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Article

Co(II) Complex of Quercetin–Spectral, Anti-/Pro-Oxidant and Cytotoxic Activity in HaCaT Cell Lines

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Abstract: In this study a cobalt(II) complex of quercetin was synthesized in the solid state with the general formula $\text{Co}(\text{C}_{15}\text{H}_9\text{O}_7)_2 \cdot 2\text{H}_2\text{O}$. The FT-IR, elemental analysis, and UV/Vis methods were used to study the composition of the complex in a solid state and in a water solution. The anti-/pro-oxidant activity of quercetin and the Co(II) complex was studied by means of spectrophotometric DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (ferric reducing antioxidant activity) and Trolox oxidation assays. The cytotoxicity of quercetin and Co(II)-quercetin complex in HaCat cell lines was then established.

Keywords: quercetin; metal complexes; Co(II); FT-IR; UV/Vis; antioxidant; pro-oxidant; cytotoxic; HaCaT



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1. Introduction

Flavonoids are a category of plant hydroxylated phenolic compounds with an aromatic ring structure and include quercetin [1–3]. The highest concentrations of quercetin were reported in unsweetened cocoa powder (20.10 mg/100 g); fruits such as apples (with skin 4.42 mg/100 g) and raw cranberries (14.00 mg/100 g); and vegetables such as raw onions (13.27 mg/100 g), raw broccoli (3.21 mg/100 g), and raw spinach (4.86 mg/100 g). The concentration of quercetin in beverages such as green tea (2.69 mg/100 g), black tea (1.99 mg/100 g), and red wine (0.84 mg/100 g) was also determined [4].

Quercetin possesses a variety of biological properties (Figure 1), but low water solubility and bioavailability limits its use as a drug or food supplement [5–9]. However, new formulations of quercetin, such as metal complexes of quercetin, may be able to overcome these limitations.

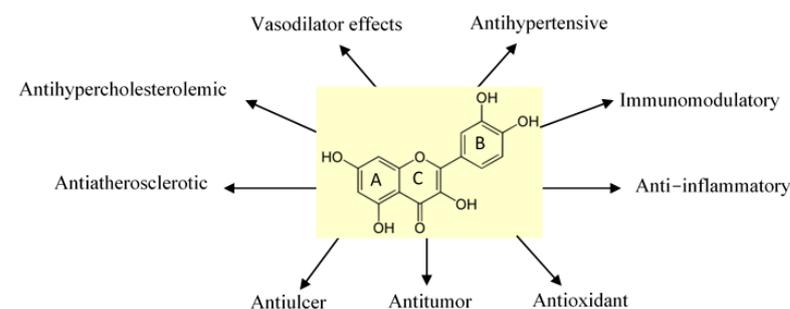


Figure 1. Biological properties of quercetin [5–9].

According to the literature, quercetin has a protective effect against oxidant damage (lipid peroxidation, DNA and protein damage). Oxidant damage can contribute to the development of many of civilization's diseases such as diabetes, coronary artery disease, Alzheimer's disease, Parkinson's disease or even cancer [10,11]. Quercetin is probably the most powerful scavenger of reactive oxygen species and reactive nitrogen species [12]. In certain cases, it is more powerful than vitamin C, due to the 3-OH and 3', 4'-catechol groups, proving to be ten times stronger for peroxy-nitrite than the known peroxy-nitrite scavenger, ebselen [13]. The mechanism of action for quercetin can include increasing antioxidant defense and the inhibition of enzymes involved in the generation of reactive oxygen species (ROS) or scavenging ROS [14]. Recently, there have been many reports in the literature regarding the high antioxidant properties of metal ion complexes with quercetin, e.g., copper(II), iron(II) [15,16], cobalt(II), cadmium(II) [17], magnesium(II) [18], and nickel(II)-quercetin complexes [19]. Cu(II), Fe(II), Co(II) and Cd(II) complexes of quercetin showed higher antioxidant properties than ligands alone [15,16,19–21], whereas for Pb(II) and Sb(II)-quercetin complexes, the opposite effect was reported [22,23]. The type of metal coordination by quercetin may explain the different antioxidant properties of these complexes compared to ligand.

Quercetin is one of the most widely investigated polyphenols with well-known anticancer properties. Its anti-proliferative mechanisms include, modulating apoptosis-inducing factors, and surviving signaling pathways. The conjugation of quercetin and transition metal ions promotes its anticancer activity due to the favorable geometric spatial orientation of the active site, with the addition of the metal ion [24]. Tan and others [24] described the anticancer activity of a Ni(II)-quercetin complex, suggesting that its mechanism of action relies on DNA intercalation. Lee and Tuyet [25] investigated the activity of a Zn(II)-quercetin complex in relation to human bladder cancer cells (BFTC-905), where the concentration of $\geq 12.5 \mu\text{M}$ effectively inhibited their invasiveness. In another study, a Ge(IV)-quercetin complex showed cytotoxicity in SPC-A-1, EC9706, HeLa and PC-3 cancer cell lines [26]. As reported in [24], the DNA intercalation of metal-quercetin complexes is probably the main mechanism of their anticancer properties. The heterobimetallic complexes, in the potential cytotoxic therapy, are noteworthy, and include Cu(II)/Sn(IV)-quercetin and Zn(II)/Sn(IV)-quercetin. Their unique mechanism of action is a dual mode of binding to DNA and presents cleavage properties. High DNA binding affinity is achieved via: (a) electrostatic interactions of Sn(IV) ions coordinating the oxygen atoms of the phosphate backbone and (b) covalent binding of Cu(II)/Zn(II) to N-3/N-7 nucleobase positions [26]. Both of the complexes worked actively against PC-3, HL-60, HCT-15, HeLa, Hop62, U373MG and A2780 human cancer cell lines [27]. The results obtained by Zhou and others [28] confirm that Zn(II) and Cu(II) complexes were more cytotoxic than quercetin alone in PC3, Skor3, BGC-823, Bel-7402, KB and HL-60 cell lines. Moreover, recent findings propose that quercetin is able to trigger apoptosis in several tumor cells via its pro-oxidant properties, due to its generation of reactive oxygen species [29]. Therefore, both anti- and pro-oxidant activities of quercetin should be considered in relation to its tumoricidal effects.

Because quercetin possesses good chelating properties many papers describe the synthesis of transition metal complexes with quercetin and their interesting biological properties, as well as their possible application in pharmacy, medicine, food technology and biotechnology [30]. Cobalt(II) stimulates the antioxidant defense and supports anti-inflammatory processes [31]. Moreover, many of the Co(II) complexes revealed interesting biological properties, including antibacterial, antiviral and antioxidant properties [32]. Although it is an essential microelement in animals, for health, e.g., as a component of Vitamin B12, an excess of cobalt ions is well-known for its toxicity [33]. For example, the exposure of rats to cobalt chloride leads to oxidative stress, observed through a significant increase of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) levels, and conversely, reduced nitric oxide (NO) levels. Stress is also observed through the reduced activity of glutathione peroxidase (GPx) and reduced glutathione (GSH) content in heart and kidney tissues [34]. However, it has been noted that treatment with quercetin

and/or vitamin C reverses the effects of cobalt chloride on MDA, H_2O_2 and NO (where a synergistic effect of Q and vitamin C was observed) and strengthens the antioxidant defense system. In addition, the treatment of rats with a combination of quercetin and vitamin C resulted in a significant ($p < 0.05$) decrease in systolic, diastolic and the mean blood pressure in rats compared to those exposed to cobalt chloride alone [34]. Therefore, the study of metal-transition–quercetin complexes are very important, in order to establish whether the physico-chemical and biological properties (e.g., solubility, bioavailability, antioxidant) of a parent ligand can be changed through its complexation with metal ions. The reported metal-quercetin complexes possessed different metal:ligand ratios of 1:1, 1:2, 2:1, and 3:2 depending on the pH of the solution and the number of reagents [27–33]. In this study the cobalt(II) complex of quercetin was synthesized in pH = 7.4. The FT-IR, elemental analysis, and the UV/Vis methods were applied to study the composition in its solid state and solution. The anti-/pro-oxidant activity of the Co(II)-quercetin complex was studied by means of DPPH, FRAP and Trolox oxidation assays and then compared with the antioxidant properties of ligand alone. The HaCaT human immortalized keratinocyte cell line has been chosen for the cellular toxicity test in mammalian cell culture. The antioxidant and cytotoxic properties of the Co(II)-quercetin complex and quercetin were compared and discussed in terms of their potential application.

2. Materials and Methods

2.1. Materials

All chemicals were analytically pure and were used without further purification. Quercetin, cobalt chloride ($CoCl_2 \cdot 6H_2O$), sodium hydroxide (NaOH), DPPH (2,2-diphenyl-1-picrylhydrazyl), iron(II), sulfate ($FeSO_4 \cdot 7H_2O$), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), hydrogen peroxide (H_2O_2), phosphate buffer pH = 7, and horseradish peroxidase (HRP) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Methanol was sourced from Merck (Darmstadt, Germany). The fetal bovine serum was purchased from Gibco (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

The HaCaT cells obtained from CLS Cell Lines Service GmbH (Eppelheim, Germany) were cultured in a DMEM medium supplemented with 4.5 g/L glucose, 2 mM L-glutamine, and fetal bovine serum, 10%. An assay based on the neutral red's (3-amino-7-dimethylamino-2-methylphenylnosine hydrochloride) absorption capacity was selected [35] was selected for the cytotoxicity assessment of the tested compounds. The neutral red (N4638, Sigma Aldrich, Darmstadt, Germany) sterile solution in PBS (5 mg/mL) was added to the cell culture medium for a final concentration of 50 μ g/mL and kept overnight at 37 °C before it was added to the cell culture.

2.2. Synthesis

Quercetin was dissolved in methanol and mixed with an aqueous solution of $CoCl_2$ (0.1M), with a stoichiometric molar ratio of 1:2 (metal ion:ligand). The pH was adjusted to 7.4 through the addition of NaOH (0.1 M). The precipitate occurred immediately. It was filtered, washed several times with distilled water and dried at room temperature. The results of the elementary analysis for the cobalt(II) complex of quercetin gave the formula $Co(C_{15}H_9O_7)_2 \cdot 2H_2O$: %C = 50.75 (calc. %C = 51.66), %H = 3.21 (calc. %H = 3.32). The yield of reaction was 42%.

2.3. Spectral Studies

The FT-IR spectra of the solid samples as KBr pellets were recorded on an Alfa Bruker spectrometer (Bremen, Germany) in the spectral range of 400–4000 cm^{-1} . UV/VIS spectra were recorded in the range of 200–500 nm using the UV/VIS/NIR Agilent Carry 5000 spectrophotometer (Santa Clara, CA, USA). The mole-ratio method was used for the determination of the metal–ligand stoichiometry. 1 mL of quercetin solution (1 mM in Tris-HCl, pH = 7.4) and an increasing amount of aqueous solution of $CoCl_2$ (10 mM; from 0 to 1 mL) were added to twelve volumetric flasks and diluted with Tris-HCl (pH = 7.4) to a

total volume 10 mL. The UV/Vis spectra of these solutions were registered in the range of 200–500 nm. All measurement were carried out at room temperature and taken in five repetitions for three independent experiments.

2.4. Anti-/Pro-Oxidant Studies

The DPPH assay was carried out according to the spectroscopic method described in [36]. The methanolic solution of quercetin or Co(II)-quercetin complex (0.1 mM) and 2 mL of methanolic solution of DPPH• (2,2-diphenyl-1-picrylhydrazyl) radical (60 µM), were mixed and incubated in the dark, at room temperature for 1 h. The final concentrations of tested substances were in the range 0.5–20 µM. The absorbance of the samples was measured by the use of UV/VIS Agilent Cary 5000 spectrophotometer (Santa Clara, CA, USA) at a 516 nm wavelength against methanol as blank. The percentage (%I) of DPPH• radical scavenging activities in the tested substances was calculated using the following formula:

$$\%I = \left(\frac{A_{control} - A_{sample}}{A_{control}} \right) \times 100\% \quad (1)$$

where %I is the percent of inhibition of DPPH• radical; $A_{control}$ is the absorbance of the control sample; and A_{sample} is the absorbance of the tested substance. The concentrations of the tested compounds were plotted against the percentages of inhibition, and the IC_{50} values were determined using a linear regression analysis.

The FRAP assay shows the ferric-reducing antioxidant activity of the substance [37]. 0.3 M acetate buffer (pH 3.6), 10 mM of 2,4,6-tripyridyl-s-triazine (TPTZ; in 40 mM HCl) and 20 mM of $FeCl_3$ (in water) were mixed in a volumetric ratio of 10:1:1. Next, 3 mL of the FRAP mixture and 0.4 mL of the tested substance (50 µM) were mixed. After 7 min the absorbance was measured at 595 nm against blank. The blank was 3 mL of FRAP mixture and 0.4 mL of methanol. The antioxidant activity was expressed as a FRAP value (Fe^{2+} equivalents) (µM) using the calibration curve prepared for $FeSO_4$ ($y = 3.296.9x - 0.0331$; $R^2 = 0.9997$).

The pro-oxidant activity was measured as the rate of oxidation of Trolox [38]. The following solutions were mixed in test tubes: 0.5 mL of Trolox ($C = 100 \mu M$), 0.5 mL of H_2O_2 ($C = 50 \mu M$), 0.5 mL of horseradish peroxide ($C = 0.01 \mu M$) in phosphate buffer (pH = 7), 0.05 mL of the tested substance (µM), and 0.495 mL of distilled. The absorbance measurements against the phosphate buffer were made every 10 min for 50 min (at 272 nm). The control sample contained 0.05 mL of pure methanol instead of the tested substance.

All anti-/pro-oxidant assays were taken in five repetitions for three independent experiments. The results were expressed as the mean of the values obtained for the replications. The averages, standard deviation calculations, and graphs were performed calculated with Microsoft Excel 2019.

2.5. Cell Viability Test

A day before the experiment, HaCaT cells were seeded in 96-well plates at a density of 10000 cells per well, in 100 µL of DMEM medium. Quercetin (Q) and its cobalt complex (Co-Q) were dissolved in DMSO, to the highest concentrations possible (10 mg Q ad 200 µL, 6 mg Co-Q ad 2000 µL). Next, the obtained DMSO solutions were mixed with a cell culture medium in the proportion of 1:100, to obtain the highest working drug solutions. The DMSO content in the cell culture medium did not exceed 1%. These solutions were further diluted by a factor of 2 to obtain the decreasing concentration of the compounds. Then, the cell medium from the 96-well plate cultures was replaced with the working solutions of Q or Co-Q, and the cells were incubated for 24 h. After this time period, the medium was removed, and cells were incubated for 4 h in a medium containing 50 µg/mL of neutral red (NR). The NR-containing medium was removed and the cultures were rinsed twice with phosphate buffered saline (PBS). Cells were treated with a solution of 50% alcohol and 1% acetic acid in water to lyse the cells and dissolve the absorbed dye. The preparations were mixed in a shaker for 10 min and the fluorescence was measured

in excitation and emission wavelengths of 530 nm and 645 nm, respectively. Experiments were performed in 4–6 repetitions. As a negative control (background), cell-culture-free wells were measured. As a positive control, wells containing untreated cell cultures were measured. The average, standard deviation calculation, and graphs were completed using Microsoft Excel 2019.

3. Results

3.1. Spectral Analysis

The assignment of selected bands, from the FT-IR spectra of the quercetin and the Co(II) complex of quercetin, are gathered in Table 1 and shown in Figure 2. The strong broad-band derived from the stretching vibrations of the -OH groups were located in the spectral range of 3426–3250 cm^{-1} . The strong band assigned to the stretches of the C=O in the quercetin molecule (1672 cm^{-1}) was slightly shifted towards a lower wavenumber in the spectra of the Co(II) complex. The stretching $\nu(\text{OH})$ and $\nu(\text{C}=\text{O})$ vibrations moved towards lower wavenumbers due to the breakdown of the inter- and intramolecular hydrogen bonds, and a metal complex formation with the participation of carbonyl and hydroxyl groups. Moreover, such an occurrence in the FT-IR spectra of the complex, in the bands assigned to the stretching vibrations of the C-O catechol group at 1460 and 1422 cm^{-1} and Co(II)-O, was caused by the participation of catechol moiety in the metal ion coordination. The other bands, which were derived from the aromatic ring of the ligand, changed their position as well when comparing the spectra of quercetin to its metal complex.

Table 1. The wavenumbers and intensity of selected bands from the FT-IR and FT-Raman spectra of studied compounds.

Assignment	Quercetin		Co(II)-Quercetin	
	IR	Int.	IR	Int.
$\nu(\text{OH})$ *	3426–3291	s	3397–3266	s
$\nu(\text{C}=\text{O})$	1672	s	1663, 1655	s
ν ring	1614	vs	1611	vs
ν ring	1514	vs	1522	vs
$\nu(\text{C}-\text{O})$ catechol group	-		1460	m
ν ring	1429	s	1449	s
$\nu(\text{C}-\text{O})$ catechol group	-		1422	s
$\beta(\text{C}-\text{OH})$	1362	vs	-	
$\beta(\text{CH})$	1317	s	1317	s
$\nu(\text{C}-\text{O}-\text{C})$	1244	vs	1261	vs
$\beta(\text{OH})$	1213	vs	1210	m
$\nu(\text{C}-\text{CO}-\text{C}) + \beta(\text{C}-\text{CO}-\text{C})$	1165	vs	1167	s
$\nu(\text{Co(II)-O})$			604	w

* symbols denote: ν —stretching vibrations, β —deforming in-plane vibrations; int—intensity, m—medium, s—strong, vs—very strong.

In the UV/Vis spectrum of quercetin, recorded in a Tris-HCl buffer solution with pH = 7.4 and concentration $C = 5 \times 10^{-5}$ M (Figure 3), two main absorption bands were observed corresponding to the $\pi-\pi^*$ transitions. Band I, present at the wavelength $\lambda_{\text{max}} = 375$ nm, corresponds to the $\pi-\pi^*$ transitions in the ring marked as B, and band II present at the wavelength $\lambda_{\text{max}} = 256$ nm, corresponds to the $\pi-\pi^*$ electron transition in ring A [39]. The intensity of the bands in the UV/Vis spectra of Co(II)-quercetin complex dropped significantly, and a slight shift of band II towards longer wavelengths and a shift of band I towards shorter wavelengths was observed.

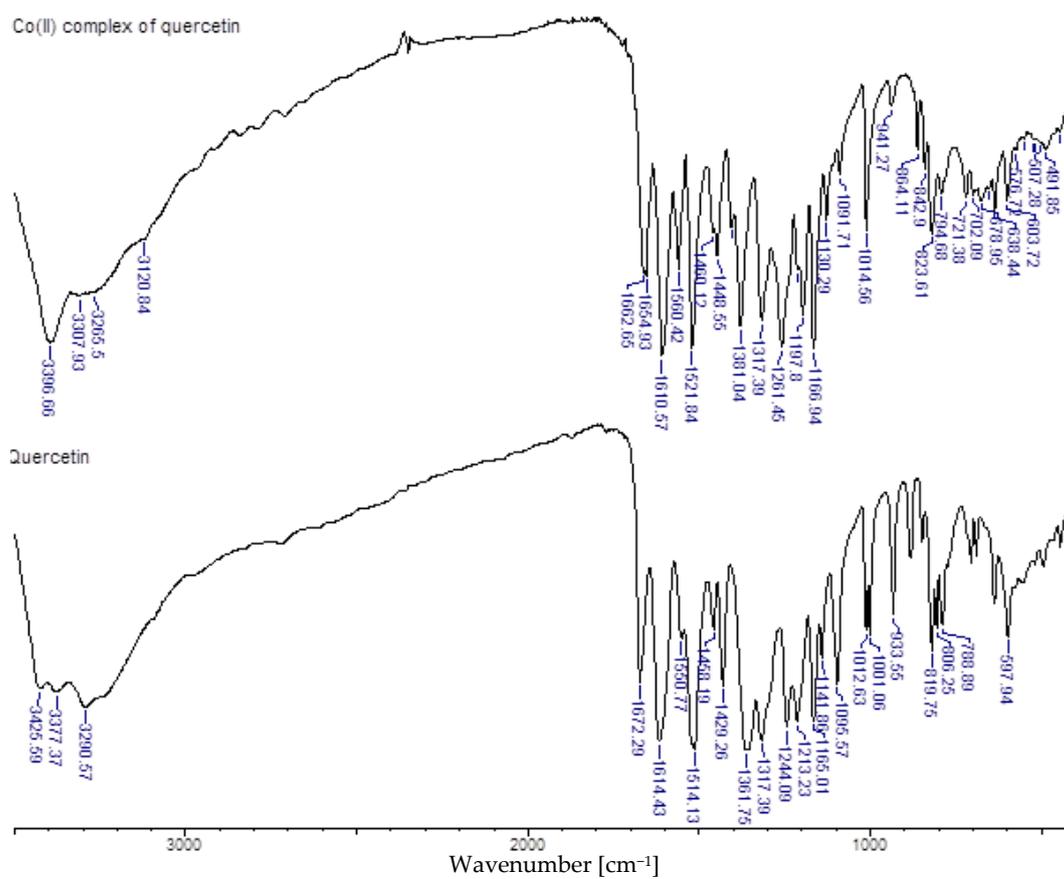


Figure 2. The FT-IR spectra of Co(II) complex of quercetin and quercetin registered in the range 4000–400 cm⁻¹.

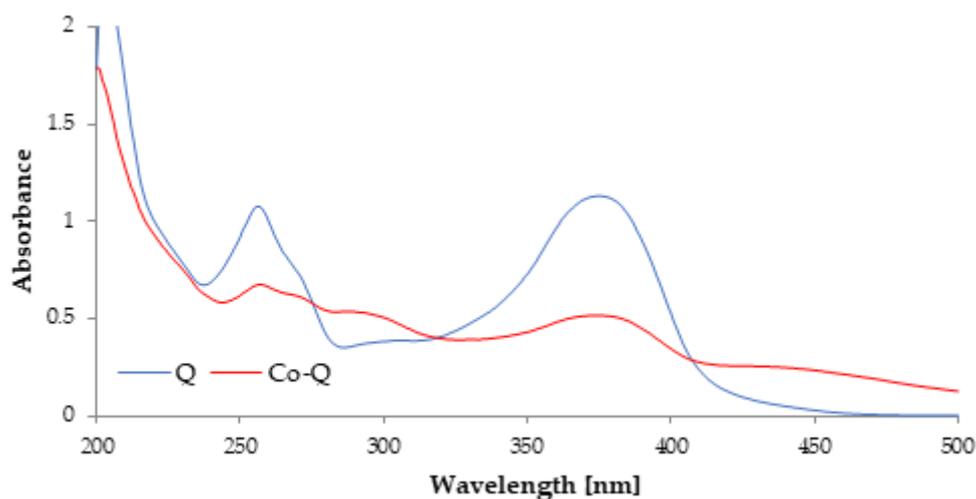


Figure 3. UV spectra for quercetin (Q) and cobalt(II) complex of quercetin (Co-Q) at the concentration $C = 5 \cdot 10^{-5}$ M registered in Tris-HCl for (pH = 7.4).

In order to establish the metal-ion–ligand ratio in the solution, the molar ratio method was applied. Figure 4 shows the dependence of the absorbance maxima (for the I band; 375 nm), on the composition of the Co(II)-quercetin complex. The analysis showed that in the solution, at pH = 7.4, the complex is present in the composition at a ratio of 1:2 (metal:ligand). Similarly in the solid state, the complex shows a molar ratio of 1:2 (metal:ligand), as was shown by an elemental analysis.

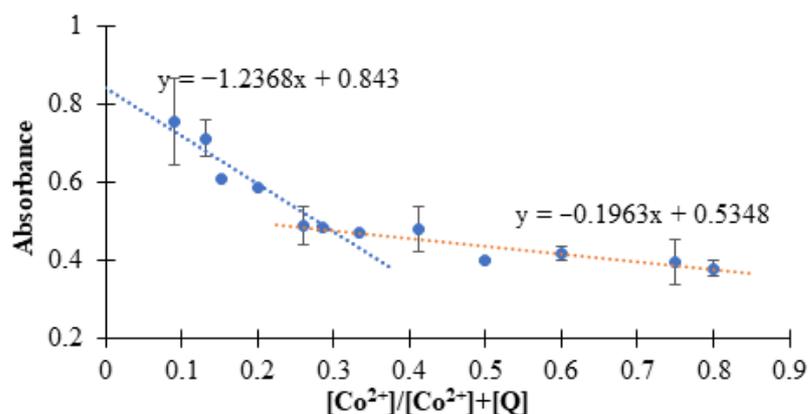


Figure 4. Curve of the dependence of the maximum absorbance in the UV/Vis electronic spectra on the composition of the cobalt(II)-quercetin complex registered in Tris-HCl buffer (pH = 7.4).

The analysis of infrared spectra of the cobalt with quercetin complex indicates the participation of the carbonyl group and the hydroxyl group, from the B ring, in the formation of the complex. Moreover, the decrease in the absorbance of band I and II in the UV/Vis spectra of the complex also suggests the participation of catechol moiety and the carboxyl group in metal ion bonding [40]. Having taken into account the results of the elemental analysis (1:2 molar ratio of metal:ligand), the proposed structure of Co(II)-quercetin is shown in Figure 5. The central ion attaches two ligand molecules via chelate bonds involving the carbonyl group and the deprotonated hydroxyl group of the quercetin pyron ring. The central ion also co-ordinates the two water molecules. The way in which the ligand coordinates metal is the same as described by other authors [41]. The catechol moiety is probably engaged in the metal bonding and may therefore lead to polymeric structures being formed.

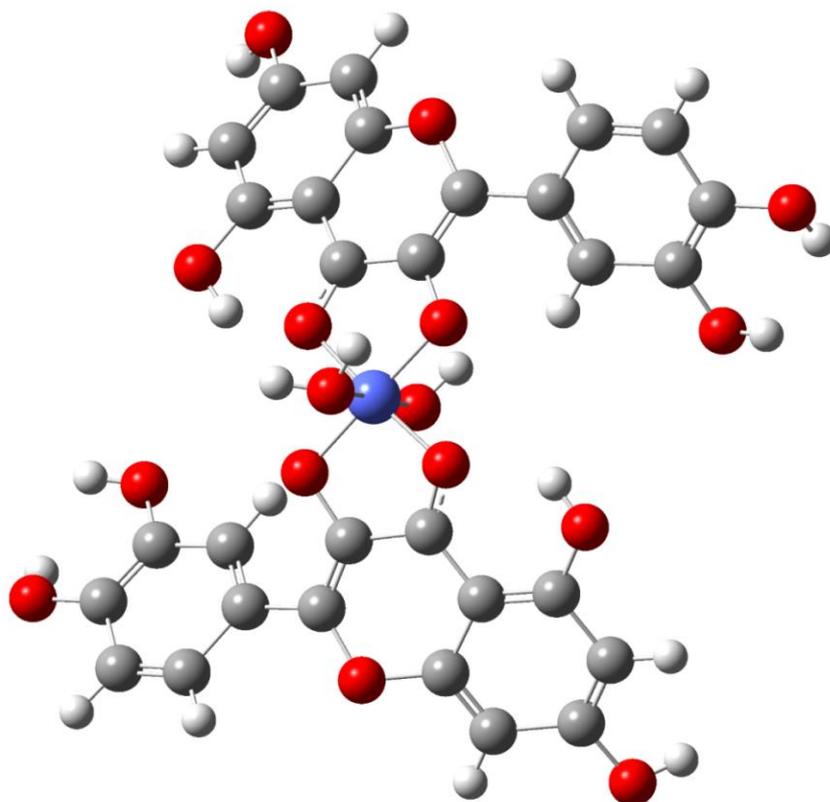


Figure 5. Proposed structure of dehydrated cobalt complex with quercetin.

3.2. Antioxidant Activity

The antioxidant activity of the Co(II) complex of quercetin was studied by means of DPPH and FRAP spectrophotometric assays. The first relied on the reduction of DPPH• radicals by antioxidants. With the increase in the concentration of quercetin and the Co(II)-quercetin complex, the antiradical activity against DPPH• increased too (Figure 6). The determined value of IC₅₀ for quercetin was 3.88 ± 0.06 μM, whereas for the Co(II) complex it was 2.01 ± 0.25 μM. This means that its complexation with Co(II) increased the antiradical properties of quercetin (measured in the DPPH assay). According to the data, both synthesized Cu(II) and Fe(II) complexes with quercetin showed stronger antioxidant activity compared to the flavonoid alone in the DPPH assay [16]. In another study, Co(II)-quercetin and Cd(II)-quercetin complexes showed a higher antioxidant activity measured in the DPPH assay than in the ligand, i.e., 74.20, 82.31, and 48.43%, respectively (after 30 min, C = 0.01 M) [17]. In the work conducted by Raza et al., the DPPH• radical scavenging activity of Fe(II)-quercetin after a 30 min reaction was approximately 97%, while for quercetin it was only about 85% (C = 20 μM) [15]. Bukhari et al. reported a greater antioxidant activity of the Co(II)-quercetin complex compared to the flavonoid alone. For example, in the DPPH assay conducted by the researchers, the % of the DPPH• remaining for quercetin was equal to around 80%, while for the complex it was around 50% (C = 4 μM) [20]. In their other work, Cu(II)-quercetin complex also showed higher antioxidant activity, measured in the DPPH assay, than the ligand, e.g., the percentage of DPPH• remaining with the complex was around 50%, and for quercetin it was around 90% (C = 4 μM) [21]. However, there are also reports in the literature of reduced antioxidant activity of quercetin after complexation with metals. For example, in the work of Ravichandran et al., quercetin showed higher DPPH• radical scavenging activity than the Pb(II)-quercetin complex. In their research, after 15 min of incubation, it was found that quercetin inhibits DPPH• radicals by 4.08, 23.90, and 30.10%, while Pb(II)-quercetin only inhibits them by 1.2, 4.69, and 5.8%, at concentrations of 5, 10, and 15 μM, respectively [22]. In a study conducted by Tong et al., a Sb(II)-quercetin complex was found to have lower antioxidant activity than the ligand alone. The IC₅₀ values obtained in the DPPH• assay were equal to 13.46 mg/L and 3.78 mg/L, for the complex and the ligand, respectively [23].

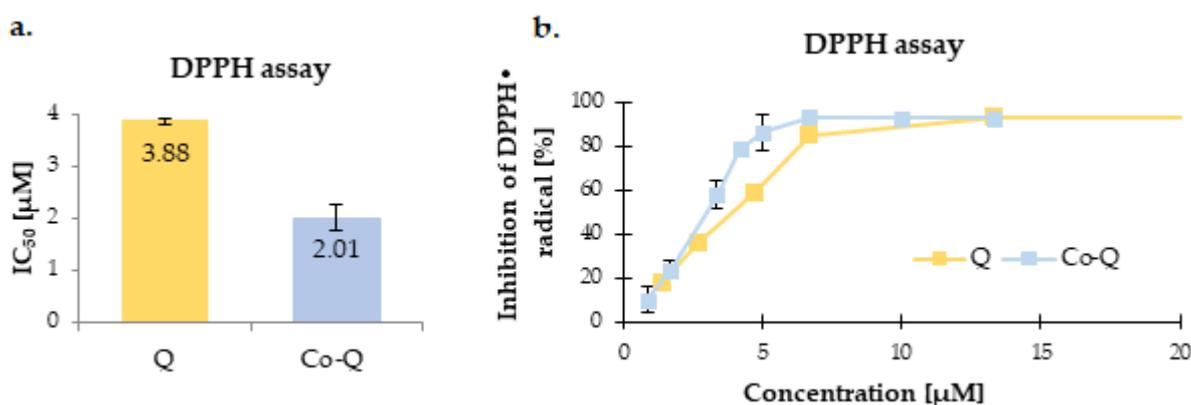


Figure 6. (a) The IC₅₀ values for quercetin (Q) and Co(II) complex of quercetin (Co-Q); (b) the scavenging of DPPH• by Q and Co-Q. Mean values from three independent experiments ± SD are shown.

Similar results as in the DPPH test were obtained in the ferric-reducing antioxidant assay FRAP (Figure 7), where the Fe(III)-TPTZ complex is reduced, using an antioxidant, to a Fe(II)-TPTZ complex. The reducing activity of studied compounds expressed in FRAP values were: 566.23 ± 18.52 and 830.16 ± 15.56 μM Fe²⁺, respectively. Both selected tests differ in their mechanism of action. The FRAP assay is described as a totally SET (single electron transfer) reaction, where one electron is transferred from an antioxidant to reduce the number of metal ions or radicals [42], whereas the reaction of antioxidants with DPPH• can be describe as having SET or hydrogen atom transfer (HAT) mechanisms (or the

combination of them) [43,44]. Moreover, the type of solvent and the pH of the environment do not affect HAT-type reactions, unlike in the SET reaction, which depends on the acidity of the environment [45]. The DPPH assay was conducted in a methanolic solution, whereas FRAP was more often in an aqueous environment, which may affect the rate of reaction and compound solubility.

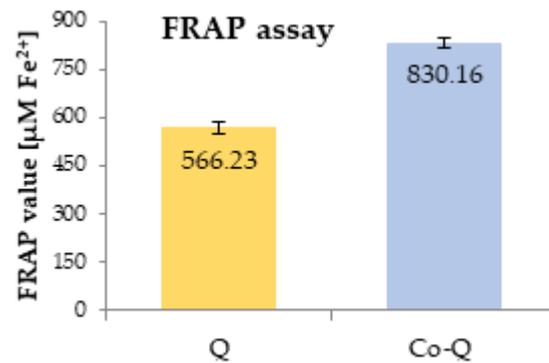


Figure 7. The FRAP values obtained for quercetin (Q) and Co(II) complex of quercetin (Co-Q). Mean values from three independent experiments \pm SD are shown.

The quercetin and the Co(II) complex of quercetin revealed pro-oxidant activity in the Trolox oxidation assay. The reaction between tested phenolic compounds and H_2O_2 catalyzed by horseradish peroxidase provide the phenoxyl radicals. The obtained phenoxyl radicals undergo a reaction with Trolox, the Trolox is oxidized to create Trolox radicals, and then transformed into Trolox quinones (with maximum absorption at 272 nm), and the phenoxyl radicals are transformed into phenolic compounds. The pro-oxidant activity of the tested substances was measured for two concentrations (0.35 and 0.70 μM) and expressed as the absorbance of the tested samples (Figure 8). The Co(II)-complex of quercetin showed slightly lower pro-oxidant activity in the Trolox assay compared with quercetin alone.

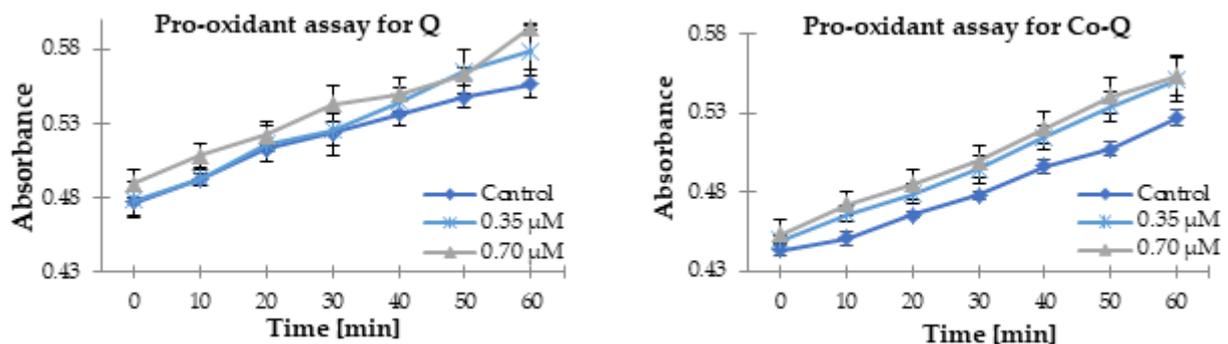


Figure 8. The absorbance ($\lambda_{\text{max}} = 272 \text{ nm}$) obtained in the Trolox oxidation assay measured every 10 min for 60 min for control sample, quercetin (Q) and Co(II)-complex of quercetin (Co-Q). Mean values from three independent experiments \pm SD are shown.

3.3. Cell Viability

The influence of quercetin and its cobalt salt on cell viability was estimated using the fluorimetric neutral red uptake test, as described previously [29,46]. Neutral red dye is uptaken by the viable cells and stains the lysosomes. The Co(II)-Q had a much lower solubility in the culture medium than the Q alone, therefore a lower range of concentrations could be studied for the Co(II)-Q complex. However, practically no antiproliferative activity was observed within the solubility range of the salt. Simultaneously, the quercetin was not toxic in HaCat cells even in the concentrations that were three orders of magnitude

higher than the maximum Co(II)-Q complex concentration (Figure 9). It can be concluded that complexing quercetin to Co(II) strongly decreases its availability in the water-based medium, which renders the Co(II)-Q non-toxic in the given solubility range.

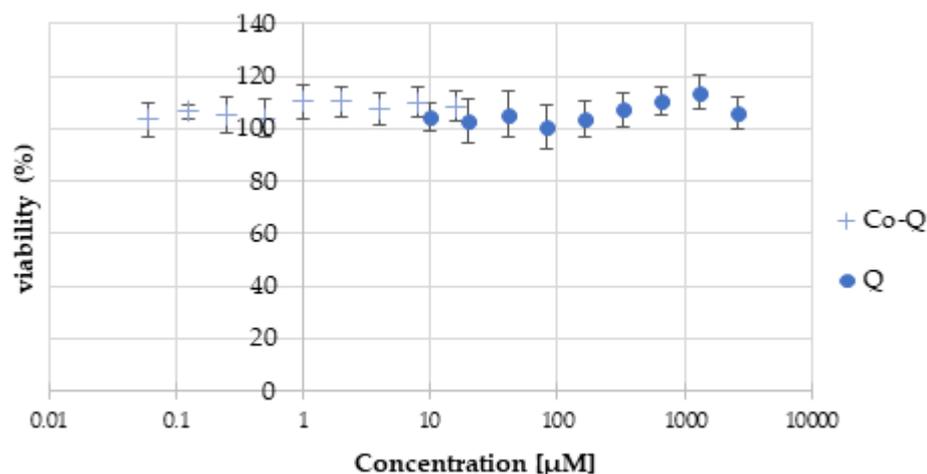


Figure 9. Toxicity of quercetin (Q) and its cobalt(II) complex of quercetin (Co-Q) against HaCaT immortalized human keratinocyte cell line, as measured by the fluorimetric neutral red uptake assay. The maximal concentration of Co-Q in the culture media was applied (15 μM). The concentration range for Co-Q was 0.06–15 μM, for Q it was 10–2 577 μM.

4. Conclusions

The cobalt(II) complex of quercetin with the formula $\text{Co}(\text{C}_{15}\text{H}_9\text{O}_7)_2 \cdot 2\text{H}_2\text{O}$ was synthesized in its solid state in $\text{pH} = 7.4$. The metal:ligand ratio of 1:2 in the solution was determined using the molar-ratio method. In line with the above-cited reports showing a protective role for quercetin in the amelioration of the oxidative stress-related toxicity of cobalt [34], the presented results indicate that the obtained Co(II)-quercetin complex showed higher antioxidant activity than quercetin alone in the DPPH and FRAP assays. Moreover, the Co(II)-quercetin complex showed slightly lower pro-oxidant activity in the Trolox oxidation assay than the ligand. It should also be noted that the Co(II) complex showed low solubility, suggesting that quercetin may also significantly reduce the concentration of toxic cobalt ions and other transition metals in biological medias. Quercetin was found to be non-toxic towards HaCaT keratinocyte cells in the wide concentration range (10–2 577 μM). The considerably lower obtainable concentrations of the Co(II) complex (0.06–15 μM) were also found to be non-toxic, with the simultaneous enhancement of antioxidative potential of Co(II)-quercetin complex. The above findings correspond well with the strengthening of the antioxidant defense system observed in CoCl_2 -treated rats upon quercetin and ascorbate administration [34] and contributes to the clarification of the reported protection provided by quercetin against oxidative stress-related and metal-related diseases [47–49]. Further research on Co(II)-quercetin action in vitro and in vivo models is needed to define the possibility of the application of Co(II)-quercetin and to determine the Q-metal chelation potential in many oxidative stress-related disorders.

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