

The inhibitory activity of linoleic acid peroxidation by 5-CQA, Cu(II) 5-CQA, and Fe(III) 5-CQA was measured for 5 days. As shown in Figure 11, there were no significant differences in the inhibitory activity between the studied compounds on the first and second days of the experiment. An amount of $55.41 \pm 2.40\%$ inhibition by Fe(III) 5-CQA, $45.49 \pm 12.50\%$ inhibition by Cu(II) 5-CQA, and $17.38 \pm 3.44\%$ inhibition by 5-CQA were observed on the third day of measurement. The greatest differences in the activity of the compounds were observed on the fourth day of measurement, where the inhibition of linoleic acid peroxidation for Fe(III) 5-CQA was $74.60 \pm 1.24\%$; for Cu(II) 5-CQA, it was $59.09 \pm 2.36\%$; and for 5-CQA, it was $23.74 \pm 6.61\%$. On the fifth day of the experiment,

Fe(III) 5-CQA and Cu(II) 5-CQA inhibited the peroxidation of linoleic acid by $75.22 \pm 0.47\%$ and $64.87 \pm 1.48\%$, respectively, while 5-CQA only inhibited it by $23.91 \pm 4.20\%$. The results of this assay indicated that the synthesized chlorogenic acid complexes inhibited the peroxidation of linoleic acid more effectively than the ligand itself. This fact could be useful for designing compounds that are capable of extending the shelf life of oleaginous food.

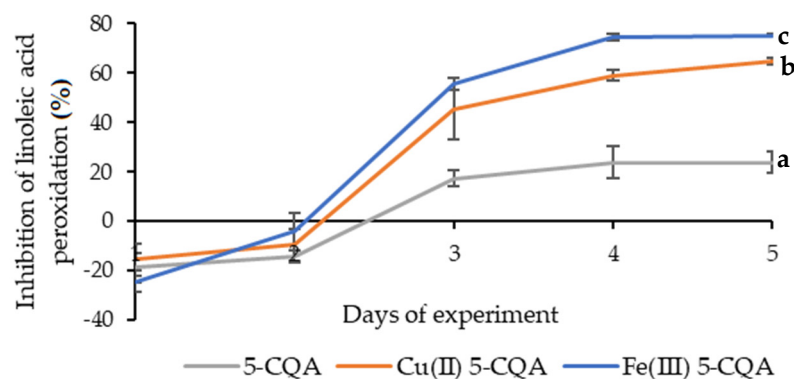


Figure 11. Linoleic acid peroxidation inhibition assay of 5-CQA, Cu(II) 5-CQA, and Fe(III) 5-CQA (0.001 M). Mean values from three independent experiments \pm SDs are shown. The same letter near the means indicates no significant difference (Tukey's test, $p < 0.05$).

3.6. Pro-Oxidant Activity Assay

The pro-oxidant activity of chlorogenic acid and its Cu(II) and Fe(III) complexes were measured for 60 min in two concentrations: 2.5 and 1.25 μ M. As shown in Figure 12, the Cu(II) chlorogenate showed the strongest pro-oxidant activity and the Fe(III) chlorogenate had the lowest activity, while the chlorogenic acid itself had pro-oxidative power between these complexes. The pro-oxidant activity increased with time, with the highest values at 60 min in almost all cases. After 60 min, 5-CQA, Cu(II) 5-CQA, and Fe(III) 5-CQA at 1.25 and 2.5 μ M concentrations increased the oxidation of Trolox by $108.46 \pm 5.31\%$ and $156.43 \pm 3.58\%$; $52.23 \pm 1.89\%$ and $93.32 \pm 13.99\%$; and $20.39 \pm 0.81\%$ and $38.81 \pm 2.30\%$, respectively. The pro-oxidant activity of Cu(II) 5-CQA and Fe(III) 5-CQA was higher than that of the 5-CQA itself, which was also observed for the Zn 5-CQA complex in the work of Kalinowska and others [7].

When subjected to certain conditions (e.g., high concentration, occurrence of metal ions), some antioxidants can have pro-oxidant properties. One of the most well-known examples would be ascorbic acid, which displays a high pro-oxidant activity at a higher concentration. This is due to the overbalance of the reducing power over its antiradical activity. Low or moderately concentrated pro-oxidants can be beneficial for the defense system, but when they occur in excess, pro-oxidants cause oxidative imbalance [60]. It was also confirmed that in an oxygen environment, the presence of Cu or Fe can lead to the formation of reactive oxygen species [61]. Furthermore, this ability can be used in cancer therapy for inducing cancer cell apoptosis, for example through hydrogen peroxide generation [62].

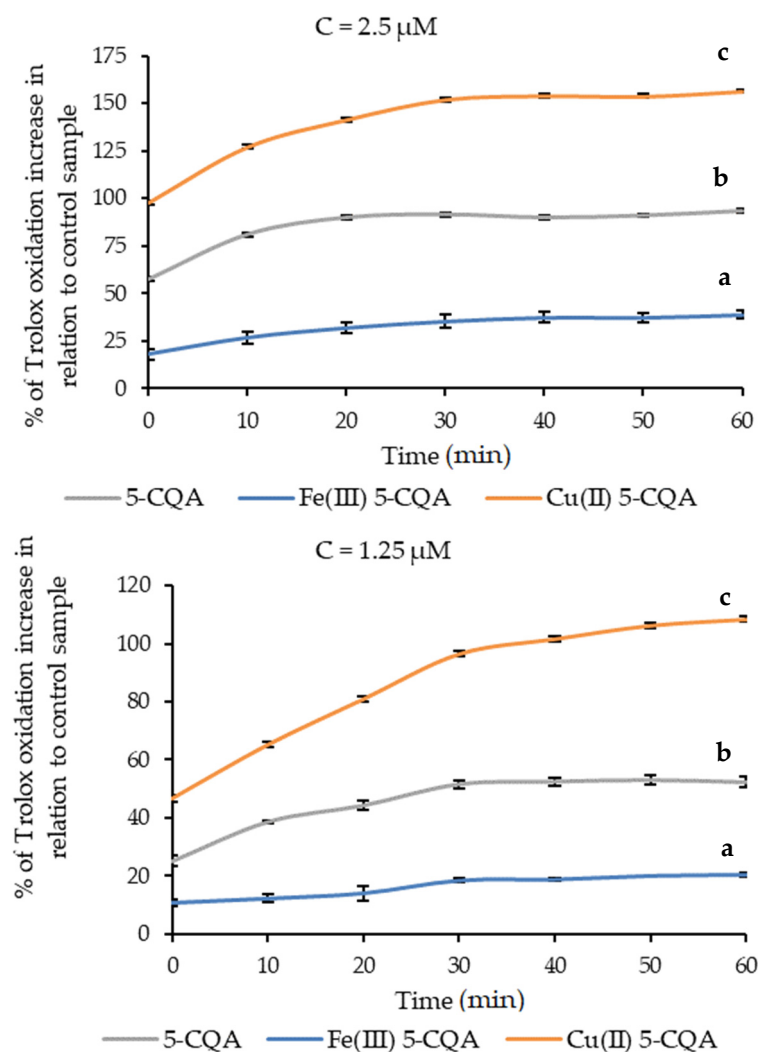


Figure 12. Pro-oxidation activity assay of 5-CQA, Cu(II) 5-CQA, and Fe(III) 5-CQA (2.5; 1.25 μ M). Mean values from three independent experiments \pm SDs are shown. The same letter near the means indicates no significant difference (Tukey's test, $p < 0.05$).

3.7. Cell Viability Assay

The influence of 5-CQA and the Fe(III) 5-CQA and Cu(II) 5-CQA complexes on the proliferation of HaCaT cells was investigated using an MTS assay (Figure 13). The obtained results indicated that 5-CQA alone does not influence the HaCaT cell viability, even in the wide concentration range of 0.15–1000 nM, and this result is comparable to other data obtained for various cell lines [54,55]. The Fe(III) 5-CQA and Cu(II) 5-CQA complexes were not toxic to cells in the tested concentration range either; however, at the highest applied concentration of 1000 nM, they reduced cell viability to $91.4 \pm 4.9\%$ and $83.9 \pm 5.1\%$, respectively, whereas CQA alone did not influence HaCaT cell viability at this concentration ($100.7 \pm 4.5\%$). The one-way ANOVA of the viability of the HaCaT cells as a function of compound concentration for 5-CQA, Fe(III) 5-CQA, and Cu(II) 5-CQA at $\alpha = 0.05$ revealed that there were no significant statistical differences in cell viability as a function of drug concentration for 5-CQA or Fe(III) 5-CQA. A statistically significant difference was found by ANOVA for the Cu(II) 5-CQA. A two-tailed unequal variance *t*-test at each drug concentration versus the control revealed that the only statistically significant difference was between the control and the highest concentration of the Cu(II) 5-CQA series. The higher reduction in cell viability caused by Cu(II) 5-CQA compared to Fe(III) 5-CQA and 5-CQA may be explained by the higher pro-oxidant properties of Cu(II) 5-CQA compared to the other studied compounds (Figure 12).

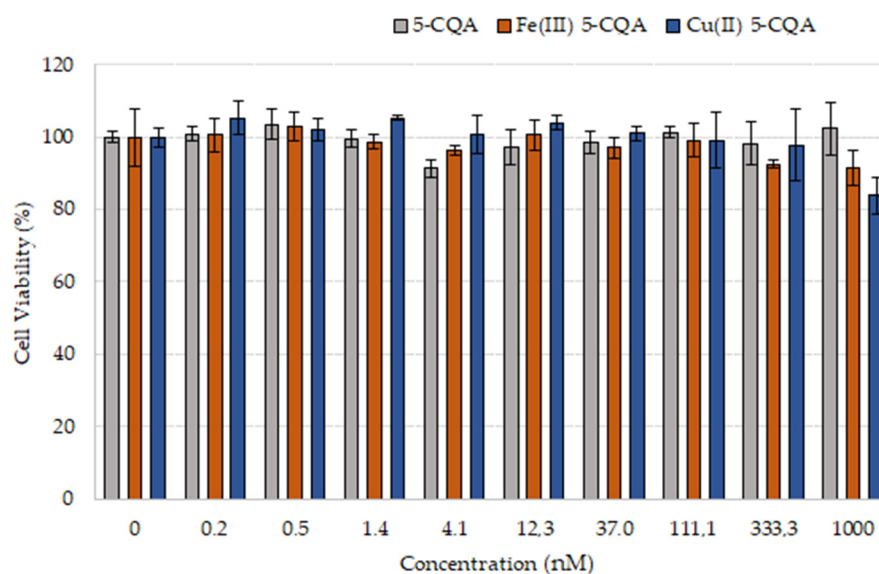


Figure 13. Viability of HaCaT cells after treatment with 5-CQA and its complexes with Fe(III) and Cu(II) ($C = 0.15$ – 1000 nM) for 24 h.

3.8. Lipophilicity Assay

The chromatographic lipophilicity parameters were collected in Table 3. Different stationary phases were selected because the mechanism of molecule separation involves different type of interactions, i.e., hydrophobic van der Waals interactions in the case of C18 and C8 alkyl-modified silica stationary phases, $\pi \rightarrow \pi^*$ interactions when the PHE phenyl-modified silica phase is applied, or hydrogen bond formation between -CN groups (in the case of the CN cyano-bonded silica phase) and HO- groups from separate molecules. On the basis of the result, it may be concluded that the Cu(II) and Fe(III) 5-CQA complexes showed significantly lower lipophilicity than the ligand alone. The compounds can be ordered according to their decreasing lipophilicity as follows: 5-CQA \rightarrow Cu(II) 5-CQA \rightarrow Fe(III) 5-CQA (determined in the C18, CN, and PHE stationary phases). The differences in the lipophilicity and solubility between 5-CQA and its Cu(II) and Fe(III) complexes may explain their different cytotoxic and even antioxidant properties as well as membrane permeability.

Table 3. Lipophilicity parameters determined by chromatographic methods (the logarithm of the retention factor, $\log k_w$) for the Cu(II) and Fe(III) chlorogenates and chlorogenic acid [20].

Compound	C18	C8	CN	PHE
	$\log k_w$			
Cu(II) 5-CQA	0.45	0.04	1.28	0.80
Fe(III) 5-CQA	0.23	0.23	0.36	0.67
5-CQA [20]	3.88	1.11	2.09	1.45

4. Conclusions

The Cu(II) and Fe(III) complexes of chlorogenic acid (5-CQA) were synthesized and studied using UV/Vis and FT-IR. The anti- and pro-oxidant properties of the obtained complexes and 5-CQA were measured using various assays (DPPH \cdot , HO \cdot , ABTS \cdot^+ , linoleic acid peroxidation inhibition, and Trolox pro-oxidation). 5-CQA and the metal complexes revealed a high antioxidant activity. The pH of the assays was an important factor determining the degree of ligand complexation by the copper and iron ions and the participation of carboxylate groups and -OH substituents from the aromatic ring in metal ion coordination. The coordination of Fe(III) and Cu(II) ions by the catechol moiety of 5-CQA decreased the radical scavenging properties of the complexes compared to those of the ligand alone. On the other hand, the Fe(III) and Cu(II) complexes with 5-CQA retained lipid peroxidation

to a greater extent than 5-CQA. Special attention should be paid to Fe(III) 5-CQA, which exhibited a lower pro-oxidant activity in the Trolox assay compared to Cu(III) 5-CQA and 5-CQA. Moreover, 5-CQA alone and its complexes with Fe(III) and Cu(II) cations were not toxic to HaCaT cells in a tested concentration range of 0.15–1000 nM after a 24 h incubation time. However, for the Fe(III) 5-CQA and Cu(II) 5-CQA compounds, a slight decrease in cell viability was observed at the highest applied concentration. Further studies are needed to explore this influence and its potential mechanism.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ma15196832/s1>, Table S1: Sources of CQA with extraction and determination methods; Table S2: Sources of different CQA isomers; Table S3: The wavenumbers, intensities and assignment of selected bands from the FT-IR spectra of Cu(II) and Fe(III) 5-CQAs and 5-CQA acid [7]; the symbols denote: ν —stretching vibrations, δ —deforming in plane and oop—out of plane bending vibrations; s—strong, m—medium, w—weak, v—very, sh—on the slope.

Author Contributions: Conceptualization, M.K. (Monika Kalinowska); methodology, M.K. (Monika Kalinowska); investigation, E.G., M.Z., M.K. (Maciej Kozłowski), G.Ś., J.S.-G., H.L. and M.P.; data curation, M.K. (Monika Kalinowska), E.G., M.Z., M.K. (Maciej Kozłowski), G.Ś., J.S.-G., H.L., M.P. and K.G.; writing—original draft preparation, E.G., M.Z., M.K. (Maciej Kozłowski), G.Ś., H.L. and J.S.-G.; writing—review and editing, W.L., M.K. (Monika Kalinowska) and K.G.; visualization, E.G., M.Z., K.G., H.L. and J.S.-G.; supervision, M.K. (Monika Kalinowska); project administration, M.K. (Monika Kalinowska); funding acquisition, W.L. All authors have read and agreed to the published version of the manuscript.

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References

1. Naveed, M.; Hejazi, V.; Abbas, M.; Kamboh, A.A.; Khan, G.J.; Shumzaid, M.; Ahmad, F.; Babazadeh, D.; FangFang, X.; Modarresi-Ghazani, F.; et al. Chlorogenic acid (CGA): A pharmacological review and call for further research. *Biomed. Pharmacother.* **2018**, *97*, 67–74. [\[CrossRef\]](#)
2. Liang, N.; Kitts, D.D. Role of Chlorogenic Acids in Controlling Oxidative and Inflammatory Stress Conditions. *Nutrients* **2015**, *8*, 16. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Yang, R.; Tian, J.; Liu, Y.; Zhu, L.; Sun, J.; Meng, D.; Wang, Z.; Wang, C.; Zhou, Z.; Chen, L. Interaction mechanism of ferritin protein with chlorogenic acid and iron ion: The structure, iron redox, and polymerization evaluation. *Food Chem.* **2021**, *349*, 129144. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Gil, M.; Wianowska, D. Chlorogenic acids—their properties, occurrence and analysis. *Ann. Univ. Mariae Curie-Skłodowska Sect. AA Chem.* **2017**, *72*, 61. [\[CrossRef\]](#)
5. Kalinowska, M.; Wróblewska, A.M.; Gryko, K. Kwasy chlorogenowe w produktach naturalnych oraz ich właściwości antyutleniające i przeciwdrobnoustrojowe. *Przem. Chem.* **2017**, *96*, 2259–2264. [\[CrossRef\]](#)
6. Wang, S.; Li, Y.; Meng, X.; Chen, S.; Huang, D.; Xia, Y.; Zhu, S. Antioxidant activities of chlorogenic acid derivatives with different acyl donor chain lengths and their stabilities during in vitro simulated gastrointestinal digestion. *Food Chem.* **2021**, *357*, 129904. [\[CrossRef\]](#)
7. Kalinowska, M.; Sienkiewicz-Gromiuk, J.; Świdorski, G.; Pietryczuk, A.; Cudowski, A.; Lewandowski, W. Zn(II) complex of plant phenolic chlorogenic acid: Antioxidant, antimicrobial and structural studies. *Materials* **2020**, *13*, 3745. [\[CrossRef\]](#)
8. Akimowicz, M.; Samsonowicz, M. Antyoksydacyjne i przeciwdrobnoustrojowe właściwości ekstraktów z suszu skórek odmian Papierówka, Kosztela oraz Gold Milenium. *Postępy Nauk. I Technol. Przem. Rolno-Spożywczego* **2021**, *75*, 5–22.
9. Zhang, Y.; Lu, Y.; Yang, Y.; Li, S.; Wang, C.; Wang, C.; Zhang, T. Comparison of non-covalent binding interactions between three whey proteins and chlorogenic acid: Spectroscopic analysis and molecular docking. *Food Biosci.* **2021**, *41*, 101035. [\[CrossRef\]](#)

10. Bajko, E.; Kalinowska, M.; Borowski, P.; Siergiejczyk, L.; Lewandowski, W. 5-O-Caffeoylquinic acid: A spectroscopic study and biological screening for antimicrobial activity. *LWT Food Sci. Technol.* **2016**, *65*, 471–479. [[CrossRef](#)]
11. Belay, A. Determination of self-associated 5-caffeoylquinic acid and its complexation with sodium hydroxide using UV-Vis spectroscopy. *Int. J. Phys. Sci.* **2010**, *5*, 459–464.
12. Suberu, J.O.; Romero-Canelón, I.; Sullivan, N.; Lapkin, A.A.; Barker, G.C. Comparative cytotoxicity of artemisinin and cisplatin and their interactions with chlorogenic acids in MCF7 breast cancer cells. *ChemMedChem* **2014**, *9*, 2791–2797. [[CrossRef](#)] [[PubMed](#)]
13. Wang, L.; Bi, C.; Cai, H.; Liu, B.; Zhong, X.; Deng, X.; Wang, T.; Xiang, H.; Niu, X.; Wang, D. The therapeutic effect of chlorogenic acid against *Staphylococcus aureus* infection through sortase A inhibition. *Front. Microbiol.* **2015**, *6*, 1031. [[CrossRef](#)] [[PubMed](#)]
14. Yun, J.E.; Lee, D.G. Role of potassium channels in chlorogenic acid-induced apoptotic volume decrease and cell cycle arrest in *Candida albicans*. *Biochim. Biophys. Acta Gen. Subj.* **2017**, *1861*, 585–592. [[CrossRef](#)]
15. Wang, W.; Wen, C.; Guo, Q.; Li, J.; He, S.; Yin, Y. Dietary Supplementation With Chlorogenic Acid Derived From *Lonicera macranthoides* Hand-Mazz Improves Meat Quality and Muscle Fiber Characteristics of Finishing Pigs via Enhancement of Antioxidant Capacity. *Front. Physiol.* **2021**, *12*, 501. [[CrossRef](#)]
16. Naem, S.; Hylands, P.; Barlow, D. Docking studies of chlorogenic acid against aldose reductase by using molgro virtual Docker software. *J. Appl. Pharm. Sci.* **2013**, *3*, 13–20.
17. Peña, Á.; Yosa, J.; Cuesta-Astroz, Y.; Acevedo, O.; Lareo, L.; García-Vallejo, F. Influence of Mg²⁺ ions on the interaction between 3,5-dicaffeoylquinic acid and HTLV-I integrase. *Univ. Sci.* **2012**, *17*, 5–15. [[CrossRef](#)]
18. Naso, L.G.; Valcarcel, M.; Roura-Ferrer, M.; Kortazar, D.; Salado, C.; Lezama, L.; Rojo, T.; González-Baró, A.C.; Williams, P.A.M.; Ferrer, E.G. Promising antioxidant and anticancer (human breast cancer) oxidovanadium(IV) complex of chlorogenic acid. Synthesis, characterization and spectroscopic examination on the transport mechanism with bovine serum albumin. *J. Inorg. Biochem.* **2014**, *135*, 86–99. [[CrossRef](#)]
19. Xu, J.; Gao, L.; Liang, H.; Zhang, S.; Lai, P.; Chen, S. Evidence for the anti-NAFLD effectiveness of chlorogenic acid as a HAT inhibitor using in vivo experiments supported by virtual molecular docking. *Phytomed. Plus* **2021**, *1*, 100055. [[CrossRef](#)]
20. Kalinowska, M.; Bajko, E.; Matejczyk, M.; Kaczyński, P.; Łozowicka, B.; Lewandowski, W. The study of anti-/pro-oxidant, lipophilic, microbial and spectroscopic properties of new alkali metal salts of 5-o-caffeoylquinic acid. *Int. J. Mol. Sci.* **2018**, *19*, 463. [[CrossRef](#)]
21. Chedea, V.S.; Tomoiagă, L.L.; Macovei, Ș.O.; Măgureanu, D.C.; Iliescu, M.L.; Bocsan, I.C.; Buzoianu, A.D.; Voşloban, C.M.; Pop, R.M. Antioxidant/Pro-Oxidant Actions of Polyphenols from Grapevine and Wine By-Products-Base for Complementary Therapy in Ischemic Heart Diseases. *Front. Cardiovasc. Med.* **2021**, *8*, 750508. [[CrossRef](#)] [[PubMed](#)]
22. Castaneda-Arriaga, R.; Pérez-González, A.; Reina, M.; Alvarez-Idaboy, J.R.; Galano, A. Comprehensive Investigation of the Antioxidant and Pro-oxidant Effects of Phenolic Compounds: A Double-Edged Sword in the Context of Oxidative Stress? *J. Phys. Chem. B* **2018**, *122*, 6198–6214. [[CrossRef](#)] [[PubMed](#)]
23. Guzman, J.D. Natural cinnamic acids, synthetic derivatives and hybrids with antimicrobial activity. *Molecules* **2014**, *19*, 19292–19349. [[CrossRef](#)] [[PubMed](#)]
24. Juan, C.A.; de la Lastra, J.M.P.; Plou, F.J.; Pérez-Lebeña, E. The Chemistry of Reactive Oxygen Species (ROS) Revisited: Outlining Their Role in Biological Macromolecules (DNA, Lipids and Proteins) and Induced Pathologies. *Int. J. Mol. Sci.* **2021**, *22*, 4642. [[CrossRef](#)]
25. Luís, Á.; Silva, F.; Sousa, S.; Duarte, A.P.; Domingues, F. Antistaphylococcal and biofilm inhibitory activities of gallic, caffeic, and chlorogenic acids. *Biofouling* **2014**, *30*, 69–79. [[CrossRef](#)]
26. Ahmed, M.A.E.; Mohanad, M.; Ahmed, A.A.E.; Aboulhoda, B.E.; El-Awdan, S.A. Mechanistic insights into the protective effects of chlorogenic acid against indomethacin-induced gastric ulcer in rats: Modulation of the cross talk between autophagy and apoptosis signaling. *Life Sci.* **2021**, *275*, 119370. [[CrossRef](#)]
27. Liu, Q.; Dong, Z.; Hao, A.; Guo, X.; Dong, W. Synthesis of highly fluorescent carbon dots as a dual-excitation ratiometric fluorescent probe for the fast detection of chlorogenic acid. *Talanta* **2021**, *221*, 121372. [[CrossRef](#)]
28. Lu, H.; Tian, Z.; Cui, Y.; Liu, Z.; Ma, X. Chlorogenic acid: A comprehensive review of the dietary sources, processing effects, bioavailability, beneficial properties, mechanisms of action, and future directions. *Compr. Rev. Food Sci. Food Saf.* **2020**, *19*, 3130–3158. [[CrossRef](#)]
29. Clifford, M.N.; Kerimi, A.; Williamson, G. Bioavailability and metabolism of chlorogenic acids (acyl-quinic acids) in humans. *Compr. Rev. Food Sci. Food Saf.* **2020**, *19*, 1299–1352. [[CrossRef](#)]
30. Santana-Gálvez, J.; Cisneros-Zevallos, L.; Jacobo-Velázquez, D.A. Chlorogenic Acid: Recent advances on its dual role as a food additive and a nutraceutical against metabolic syndrome. *Molecules* **2017**, *22*, 358. [[CrossRef](#)]
31. Joly, N.; Souidi, K.; Depraetere, D.; Wils, D.; Martin, P. Potato By-Products as a Source of Natural Chlorogenic Acids and Phenolic Compounds: Extraction, Characterization, and Antioxidant Capacity. *Molecules* **2020**, *26*, 177. [[CrossRef](#)] [[PubMed](#)]
32. Chan, E.W.C.; Lim, Y.Y.; Ling, S.K.; Tan, S.P.; Lim, K.K.; Khoo, M.G.H. Caffeoylquinic acids from leaves of *Etlingera* species (Zingiberaceae). *LWT Food Sci. Technol.* **2009**, *42*, 1026–1030. [[CrossRef](#)]
33. Awad, M.A.; de Jager, A.; van Westing, L.M. Flavonoid and chlorogenic acid levels in apple fruit: Characterisation of variation. *Sci. Hortic.* **2000**, *83*, 249–263. [[CrossRef](#)]
34. Jeszka-Skowron, M.; Sentkowska, A.; Pyrzyńska, K.; De Peña, M.P. Chlorogenic acids, caffeine content and antioxidant properties of green coffee extracts: Influence of green coffee bean preparation. *Eur. Food Res. Technol.* **2016**, *242*, 1403–1409. [[CrossRef](#)]

35. Fujioka, K.; Shibamoto, T. Chlorogenic acid and caffeine contents in various commercial brewed coffees. *Food Chem.* **2008**, *106*, 217–221. [\[CrossRef\]](#)
36. Duarte, G.S.; Pereira, A.A.; Farah, A. Chlorogenic acids and other relevant compounds in Brazilian coffees processed by semi-dry and wet post-harvesting methods. *Food Chem.* **2010**, *118*, 851–855. [\[CrossRef\]](#)
37. Perrone, D.; Farah, A.; Donangelo, C.M.; de Paulis, T.; Martin, P.R. Comprehensive analysis of major and minor chlorogenic acids and lactones in economically relevant Brazilian coffee cultivars. *Food Chem.* **2008**, *106*, 859–867. [\[CrossRef\]](#)
38. Cheng, D.; Wang, G.; Wang, X.; Tang, J.; Yu, Q.; Zhang, X.; Wang, S. Neuro-protection of Chlorogenic acid against Al-induced apoptosis in PC12 cells via modulation of Al metabolism and Akt/GSK-3 β pathway. *J. Funct. Foods* **2020**, *70*, 103984. [\[CrossRef\]](#)
39. Yoshida, K.; Ito, D.; Kondo, T. Formation of an aluminum complex of 5-O-caffeoylquinic acid with chiral molecular stacking under vacuolar condition. *Heterocycles* **2019**, *99*, 1322–1329. [\[CrossRef\]](#)
40. Kono, Y.; Kashine, S.; Yoneyama, T.; Sakamoto, Y.; Matsui, Y.; Shibata, H. Iron chelation by chlorogenic acid as a natural antioxidant. *Biosci. Biotechnol. Biochem.* **1998**, *62*, 22–27. [\[CrossRef\]](#)
41. Hynes, M.J.; O’Coinceanainn, M. The kinetics and mechanisms of reactions of iron(III) with caffeic acid, chlorogenic acid, sinapic acid, ferulic acid and naringin. *J. Inorg. Biochem.* **2004**, *98*, 1457–1464. [\[CrossRef\]](#)
42. Kiss, T.; Kozłowski, H.; Micera, G.; Erre, L.S. Cupric ion binding by dihydroxybenzoic acids. *Polyhedron* **1989**, *8*, 647–651. [\[CrossRef\]](#)
43. Kiss, T.; Nagy, G.; Pécsi, M.; Kozłowski, H.; Micera, G.; Erre, L.S. Complexes of 3,4-dihydroxyphenyl derivatives-X. Copper(II) complexes of chlorogenic acid and related compounds. *Polyhedron* **1989**, *8*, 2345–2349. [\[CrossRef\]](#)
44. van Eck, N.J.; Waltman, L. Software Survey: VOSviewer, a Computer Program for Bibliometric Mapping. *Scientometrics* **2010**, *84*, 523–538. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Kedare, S.B.; Singh, R.P. Genesis and development of DPPH method of antioxidant assay. *J. Food Sci. Technol.* **2011**, *48*, 412–422. [\[CrossRef\]](#)
46. Saravanakumar, A.; Ganesh, M.; Peng, M.M.; Aziz, A.S.; Jang, H.T. Comparative antioxidant and antimycobacterial activities of *Opuntia ficus-indica* fruit extracts from summer and rainy seasons. *Front. Life Sci.* **2015**, *8*, 182–191. [\[CrossRef\]](#)
47. Dong, J.-W.; Cai, L.; Xing, Y.; Yu, J.; Ding, Z.-T. Re-evaluation of ABTS Assay for Total Antioxidant Capacity of Natural Products. *Nat Prod Commun* **2015**, *10*, 2169–72. [\[CrossRef\]](#)
48. Kikuzaki, H.; Nakatani, N. Antioxidant Effects of Some Ginger Constituents. *J. Food Sci.* **1993**, *58*, 1407–1410. [\[CrossRef\]](#)
49. Zeraik, M.L.; Petrônio, M.S.; Coelho, D.; Regasini, L.O.; Silva, D.H.S.; da Fonseca, L.M.; Machado, S.A.S.; Bolzani, V.S.; Ximenes, V.F. Improvement of pro-oxidant capacity of protocatechuic acid by esterification. *PLoS ONE* **2014**, *9*, e110277. [\[CrossRef\]](#)
50. Cedrowska, E.; Pruszyński, M.; Gawęda, W.; Zuk, M.; Krysiński, P.; Bruchertseifer, F.; Morgenstern, A.; Karageorgou, M.A.; Bouziotis, P.; Bilewicz, A. Trastuzumab Conjugated Superparamagnetic Iron Oxide Nanoparticles Labeled with ²²⁵Ac as a Perspective Tool for Combined α -Radioimmunotherapy and Magnetic Hyperthermia of HER2-Positive Breast Cancer. *Molecules* **2020**, *25*, 1025. [\[CrossRef\]](#)
51. Ameziane, J.; Aplincourt, M.; Dupont, L.; Heirman, F.; Pierrard, J.-C. Thermodynamic stability of copper (II), manganese (II), zinc (II) and iron. (III) complexes with chlorogenic acid. *Bull. Soc. Fr.* **1996**, *133*, 243–249.
52. Mahal, H.S.; Kappor, S.; Satpati, S.K.; Mukherjee, T. Radical scavenging and catalytic activity of metal-phenolic complexes. *J. Phys. Chem. B* **2005**, *109*, 24197–24202. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Milić, S.Z.; Potkonjak, N.I.; Gorjanović, S.Ž.; Veljović-Jovanović, S.D.; Pastor, F.T.; Sužnjević, D.Ž. A polarographic study of chlorogenic acid and its interaction with some heavy metal ions. *Electroanalysis* **2011**, *23*, 2935–2940. [\[CrossRef\]](#)
54. Zheng, C.D.; Li, G.; Li, H.Q.; Xu, X.J.; Gao, J.M.; Zhang, A.L. DPPH-scavenging activities and structure-activity relationships of phenolic compounds. *Nat. Prod. Commun.* **2010**, *5*, 1759–1765. [\[CrossRef\]](#)
55. Liang, N.; Kitts, D.D. Antioxidant property of coffee components: Assessment of methods that define mechanism of action. *Molecules* **2014**, *19*, 19180–19208. [\[CrossRef\]](#) [\[PubMed\]](#)
56. Mader, E.A.; Davidson, E.R.; Mayer, J.M. Large ground-state entropy changes for hydrogen atom transfer reactions of iron complexes. *J. Am. Chem. Soc.* **2007**, *129*, 5153–5166. [\[CrossRef\]](#) [\[PubMed\]](#)
57. Ozgen, M.; Reese, R.N.; Tulio, A.Z.; Scheerens, J.C.; Miller, A.R. Modified 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2,2'-diphenyl-1-picrylhydrazyl (DPPH) methods. *J. Agric. Food Chem.* **2006**, *54*, 1151–1157. [\[CrossRef\]](#) [\[PubMed\]](#)
58. Kostyuk, V.A.; Potapovich, A.I.; Strigunova, E.N.; Kostyuk, T.V.; Afanas’ev, I.B. Experimental evidence that flavonoid metal complexes may act as mimics of superoxide dismutase. *Arch. Biochem. Biophys.* **2004**, *428*, 204–208. [\[CrossRef\]](#) [\[PubMed\]](#)
59. Dowling, S.; Regan, F.; Hughes, H. The characterisation of structural and antioxidant properties of isoflavone metal chelates. *J. Inorg. Biochem.* **2010**, *104*, 1091–1098. [\[CrossRef\]](#)
60. Dlamini, L.M.; Tata, C.M.; Djuidje, M.C.F.; Ikhile, M.I.; Nikolova, G.D.; Karamalakova, Y.D.; Gadjeva, V.G.; Zheleva, A.M.; Njobeh, P.B.; Ndinteh, D.T. Antioxidant and prooxidant effects of *Piptadeniastrum africanum* as the possible rationale behind its broad scale application in African ethnomedicine. *J. Ethnopharmacol.* **2019**, *231*, 429–437. [\[CrossRef\]](#)
61. Sakihama, Y.; Cohen, M.F.; Grace, S.C.; Yamasaki, H. Plant phenolic antioxidant and prooxidant activities: Phenolics-induced oxidative damage mediated by metals in plants. *Toxicology* **2002**, *177*, 67–80. [\[CrossRef\]](#)
62. Forester, S.C.; Lambert, J.D. The role of antioxidant versus pro-oxidant effects of green tea polyphenols in cancer prevention. *Mol. Nutr. Food Res.* **2011**, *55*, 844–854. [\[CrossRef\]](#) [\[PubMed\]](#)