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ESI-TOF MS analysis of complexes formed between quercetin and five metal ions in hot water and a study into their DNA cleavage activity

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ABSTRACT

Quercetin is a flavonoid compound widely found in vegetables, fruits, and medicinal plants. It has carbonyl and phenolic hydroxyl groups in its structure that can easily form complexes with metal ions. In this study, we reacted quercetin in water at 95 °C with five metal trace elements commonly found in plants, namely calcium, magnesium, zinc, iron(III), and copper. Each supernatant was then examined by electrospray ionization-time-of-flight mass spectrometry (ESI-TOF MS). The results showed that quercetin can form complexes with Ca^{2+}, Mg^{2+}, Zn^{2+}, and Cu^{2+} in ratios of 2:1, 3:1, and 3:2. Interestingly, after reaction with Fe^{3+} in water at 95 °C, not only can quercetin-iron(III) complexes be formed in ratios of 2:1 and 3:1, but small amounts of quercetin-iron(II) complexes can also be formed in the same ratios. Furthermore, DNA cleavage experiments showed that when acting alone, quercetin and Cu^{2+} have weak or no cleavage effects on DNA, but the complex formed after reaction in hot water cleaves DNA in a time- and concentration-dependent manner. These results indicate that complexes may form between quercetin and a variety of metal trace elements in a water decoction of plants, and that these metal complexes may be the material basis for the health-promoting and therapeutic effects of edible or medicinal plants.

1. Introduction

Quercetin, one of the most widely distributed flavonoids in nature, is found in a variety of fruits, vegetables, and medicinal plants [1]. It has been extensively studied for its potential role in the prevention and treatment of various chronic diseases, including cardiovascular disease, neurodegenerative disease, and cancer [2–8]. The structure of quercetin (Fig. 1) contains five phenolic hydroxyl groups and one carbonyl group, which allow it to easily react with metal ions [9]. To date, complexes between quercetin and a wide range of metal ions such as magnesium [10], cobalt [11], copper [12–14], chromium [15], aluminum [16], tin [17], palladium [18], iron(III) [19,20], iron(II) [9], manganese [21], and rare earth elements [22,23] have been synthesized. Interestingly, the activity of quercetin-metal complexes is significantly higher than that of quercetin itself. For example, a quercetin-cobalt complex [11] and a quercetin-chromium(III) complex [15] both have higher free-radical scavenging activities against DPPH than quercetin, and the antitumor activity of quercetin has been found to be significantly enhanced by the formation of complexes with six metal ions, namely Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Pb^{2+} [21].

In addition, studies into the structures of quercetin-metal complexes have shown that different reaction solvents have a significant effect on the coordination forms of quercetin and the metal ions. For example, quercetin and Cu^{2+} form a complex in a ratio of 1:1 in methanol solution [13], while in an alkaline aqueous solution of pH 10, they can either coordinate at the 4═O and 5─OH positions in a quercetin:Cu^{2+} ratio of 2:1, or coordinate at the 3′─OH and 4′─OH positions to form a 1:1 complex [14]. In addition, quercetin and Co^{2+} form a 1:2 complex in methanol [11], but form a 2:1 complex in ethanol [21]. As the majority of edible and medicinal plants are ingested after boiling or decocting in water, we herein investigated an induced reaction between quercetin and five metal ions commonly found in plants, namely Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{3+}, and Cu^{2+}, by heating them in water. The obtained supernatants will be examined by electrospray ionization-time-of-flight mass spectrometry (ESI-TOF MS) and the precise molecular weights and isotopic distributions will be analyzed to investigate whether complex formation was successful. The reaction products will then be tested to determine if the resulting quercetin-metal complexes exhibit DNA cleavage activity.

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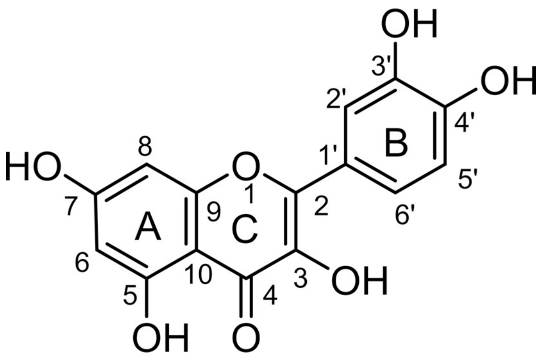

Fig. 1. The structure of quercetin.

2. Experimental

2.1. General experimental procedures

2.1.1. Materials and reagents

Quercetin (> 98%, batch number HM150815) was obtained from Chengdu Alfa Biotechnology Co., Ltd., and the quercetin control (> 98%, batch number YA0806YB13) was obtained from Shanghai Yuanye Bio-Technology Co., Ltd. Methanol (chromatographically pure and mass spectrometrically pure) was obtained from Merck (Germany), and formic acid (chromatographically pure) was obtained from Aladdin Chemical Co., Ltd. (US). Analytically pure 1,10-phenanthroline, ethanol, magnesium chloride hexahydrate, anhydrous calcium chloride, anhydrous zinc chloride, ferric chloride hexahydrate, copper chloride dihydrate, and dimethyl sulfoxide (DMSO) were obtained from Xilong Chemical Co., Ltd. pBR322 DNA was obtained from Sangon Biotech (Shanghai) Co., Ltd.

2.1.2. Instruments and equipment

Mass spectrometry was performed on a Xevo G2-XS ESI-TOF MS with a Masslynx 4.1 data processing system (Waters, US). All sample masses were measured on an XS203S precision electronic balance (Mettler Toledo, Switzerland). A Hei-VAP rotary evaporator (Heidolph, Germany) was used to remove volatiles. The reaction temperature was maintained by a DF-101S heat collection constant temperature magnetic stirrer (Gongyi Yuhua Co., Ltd., Henan, China). Electrophoresis was performed using a Mini-PROTEAN4 electrophoresis tank (with PowerPac Basic power supply, Biorad, US) and a JY04s-3c gel imaging analysis system (Beijing Junyi Dongfang Electrophoresis Co., Ltd., Beijing, China).

2.1.3. Reaction between quercetin and metal ions

A mixture of quercetin (0.02 mmol) and $CaCl_2$, $MgCl_2{\cdot}6H_2O$, $ZnCl_2$, $FeCl_3{\cdot}6H_2O$, or $CuCl_2{\cdot}2H_2O$ (0.01 mmol) was placed in a round-bottom flask, distilled water (20 mL) was added, and the resulting mixture was heated under reflux at 95 °C with magnetic stirring for 3 h to induce the reaction.

2.1.4. ESI-TOF MS analysis of the quercetin-metal complexes

Immediately after the reaction outlined in Section 2.1.3 was complete, an aliquot (2 mL) of the reaction solution was removed, centrifuged at 10,000 ×g for 2 min, and analyzed by ESI-TOF MS. ESI was conducted in positive ion mode, the mass spectrometry sample injection method was infusion with an injection flow rate of 20 μL/min, and the molecular weight detection range was 200–1200.

2.1.5. DNA cleavage activity of the quercetin-metal complexes

Following complex formation as described in Section 2.1.3, the reaction solution was concentrated to dryness by vacuum decompression and dissolved in DMSO. The solution was prepared as a stock solution with a metal ion concentration of 1 mmol/L (whereupon the concentration of quercetin was 2 mmol/L). Equal amounts of quercetin and the five metal salts were taken and formulated with DMSO to give stock solutions of the same concentrations.

Triple distilled water (8 μL), phosphate buffer (Buffer 1, 10 μL), the test sample (1 μL), and pBR 322 DNA (1 μL) were added in sequence to a 250 μL centrifuge tube to form a 20 μL reaction system with a quercetin concentration of 100 μmol/L and a metal ion concentration of 50 μmol/L. The solution was blown with a 10 μL pipette to ensure sufficient mixing, and incubated in a 37 °C constant temperature incubator for 4 h. After this time, the stop solution (Buffer 2, 4 μL) was added, the solution was mixed thoroughly, and an aliquot (5 μL) was added to the corresponding agarose gel well (0.16 g agarose, 20 mL Buffer 3). Electrophoresis was performed at 120 mV for 60 min until bromophenol blue migrated to the back of the gel. The solution was then stained with 0.5 mg/L Gel Red solution for 1 h and observed on the gel imaging apparatus.

Buffer 1: 10 mM KH_2PO_4, 10 mM NaCl, 10 mM EDTA, pH = 7.2
Buffer 2: 0.05% bromophenol blue, 36% glycerol, 30 mM Na_2H_2EDTA
Buffer 3: (TBE 1 ×): 89 mM Tris-borate, 2 mM Na_2H_2EDTA

3. Results

3.1. ESI-TOF MS analysis of the quercetin-metal complexes

During ESI-TOF MS analysis of the supernatants obtained from the reaction of quercetin with the five different metal ions, both ultra performance liquid chromatography (UPLC) injection and direct injection (infusion) techniques were investigated. It was found that when using UPLC injection (10 μL injection volume), only quercetin was detected, likely due to adsorption of the complexes on the column, or decomposition of the metal complexes under the analysis conditions. Therefore, subsequent analyses of the reaction supernatants were carried out by direct injection, and the obtained ESI-TOF MS results are outlined in Table 1. Comparisons between the measured and theoretical isotopic distributions of the newly formed quercetin-metal complexes are shown in Figs. 2-1–6-1.

3.1.1. Inference of reaction products between quercetin and Ca^{2+}, Mg^{2+}, Zn^{2+}, and Cu^{2+}

In the supernatant obtained from the reaction of quercetin with Ca^{2+}, the $[M + H]^+$ quasi-molecular ion of quercetin and those of three compounds with *m/z* values of 643.0406, 945.0857, and 983.0309 were detected by ESI-TOF MS in positive ion mode. Upon comparison of the molecular weights and isotopic distributions (see Fig. 2-1), the molecular formula of the compound with m/z = 643.0406 was assumed to be $C_{30}H_{19}O_{14}Ca$, i.e., the M^+ or $[M + H]^+$ of the complex formed by a 2:1 ratio of quercetin and Ca^{2+}. The measured isotopic distributions of m/z = 945.0857 and 983.0309 were in complete agreement with the theoretical isotopic distributions of $C_{45}H_{29}O_{21}Ca$ and $C_{45}H_{27}O_{21}Ca_2$, respectively (see Fig. 2- and -3), i.e., these compounds were presumably $[M + H]^+$ of the complexes formed between quercetin and Ca^{2+} in ratios of 3:1 and 3:2, respectively. The metal complexes formed by the reaction of Mg^{2+}, Zn^{2+}, and Cu^{2+} with quercetin in hot water were similar to those formed by the reaction with Ca^{2+}.

3.1.2. Inference of reaction products between quercetin and Fe^{3+}

From the data outlined in Table 1, it is apparent that four compounds formed between quercetin and Fe^{3+} in the reaction supernatant, i.e., m/z = 658.0049, 659.0118, 960.0518, and 961.0599. The measured isotopic distribution of m/z = 658.0049 was consistent with the theoretical isotopic distribution of $C_{30}H_{18}O_{14}Fe$ (Fig. 6-1A and -1C), and so this species was presumed to be $[2(L–H) + Fe^{3+}]^+$, formed from

Table 1
ESI-TOF MS analysis of the supernatants obtained following reaction of quercetin with the five metal ions (ESI in positive ion mode, L represents quercetin).

Source of reaction supernatant	*m/z*		Molecular formula	Possible structure	Comparison between theoretical and measured isotopic distribution
	Theoretical value	Measured value			
All samples	303.0505	303.0508	$C_{15}H_{11}O_7$	$[L + H]^+$	
Quercetin and Ca^{2+}	643.0401	643.0406	$C_{30}H_{19}O_{14}Ca$	$[2L + Ca^{2+} - H]^+$	Fig. 2-1
	945.0827	945.0857	$C_{45}H_{29}O_{21}Ca$	$[3L + Ca^{2+} - H]^+$	Fig. 2-2
	983.0297	983.0309	$C_{45}H_{27}O_{21}Ca_2$	$[3L + 2Ca^{2+} - 3H]^+$	Fig. 2-3
Quercetin and Mg^{2+}	627.0625	627.0617	$C_{30}H_{19}O_{14}Mg$	$[2L + Mg^{2+} - H]^+$	Fig. 3-1
	929.1052	929.1082	$C_{45}H_{29}O_{21}Mg$	$[3L + Mg^{2+} - H]^+$	Fig. 3-2
	951.0746	951.0730	$C_{45}H_{27}O_{21}Mg_2$	$[3L + 2\,Mg^{2+} - 3H]^+$	Fig. 3-3
Quercetin and Zn^{2+}	667.0067	667.0057	$C_{30}H_{19}O_{14}Zn$	$[2L + Zn^{2+} - H]^+$	Fig. 4-1
	969.0493	969.0488	$C_{45}H_{29}O_{21}Zn$	$[3L + Zn^{2+} - H]^+$	Fig. 4-2
	1034.9595	1034.9595	$C_{45}H_{27}O_{21}Zn_2$	$[3L + 2Zn^{2+} - 3H]^+$	Fig. 4-3
Quercetin and Cu^{2+}	666.0071	666.0098	$C_{30}H_{19}O_{14}Cu$	$[2L + Cu^{2+} - H]^+$	Fig. 5-1
	968.0497	968.0504	$C_{45}H_{29}O_{21}Cu$	$[3L + Cu^{2+} - H]^+$	Fig. 5-2
	1030.9631	1030.9647	$C_{45}H_{27}O_{21}Cu_2$	$[3L + 2Cu^{2+} - 3H]^+$	Fig. 5-3
Quercetin and Fe^{3+}	658.0046	658.0049	$[C_{30}H_{18}O_{14}Fe(III)]^+$	$[2L + Fe^{3+} - 2H]^+$	Fig. 6-1
	659.0124	659.0118	$C_{30}H_{19}O_{14}Fe(II)$	$[2L + Fe^{2+} - H]^+$	
	960.0474	960.0518	$[C_{45}H_{28}O_{21}Fe(III)]^+$	$[3L + Fe^{3+} - 2H]^+$	Fig. 6-2
	961.0552	961.0599	$C_{45}H_{29}O_{21}Fe(II)$	$[3L + Fe^{2+} - H]^+$	

the reaction of quercetin and Fe^{3+} in a 2:1 ratio and detected as M^+ under in the positive ESI mode. In addition, the abundances of the isotopic peaks corresponding to $m/z = 657.0137$ and 659.0118 were significantly higher than the theoretical values in the analysis of the measured isotopic distribution. Considering that Fe^{3+} exhibits strong oxidizing properties and quercetin is a natural antioxidant, it is assumed that a small amount of Fe^{3+} was reduced to Fe^{2+} during the reaction, and that Fe^{2+} formed the complex $C_{30}H_{18}O_{14}Fe(II)$ with quercetin in a ratio of 1:2, which then resulted in the formation of $[M + H]^+$ quasi-molecular ions under positive ion ESI mode ($m/z = 659.0118$, see Fig. 6-1B for theoretical isotopic distribution). It should be noted that Fernandez et al. [24] detected the presence of a divalent iron complex in the reaction products between various flavonoids, including quercetin and Fe^{3+}, by ESI-MS using methanol-water (v:v = 1:1) as a solvent. It is thus inferred that the reaction products of quercetin and Fe^{3+} in hot water contain both $[C_{30}H_{18}O_{14}Fe(III)]^+$ and $C_{30}H_{18}O_{14}Fe(II)$. In the ESI-TOF MS results, multiple isotope peaks of the two compounds with similar *m/z* values overlap with one another, resulting in a discrepancy between the measured isotope peak abundance and the theoretical abundance. Thus, to confirm the presence of Fe^{2+} in the reaction products, 1,10-phenanthroline (1 mL, dissolved in a small amount of ethanol and diluted in distilled water to give a solution with a mass fraction of 0.1%) and an acetic acid-sodium acetate buffer solution (1 mL, pH = 4.5) were added to aliquots of quercetin, $FeCl_3$, $FeCl_2$, and the quercetin-$FeCl_3$ reaction solution (2 mL) treated under the same conditions, and the solution was mixed by vortexing and allowed to stand for 150 s. The obtained results (Fig. 7) show that the quercetin and $FeCl_3$ solutions that were treated by heating in water did not change color, while the $FeCl_2$ solution became red, and the quercetin-$FeCl_3$ solution became orange-red. This phenomenon indicates that a small amount of Fe^{2+} was produced after heating quercetin and Fe^{3+} in water, thereby confirming the presence of complex $C_{30}H_{18}O_{12}Fe(II)$. Similarly, the peaks with *m/z* values of 960.0518 and 961.0599 were assigned to quasi-molecular ions corresponding to $C_{45}H_{28}O_{21}Fe(III)^+$ and $C_{45}H_{28}O_{21}Fe(II)$ formed by the reaction of quercetin with Fe^{3+} and Fe^{2+} in a 3:1 ratio under the positive ion ESI mode.

3.2. DNA cleavage activity of quercetin and its metal complexes

Since an effective method for the separation and purification of the quercetin-metal complexes has not yet been identified, following completion of the reaction, the reaction solution was concentrated to dryness by vacuum decompression and dissolved in DMSO for the activity experiments. Quercetin and the five metal salts treated under the same conditions were used as the controls. The results are shown in Fig. 8.

As can be seen from Fig. 8, under the dose chosen for this study, DMSO, quercetin, Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{3+}, and the products from the reaction of quercetin with Ca^{2+}, Mg^{2+}, Zn^{2+}, and Fe^{3+} exhibited no obvious effect on DNA after 4 h. In contrast, Cu^{2+} exhibited a weak cleavage effect when acting alone, and the reaction product formed between quercetin and Cu^{2+} exhibited significant DNA cleavage activity, with all bands representing supercoiled conformation (SC) DNA disappearing in the electropherogram.

The temporal relationship (Fig. 9) and concentration relationship (Fig. 10) of the DNA cleavage effect were then examined for the quercetin-Cu^{2+} complex. As shown in Fig. 9, DNA cleavage activity was initially apparent after 0.25 h, at which time nicked circular (NC) DNA began to form and the levels of SC DNA began to decrease. After 1 h of action, all SC DNA had disappeared and linear (L) DNA began to appear; after 2 h, the NC DNA content was significantly reduced while the L DNA content significantly increased; and after 4 h, the DNA was cleaved into even smaller fragments, causing NC DNA and L DNA to disappear completely.

As shown in Fig. 10, when the Cu^{2+} concentration in the reaction system reached 17.5 μmol/L, the NC DNA content clearly increased, suggesting that DNA cleavage began at this concentration. When the Cu^{2+} concentration reached 20 μmol/L, SC DNA completely disappeared, the NC DNA content increased significantly, and a small amount of L DNA began to appear. Furthermore, when the Cu^{2+} concentration increased to 32.5 μmol/L, the NC DNA content gradually decreased until the corresponding band essentially disappeared, while the L DNA band initially increased prior to decreasing. Indeed, when the Cu^{2+} concentration was ≥35 μmol/L, L DNA was cleaved into even smaller fragments, causing it to disappear completely.

4. Discussion

To date, the complexes formed between quercetin and various metal ions have been obtained only in organic solvents or alkaline aqueous solutions. Thus, to investigate the formation of quercetin-metal complexes in plant water decoctions, distilled water (Watsons, pH = 7.0) was selected as the reaction solvent for the purpose of our study. Interestingly, the ESI-TOF-MS results showed that quercetin can form complexes with five metal ions, namely Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{3+}, and Cu^{2+}, in hot water in a ratio of 2:1 (quercetin:metal ion), which is in agreement with the complex ratios formed in other solvents. As the

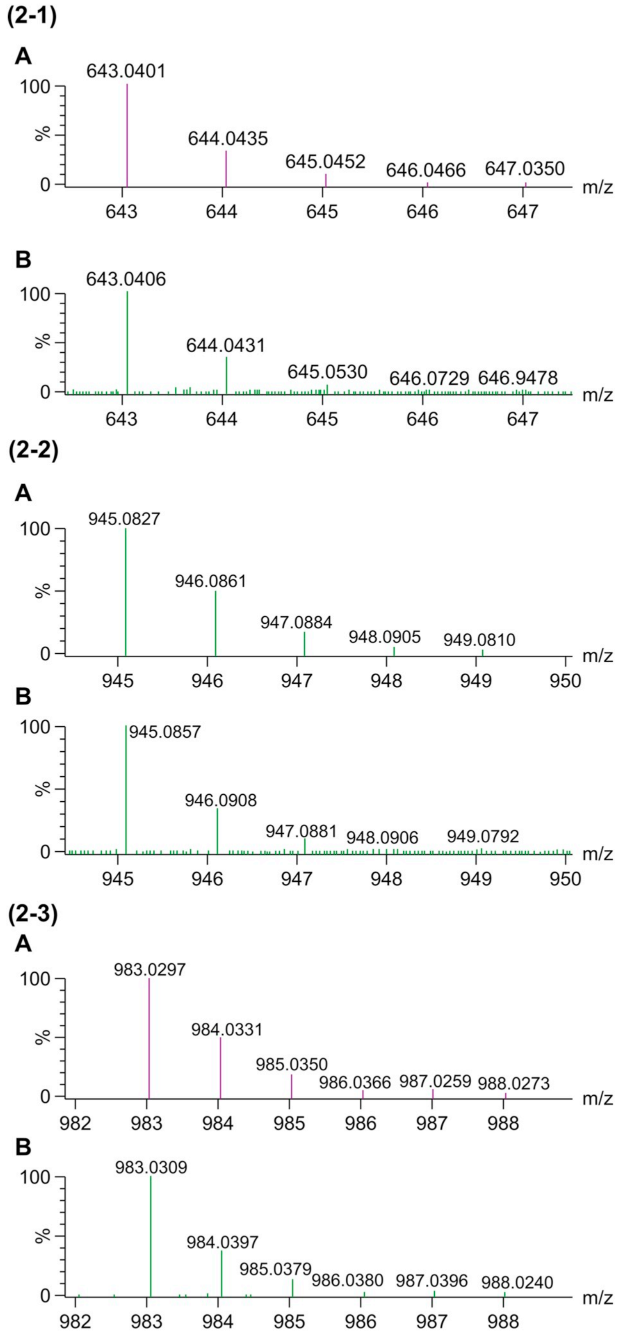

Fig. 2. 2-1. Theoretical (A) and measured (B) isotopic distributions of $C_{30}H_{19}O_{14}Ca$; 2-2. Theoretical (A) and measured (B) isotopic distributions of $C_{45}H_{29}O_{21}Ca$; 2-3. Theoretical (A) and measured (B) isotopic distributions of $C_{45}H_{27}O_{21}Ca_2$.

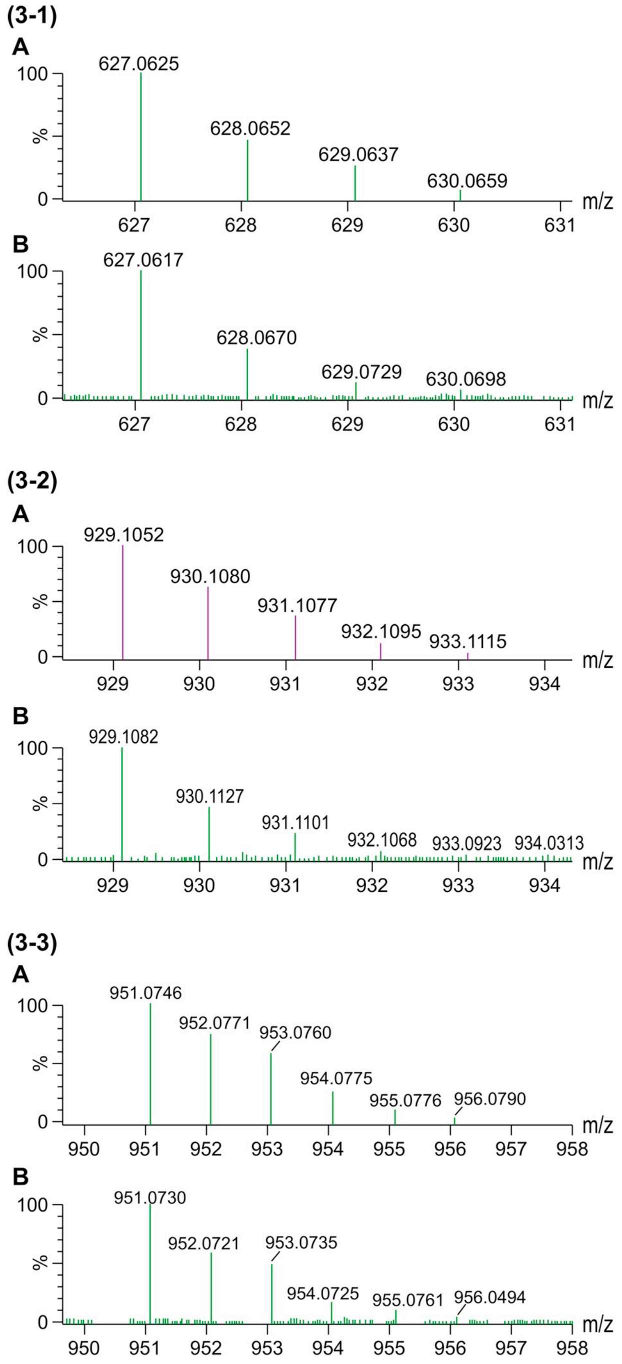

Fig. 3. 3-1. Theoretical (A) and measured (B) isotopic distributions of $C_{30}H_{19}O_{14}Mg$; 3-2. Theoretical (A) and measured (B) isotopic distributions of $C_{45}H_{29}O_{21}Mg$; 3-3. Theoretical (A) and measured (B) isotopic distributions of $C_{45}H_{27}O_{21}Mg_2$.

quercetin molecule contains two locations that can easily coordinate with metal ions, i.e., the 4-carbonyl and 5-hydroxy groups and the 3′,4′-dihydroxyl group [9], the most probable structure of this 2:1 complex, using Ca^{2+} as an example, is $[2(L - H) + Ca^{2+}]$, which is formed by the coordination of two quercetin molecules with Ca^{2+} at both the 4-carbonyl and 5-hydroxy groups, which is observed as $[M + H]^+$ under the positive ion ESI mode. However, the following two possibilities cannot be ruled out: (1) One quercetin molecule is coordinated with Ca^{2+} at the 3′,4′-dihydroxyl group, and the other quercetin molecule is coordinated with Ca^{2+} only at the 4-carbonyl group, giving $[L + (L - 2H) + Ca^{2+}]$; and (2) one quercetin molecule is coordinated with Ca^{2+} at the 4-carbonyl and 5-hydroxyl groups, and the other quercetin molecule is coordinated with Ca^{2+} only at the 4-carbonyl group, giving $[L + (L - H) + Ca^{2+}]^+$. In addition, quercetin and the four metal ions were also found to form complexes in ratios of 3:1 and 3:2, which have not been previously reported. This indicates that the reaction solvent has a large influence on the coordination pattern in the formation of complexes between quercetin and metal ions.

Unfortunately, the structures of these quercetin-metal complexes could not be fully determined as no effective method has been identified to separate and purify the reaction products. Indeed, when we attempted purification of the reaction products by column chromatography (silica gel, Sephadex LH-20, polyamide, and RP-18), only

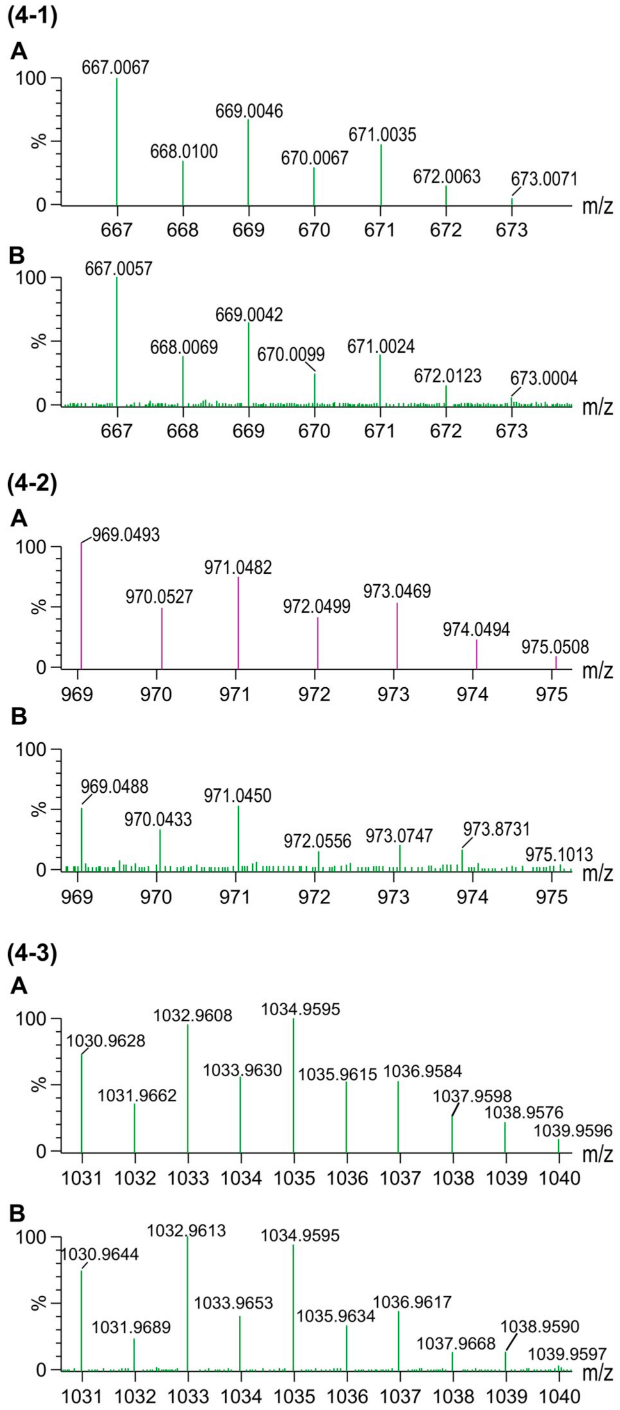

Fig. 4. 4-1. Theoretical (A) and measured (B) isotopic distributions of $C_{30}H_{19}O_{14}Zn$; 4-2. Theoretical (A) and measured (B) isotopic distributions of $C_{45}H_{29}O_{21}Zn$; 4-3. Theoretical (A) and measured (B) isotopic distributions of $C_{45}H_{27}O_{21}Zn_2$.

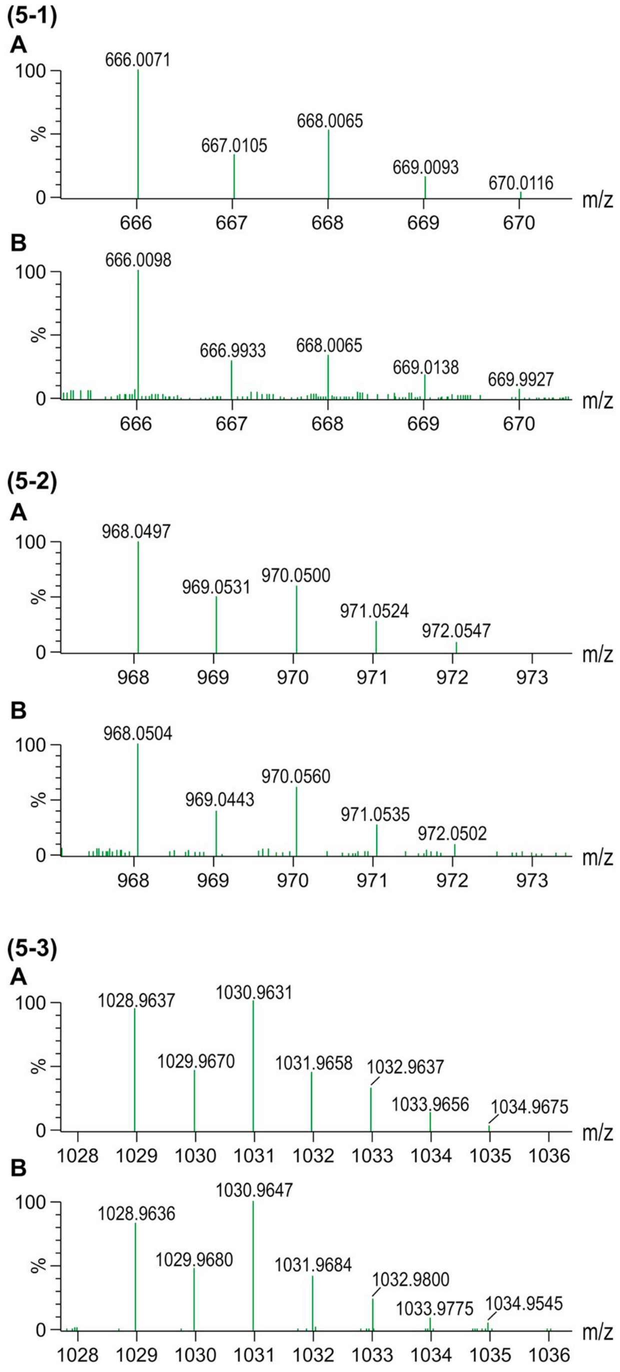

Fig. 5. 5-1. Theoretical (A) and measured (B) isotopic distributions of $C_{30}H_{19}O_{14}Cu$; 5-2. Theoretical (A) and measured (B) isotopic distributions of $C_{45}H_{29}O_{21}Cu$; 5-3. Theoretical (A) and measured (B) isotopic distributions of $C_{45}H_{27}O_{21}Cu_2$.

quercetin was detected in the eluted products. This may be because either the quercetin-metal complexes are adsorbed on the column adsorbent, or they may undergo decomposition on the column. We then attempted purification by recrystallization, and the ESI-TOF-MS results of the recrystallized products dissolved in hot water consistently showed the presence of both quercetin and the quercetin-metal complexes. These results therefore suggest that the complexes formed by quercetin and the five metal ions are not sufficiently stable to maintain a fixed structure in solution, but can be present simultaneously in aqueous solution. In recent years, the detection of metal complexes of various organic components from plant extracts has been reported using advanced analytical instruments such as ESI-MS/MS [25–27], although the isolation and purification of high-purity metal complexes from plant extracts has only been reported a few times. This may be due to the issues mentioned above.

DNA is the main target for the action of many anticancer drugs [28,29]. pBR322 DNA is a supercoiled double-stranded closed-loop molecule that has been widely used to study of the effects of metal complexes on DNA. pBR322 DNA exists in three forms. More specifically, the intact DNA is usually found in its SC conformation, which has the most compact structure and the fastest migration rate in gel

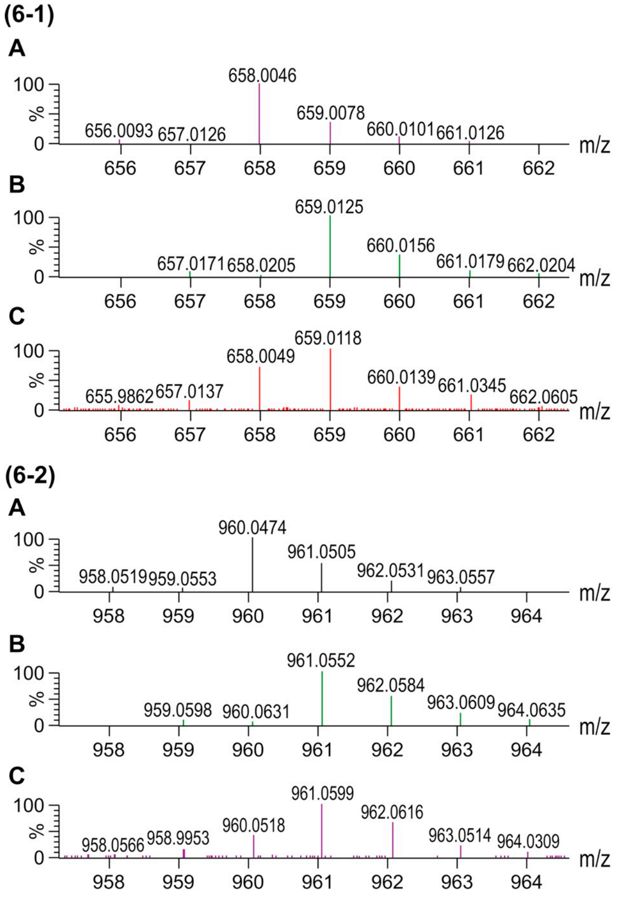

Fig. 6. 6-1. Theoretical (A) isotopic distribution of $C_{30}H_{18}O_{14}Fe(III)$, and theoretical (B) and measured (C) isotopic distributions of $C_{30}H_{19}O_{14}Fe(II)$; 6-2. Theoretical (A) isotopic distribution of $C_{45}H_{28}O_{21}Fe(III)$, and theoretical (B) and measured (C) isotopic distributions of $C_{45}H_{29}O_{21}Fe(II)$.

electrophoresis. When a nick (i.e., a single-strand break) appears on an intact DNA strand, it becomes NC DNA, which has the slowest migration rate in gel electrophoresis due to its loose structure. When both strands break at the same site, they become linear in form, i.e., L DNA, which has a migration speed in gel electrophoresis between those of SC DNA and NC DNA. The DNA cleavage experiments carried out herein demonstrated that quercetin did not exhibit any DNA cleavage effects within the ranges of concentration and duration examined herein, while Cu^{2+} exhibited a weak DNA cleavage effect at 50 μmol/L. However, the quercetin-copper complex formed in hot water produced a significant DNA cleavage effect. Upon increasing either the concentration of the quercetin-copper complex or the action duration, pBR322 DNA was cleaved from SC DNA into NC DNA, then into L DNA, and finally, L DNA was cleaved into smaller fragments that could not be observed by gel electrophoresis.

The obtained results therefore confirm that complexes between quercetin and various metal ions may form in plant decoctions, and it is

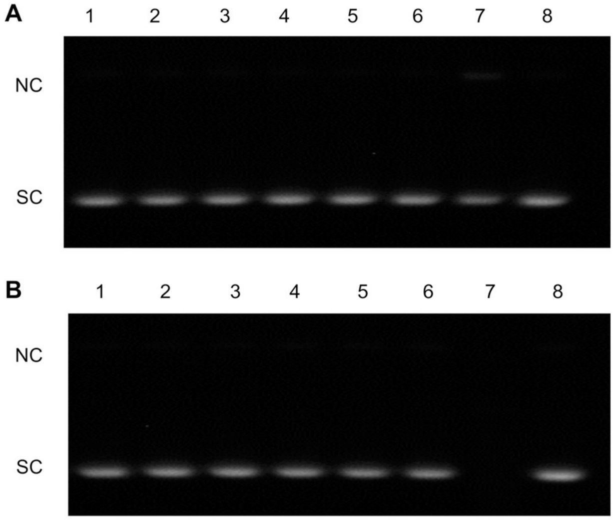

Fig. 8. DNA cleavage effects of quercetin, the five metal salts, and the five reaction products formed from quercetin and the five metal ions (with an action duration of 4 h). A: Lanes 1 and 8: DNA control, lane 2: DMSO, lanes 3–7: 50 μmol/L Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{3+}, and Cu^{2+}, respectively. B: Lanes 1 and 8: DNA control, lane 2: 100 μmol/L quercetin, lanes 3–7: metal complexes (100 μmol/L quercetin and 50 μmol/L Ca^{2+}, Mg^{2+}, Zn^{2+}, Fe^{3+}, and Cu^{2+}, respectively).

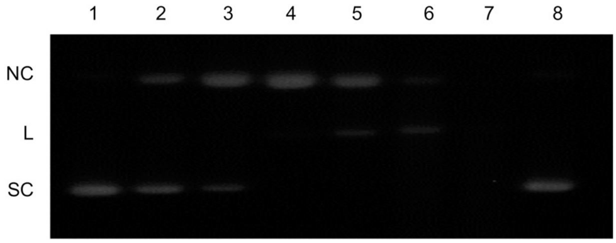

Fig. 9. Temporal relationship of the DNA cleavage effect of the reaction products formed between quercetin and Cu^{2+} (100 μmol/L quercetin and 50 μmol/L Cu^{2+}). Lanes 1 and 8: DNA control, lanes 2–7: the reaction products formed between quercetin and Cu^{2+} acting on DNA for 0.25, 0.5, 1, 2, 3, and 4 h, respectively.

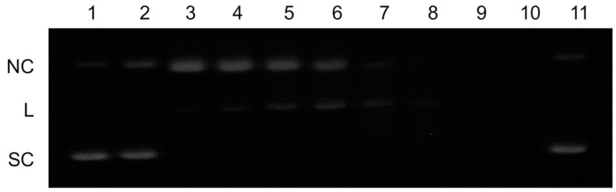

Fig. 10. Concentration relationship of the DNA cleavage effect of the reaction products formed between quercetin and Cu^{2+} (with an action duration of 4 h on DNA). Lanes 1 and 11: DNA control, lanes 2–10: DNA cleavage from Cu^{2+} concentrations of 17.5, 20, 22.5, 25, 27.5, 30, 32.5, 35, and 37.5 μmol/L, respectively (The molar ratio of quercetin to Cu^{2+} was 2:1).

Fig. 7. Color development of each sample after mixing with 1,10-phenanthroline (Position 1: Fe^{2+} solution after reaction; 2: Fe^{2+} solution after reaction + 1,10-phenanthroline; 3: Fe^{3+} solution after reaction; 4: Fe^{3+} solution after reaction + 1,10-phenanthroline; 5: quercetin solution after reaction; 6: quercetin solution after reaction + 1,10-phenanthroline; 7: quercetin-Fe^{3+} solution after reaction; 8: quercetin-Fe^{3+} solution after reaction + 1,10-phenanthroline).

possible that these complexes exhibit new activities that are not observed when the ligand or metal ions act alone. This indicates that during study of the active ingredients of edible and medicinal plants, in addition to focusing on the organic or trace elements therein, the complexes formed by the two cannot be ignored.

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Declarations of interest

None.

Author contributions

Han Manman: Data curation, Formal analysis, Methodology, Roles/Writing - original draft. Cai Weilan: Formal analysis, Methodology, Validation. Li Zhimin: Data curation, Formal analysis, Investigation, Writing - review & editing. Peng Liang: Funding acquisition, Project administration, Supervision, Writing - review & editing. He Lixia: Methodology, Validation. Chen Min: Methodology, Validation.

References

[1] M.G.L. Hertog, P.C.H. Hollman, M.B. Katan, Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands, J. Agric. Food Chem. 40 (1992) 2379–2383 https://doi.org/10.1021/jf00024a011.

[2] A.W. Boots, G.R.M.M. Haenen, A. Bast, Health effects of quercetin: from antioxidant to nutraceutical, Eur. J. Pharmacol. 585 (2008) 325–337 https://doi.org/10.1016/j.ejphar.2008.03.008.

[3] M.D. Catarino, O. Talhi, A. Rabahi, A.M.S. Silva, S.M. Carsdoso, Chapter 3 - the antiinflammatory potential of flavonoids: mechanistic aspects, Stud. Nat. Prod. Chem. 48 (2016) 65–99 https://doi.org/10.1016/B978-0-444-63602-7.00003-5.

[4] Y.C. Chen, S.C. Shen, W.R. Lee, W.C. Hou, L.L. Yang, T.J.F. Lee, Inhibition of nitric oxide synthase inhibitors and lipopolysaccharide induced inducible NOS and cyclooxygenase-2 gene expressions by rutin, quercetin, and quercetin pentaacetate in RAW 264.7 macrophages, J. Cell. Biochem. 82 (2001) 537–548 https://doi.org/10.1002/jcb.1184.

[5] J.M. Cho, S.Y. Chang, D.B. Kim, P.W. Needs, Y.H. Jo, M.J. Kim, Effects of physiological quercetin metabolites on interleukin-1β-induced inducible NOS expression, J. Nutr. Biochem. 23 (2012) 1394–1402 https://doi.org/10.1016/j.jnutbio.2011.08.007.

[6] C.H. Chuang, C.L. Yeh, S.L. Yeh, E.S. Lin, L.Y. Wang, Y.H. Wang, Quercetin metabolites inhibit MMP-2 expression in A549 lung cancer cells by PPAR-γ associated mechanisms, J. Nutr. Biochem. 33 (2016) 45–53 https://doi.org/10.1016/j.jnutbio.2016.03.011.

[7] L. Cialdella-Kam, D.C. Nieman, W. Sha, M.P. Meany, Dose–response to 3 months of quercetin-containing supplements on metabolite and quercetin conjugate profile in adults, Br. J. Nutr. 109 (2013) 1923–1933 https://doi.org/10.1017/S0007114512003972.

[8] G. D'Andrea, Quercetin: a flavonol with multifaceted therapeutic applications? Fitoterapia 106 (2015) 256–271 https://doi.org/10.1016/j.fitote.2015.09.018.

[9] R. Ravichandran, M. Rajendran, D. Devapiriam, Antioxidant study of quercetin and their metal complex and determination of stability constant by spectrophotometry method, Food Chem. 146 (2014) 472–478 https://doi.org/10.1016/j.foodchem.2013.09.080.

[10] N. Ghosh, T. Chakraborty, S. Mallick, S. Mana, D. Singha, B. Ghosh, S. Roy, Synthesis, characterization and study of antioxidant activity of quercetin–magnesium complex, Spectrochim. Acta, Part A 151 (2015) 807–813 https://doi.org/10.1016/j.saa.2015.07.050.

[11] S.B. Bukhari, S. Memon, M. Mahroof-Tahir, M.I. Bhanger, Synthesis, characterization and investigation of antioxidant activity of cobalt–quercetin complex, J. Mol. Struct. 892 (2008) 39–46 https://doi.org/10.1016/j.molstruc.2008.04.050.

[12] S.B. Bukhari, S. Memon, M. Mahroof-Tahir, M.I. Bhanger, Synthesis, characterization and antioxidant activity copper–quercetin complex, Spectrochim. Acta, Part A 71 (2009) 1901–1906 https://doi.org/10.1016/j.saa.2008.07.030.

[13] S. Vimalraj, S. Rajalakshmi, D.R. Preeth, S.V. Kumar, T. Deepak, V. Gopinath, K. Murugan, S. Chatterjee, Mixed-ligand copper(II) complex of quercetin regulate osteogenesis and angiogenesis, Mater. Sci. Eng., C 83 (2018) 187–194 https://doi.org/10.1016/j.msec.2017.09.005.

[14] A. Torreggiani, M. Tamba, A. Trinchero, S. Bonora, Copper(II)–quercetin complexes in aqueous solutions: spectroscopic and kinetic properties, J. Mol. Struct. 744 (2005) 759–766 https://doi.org/10.1016/j.molstruc.2004.11.081.

[15] W. Chen, S. Sun, W. Cao, Y. Liang, J. Song, Antioxidant property of quercetin–Cr(III) complex: the role of Cr(III) ion, J. Mol. Struct. 918 (2009) 194–197 https://doi.org/10.1016/j.molstruc.2008.08.008.

[16] J.P. Cornard, J.C. Merlin, Spectroscopic and structural study of complexes of quercetin with Al(III), J. Inorg. Biochem. 92 (2002) 19–27 https://doi.org/10.1016/S0162-0134(02)00469-5.

[17] G. Dehghan, Z. Khoshkam, Tin(II)–quercetin complex: synthesis, spectral characterisation and antioxidant activity, Food Chem. 131 (2012) 422–426 https://doi.org/10.1016/j.foodchem.2011.08.074.

[18] V. Kuntić, S. Blagojević, D. Malešev, Z. Radović, M. Bogavac, Spectrophotometric investigation of the Pd(II)-quercetin complex in 50% ethanol, Monatsh. Chem. 129 (1998) 41–48 https://doi.org/10.1007/PL00010103.

[19] J. Ren, S. Meng, Ch.E. Lekka, E. Kaxiras, Complexation of flavonoids with iron: structure and optical signatures, J. Phys. Chem. B 112 (2008) 1845–1850 https://doi.org/0.1021/jp076881e.

[20] L. Xiao, G. Luo, Y. Tang, P. Yao, Quercetin and iron metabolism: what we know and what we need to know, Food Chem. Toxicol. 114 (2018) 190–203 https://doi.org/10.1016/j.fct.2018.02.022.

[21] J. Zhou, L. Wang, J. Wang, N. Tang, Antioxidative and anti-tumour activities of solid quercetin metal(II) complexes, Transit. Met. Chem. 26 (2001) 57–63 https://doi.org/10.1023/A:1007152927167.

[22] G. Rusak, I. Piantanida, S. Bretschneider, J. Ludwig-Müller, Complex formation of quercetin with lanthanum enhances binding to plant viral satellite double stranded RNA, J. Inorg. Biochem. 103 (2009) 1597–1601 https://doi.org/10.1016/j.jinorgbio.2009.08.008.

[23] D. Nowak, E. Woźnicka, A. Kuźniar, M. Kopacz, Magnetism of the lanthanides(III) complexes with some polihydroxyflavones, J. Alloys Compd. 425 (2006) 59–63 https://doi.org/10.1016/j.jallcom.2005.11.091.

[24] M.T. Fernandez, M.L. Mira, M.H. Florêncio, K.R. Jennings, Iron and copper chelation by flavonoids: an electrospray mass spectrometry study, J. Inorg. Biochem. 92 (2002) 105–111 https://doi.org/10.1016/S0162-0134(02)00511-1.

[25] J. Rybak, L. Ruzik, Application of chromatography and mass spectrometry to the characterization of cobalt, copper, manganese and molybdenum in *Morinda Citrifolia*, J. Chromatogr. A 1281 (2013) 19–25 https://doi.org/10.1016/j.chroma.2013.01.040.

[26] J. Wojcieszek, P. Kwiatkowski, L. Ruzik, Speciation analysis and bioaccessibility evaluation of trace elements in goji berries (*Lycium Barbarum* L.), J. Chromatogr. A 1492 (2017) 70–78 https://doi.org/10.1016/j.chroma.2017.02.069.

[27] G. Weber, P. Konieczyński, Speciation of Mg, Mn and Zn in extracts of medicinal plants, Anal. Bioanal. Chem. 375 (2003) 1067–1073 https://doi.org/10.1007/s00216-002-1706-z.

[28] M. Samsonowicz, E. Regulska, Spectroscopic study of molecular structure, antioxidant activity and biological effects of metal hydroxyflavonol complexes, Spectrochim. Acta, Part A 173 (2017) 757–771 https://doi.org/10.1016/j.saa.2016.10.031.

[29] K.S. Park, C.Y. Lee, H.G. Park, Target DNA induced switches of DNA polymerase activity, Chem. Commun. 51 (2015) 9942–9945 https://doi.org/10.1039/C5CC02060C.