doi.org/10.52568/000632/JCSP/42.02.2020

Novel Synthesis Method, Characterization and Bioactivities of a Copper(II)-Hesperetin Complex Using Ion Exchange Column

Huan Wang[1,2], Boru Chen[1,2], and Siming Zhu[1,2,*]

[1]*School of Food Science and Engineering, South China University of Technology, Guangzhou 510640, China.*

[2]*Guangdong Province Key Laboratory for Green Processing of Natural Products and Product Safety, Guangzhou 510640, China.*

lfsmzhu@scut.edu.cn*

(Received on 8th May 2018, accepted in revised form 18th June 2019)

Summary: A copper(II)-hesperetin complex formulated as $[Cu(L)_2(H_2O)_2]\cdot H2O$ ("L" represented the ligand hesperetin) was synthesized using a novel ion-exchanging resin column method, based on the integration of the separation of hesperetin on hesperetin-loaded resins and the reaction of hesperetin with Cu^{2+} using $CuCl_2$ as an eluent and reacting reagent. The feasibility of this new method was testified firstly. Results indicated the D296 macroporous strong basic anion exchange resin could effectively adsorb hesperetin and the saturated adsorption capacity was 143.5 mg per mL resin. The favorable concentration of $CuCl_2$ was 0.4 mol/L for the elution and the coordination process of hesperetin. The characterization of the copper(II) complex was done by theoretical quantum chemical calculation, elemental and thermal analyses, UV-Vis absorption spectra, FTIR spectra and XRD analysis, results testified that the 4-carbonyl group and 5-hydroxyl group of hesperetin were involved in the coordination reaction. The comparison between hesperetin and its copper(II) complex about their bioactivities like the inhibitory effect of α-amylase, antioxidant activities and solubility were evaluated. Results showed the bioactivities of the copper(II) complex was better than that of hesperetin.

Keywords: Integration of separation and reaction, Ion exchange column, The copper(II)-hesperetin complex, Synthesis, Bioactivity.

Introduction

Flavonoids are a general term for a class of phenolic compounds, belonging to chromone or chroman derivatives. According to statistics, more than 4000 flavonoids have been isolated and identified up to now. They are always in the form of glycosides or free aglycone widely distributed in plants [1-3]. Most of these compounds have received much attention because of their important parts in defining the organoleptic properties of various foods (including color, flavor) and their pharmacological activities, including antioxidant, antitumor, antimicrobial, enzymatic inhibition, anti-inflammatory activities, usually used in vitro or vivo assay [4, 5]. They also have many physiological functions [6], such as reduce blood sugar, blood fat, cholesterol, reduce platelet aggregation, reduce capillary fragility and protect cardiovascular and cerebrovascular. So, these compounds play an important role in our body.

Copper is one of the essential trace elements in human body. The bioavailability of trace elements can be generally improved by complexation reaction. Some trace elements show specific activity only when they were coordinated with ligand of specific structure [7] and the flavonoids can act as metallic ligands due to their peculiar structures [8]. Therefore, it is of great significance to study trace metal elements-flavonoids compound drugs for the development of functional food and health care drugs [9]. And the application of metal complex as therapeutic compound has become more widespread.

Hesperetin (structure shown in Fig. 1), an aglycone of hesperidin, is a kind of bioactive flavonoids, commonly found in citrus fruits such as sweet orange and lemon [10-12]. It has been reported to exhibit pharmacological properties with human hemoglobin and better antitumor activities against human cancer cell lines of hepatocellular carcinoma (HepG-2) and gastric carcinomas (SGC-7901) [13]. Furthermore, hesperetin is one of the effective metal-chelators to coordinate with various metal ions [2, 14, 15]. The chelation of flavonoids with metal

*To whom all correspondence should be addressed.

ions affects the pharmacological activity of the compounds to a great extent. It has already been reported that most of metal flavonoids complexes are shown higher solubility, antioxidant and anticancer activities than free flavonoids [16-18]. Thus, these reports support and strengthen an idea that the coordination of flavonoid with metal ions can improve flavonoid's biological activities and widen its application field. However, the poor solubility of hesperetin limit its coordination reaction with Cu^{2+} to a large scale. Hesperidin, just like hesperetin, possesses poor solubility in water. In our previous work, we found that hesperidin possesses good solubility in alkali water, hesperidin in alkali crude hesperidin-extracting solution can be adsorbed on D296 resin in aionic form, and hesperidin can be easily eluted using $CuCl_2$ solution, making it true to integrate the purification and coordinating reaction of hesperidin with Cu^{2+}. In addition, the copper(II)-hesperidin complex possesses better bioactivities and solubility than hesperidin does [19]. Hesperetin is the aglycone of hesperidin. The above-mentioned findings give us a hint of trying the integration of separation and coordinating reaction of hesperetin with Cu^{2+}, to overcome the poor solubility of hesperetin in coordinating reaction process and to better the bio-activity or bioavailability of hesperetin.

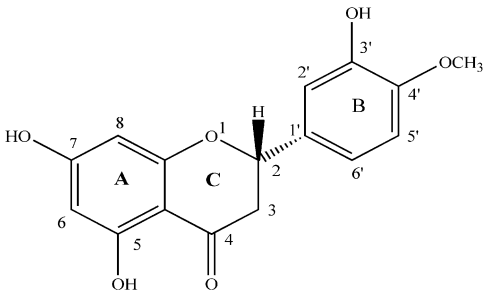

Fig. 1: Structure of hesperetin.

Present work tried a novel method to synthesize the copper(II)-hesperetin complex on an ion exchange column, and the feasibility and mechanism of the chelation reactions of hesperetin with copper ions on the column were investigated. The synthesized copper(Ⅱ) complex was characterized by Elemental analyses, UV-Vis, FTIR, XRD, TG analysis and theoretical quantum chemistry calculation to elucidate the coordination mechanism. And the solubility, α-amylase inhibitory effect and antioxidation activity of the copper(Ⅱ)-hesperetin complex were also studied.

Experimental

Materials and Chemicals

Hesperetin was purchased from Sigma Chemicals Co. and the product (its purity is greater than 95%) was identified using UV-Vis spectra. D296 macroporous strong basic anionic exchange resin (referred to as D296 resin) was provided by Zhengzhou Qinshi Technology Co. Ltd. (Zhengzhou, Henan, China). Methanol, copper dichloride, sodium hydrate, soluble starch, α-amylase, DPPH and Vitamin C were of analytical grade.

Feasibility of the Cu(II)-hesperetin complex synthesis on ion exchange column

Static adsorption curve of hesperetin

D296 resin was pretreated according to the method provided by Zhengzhou Qinshi Technology Co. Ltd. Then 1 mL pretreated wet resins were added into alkali hesperetin solution (200 mL, 1 mg/mL, pH 11.5) with constant oscillation under 60 ℃. In the process of resin adsorption, 0.2 mL solution was sampled every hour and diluted to 4 mL with caustic soda solution (pH 11.5), till the adsorption process reached an equilibrium. The content of hesperetin was determined by UV-Vis spectrophotometry method, and the adsorption capacity of D296 resin to hesperetin was calculated.

Integration of the separation of hesperetin and its coordination with Cu^{2+}

The hesperetin-loaded D296 resin was filled into an 60 ℃ ion exchange column (22 × 480 mm). 1 L of $CuCl_2$ (0.005 mol/L) prepared with 75% methanol was used as eluent. The eluates flew out at a speed of 4 mL/min and were collected or sampled using an automatic partial collector (40 mL per tube). The pH value of each sample was adjusted to pH 5.5. Each tube of eluate was sampled 1 mL and was diluted to 10 mL using 75% methanol and 0.1 mL of NaCl solution (1 mol/L) was added to make the ionic strength of the solution was 0.01mol/L. The elution curve of hesperetin was obtained by measuring the absorbance of each elution sample at 381 nm.

Effect of Cu^{2+} concentration on the static elution of hesperetin

Hesperetin-loaded resins of 1 mL were added to a series of concentrations of $CuCl_2$ solutions

(0.1, 0.2, 0.3, 0.4 and 0.5 mol/L), respectively. The mixture was oscillated in water bath oscillator at a rotational speed of 100 r/min for 2 h. 0.2 mL of each sample (adjusted to pH 5.5) was diluted to 4 mL and the absorbance was measured at 381nm.

Synthesis of the Cu(II)-hesperetin complex

40 mL saturated resins which adsorbed hesperetin were poured into the ion exchange column (22 mm×480 mm). According to the elution curve of hesperetin obtained in the previous step, the samples with the highest absorbency were collected. Each sample was adjusted to its natural pH value and was concentrated at 50 ℃ in a rotary evaporator (RE-52AA, Shanghai, China), and the concentrate liquid was cooled to room temperature and filtered. Precipitation was dried in a freeze drier (SCIENTZ-18N, Ningbo, China) for 24 h. A brown yellowish product Cu(II)-hesperetin complex was then obtained in 78% yield. Calc. for $C_{32}H_{34}O_{15}Cu$: C, 53.14%; H, 4.71%, Cu, 8.86%. Found: C, 51.44%; H, 4.94%, Cu, 9.22% (Elemental Analyzer: Vario EL cube, Germany; ICP-OES: Optima 8300, PerkinElmer).

Theoretical quantum chemistry calculation

In order to acquire the structure information of ligand related to the coordination reaction, the charge of all carbon, hydrogen and oxygen atoms in hesperetin were taken into account. The structural optimization of hesperetin was carried out by the method of CNDO (Complete neglect differential overlap) in Hyperchem software.

Structure characterization of hesperetin and the Cu(II)-hesperetin complex

UV-Vis spectra

UV–Vis spectral scans of hesperetin and the Cu(α)-hesperetin complex were recorded using UV-visible spectrophotometer (TU-1901, Beijing, China) from 275 to 500 nm during each run.

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

In order to confirm the possible chelation site of the Cu(II)-hesperetin complex, FTIR spectra of hesperetin and its copper(II) complex were recorded using a Bruker spectrophotometer (KBr pellets, VERTEX 33, Bruker, Germany) among the wave number region from 500 to 4000 cm^{-1} with a blank KBr disk as background. Both samples were mixed with dry potassium bromide (KBr) powder respectively and then compressed into 1 mm disk. The IR spectra difference can be used to characterize the structure difference of hesperetin and its complex.

X-ray Diffractometry (XRD) Analysis

The flavonoid-metal complex may be either crystalline or amorphous. In order to study the structure of the Cu(II)-hesperetin complex, the crystal morphology of the $CuCl_2 \cdot 2H_2O$, hesperetin and Cu(II)-hesperetin complex were studied by means of XRD (Bruker, Germany). The scanning regions of the diffraction angle of 2θ were 2–90˚.

Thermogravimetric (TG) Analysis

The TG-DTG curves of the Cu(II)-hesperetin complex were recorded using a Thermal gravimetric analyzer (NETZSCH TG 209 F1 Libra, Germany) with a sample weight 15 mg over the temperature range of 30–500 °C and a heating rate of 10 °C/min. The measurements were carried out in nitrogen atmosphere.

Determination of the solubility of hesperetin and its complex

Different concentrations of hesperetin (5, 10, 20, 40 and 60 μg/mL) and its copper complex solutions (10, 30, 50, 70 and 90 μg/mL) were dissolved completely in 75% methanol by stirring in a magnetic stirrer with water bath for 6 h at 60 ℃. Then the calibration curves were drawn according to the absorbance measured at the maximum wavelength. In order to determine the solubility of hesperetin and its complex in 75% methanol, 10 mg of hesperetin and its complex were placed in the cuvettes separately and 5 mL of 75% methanol were added to each cuvette. Then the cuvettes were left in a shaker overnight at 25 ℃. The solutions were centrifuged at 4000 rpm for 10 min, 1 mL supernatant of these samples were diluted to 5 mL with 75% methanol. After mixing evenly, the absorbance of hesperetin and its copper complex solutions were measured at 358 and 381 nm, respectively.

Inhibition assay for α-amylase activity

The inhibition of α-amylase assay was a slight modification of the method previously reported

by Wang, Du, and Song [20]. In this study, 1% soluble starch solution was prepared with 0.05 M sodium phosphate buffer (pH 6.9), which was used as the substrate. And 400 μL of α-amylase solution (enzyme activity 40 U/mL) was mixed with 1 mL of sodium phosphate buffer (pH 6.9), followed by the addition of 200 μL different concentrations of hesperetin and its copper complex solutions (100-500 μg/mL), respectively. After reaction at 37 ℃ for 10 min, 1% soluble starch solution (300 μL) was added and the mixture re-incubated at 37 ℃ for 30 min. The reaction was terminated by adding 0.05 M dinitrosalicylic acid solution (0.2 mL) and boiled for 15 min in boiling water. The final volume of the mixture is up to 8 mL with distilled water. In the blank group, sodium phosphate buffer solution was used instead of α-amylase solution. Acarbose (100 μg/mL) was used as a positive control. All samples were measured at 540 nm. The assay has been done in triplicate. The percentage of α-amylase inhibition was calculated as follows:

$$\text{inhibition } (\%) = \left(1 - \frac{\Delta A_{sample}}{\Delta A_{enzyme}}\right) \times 100$$

Antioxidant activity of the Cu(II)-hesperetin complex by DPPH method

The scavenging of the DPPH radicals by hesperetin and its copper complex were measured in triplicate using a modified method raised by Lalhminghlui *et al* [21]. Hesperetin and its copper complex of 2 mL were added into 100 μmol/L 1,1-diphenyl-2-picrylhydrazyl (DPPH·) (2mL) in methanol to give final antioxidant concentrations of 0.1, 0.2, 0.3, 0.4, 0.5 mg/mL, respectively. The absorbance of testing samples were measured and recorded at 517 nm by a UV-Visible spectrophotometer after reacting 60 min in the dark and the results were expressed as the reduction amount of DPPH· radicals. Vitamin C was used as a positive control. Results are expressed as IC 50 (50% inhibition) calculated via a diagram of concentration and inhibition rate.

Results and discussion

Static adsorption curve of hesperetin

As shown in Fig. 2, the absorbance of solution decreased with the time increased. It could also be seen that after 10 h the absorbency was almost invariable, indicating the D296 resin was saturated at this time. In addition, the standard curve of basic hesperetin solution was Y=11.27x + 0.0057 (R^2=0.9997, the unit of x was 1 mg/mL). According to the regression equation, the saturated adsorption capacity of the D296 resin for hesperetin was 143.5 mg/mL.

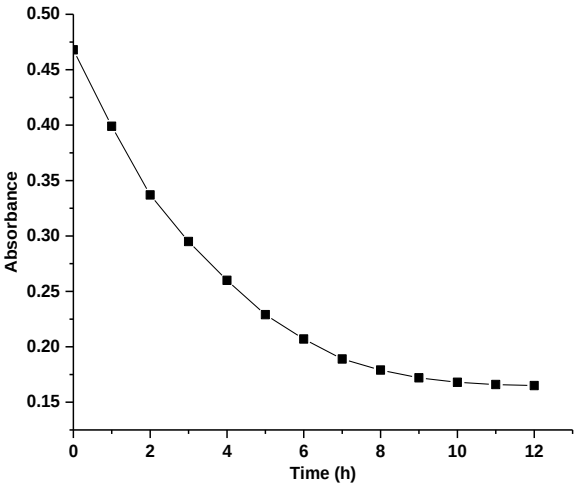

Fig. 2: Static adsorption curve of hesperetin using D296 macroporous strong basic anionic exchange resin.

Dynamic elution curve of hesperetin

As shown in Fig. 3, the dynamic elution curve was a parabolic type, indicating that $CuCl_2$ solution could effectively elute hesperetin from the resin and realize the integration of ligand separation and coordination reaction. The absorbency of eluant increased first and then decreased with the time increased, and the highest absorbency appeared at the eluate volume of 320 mL.

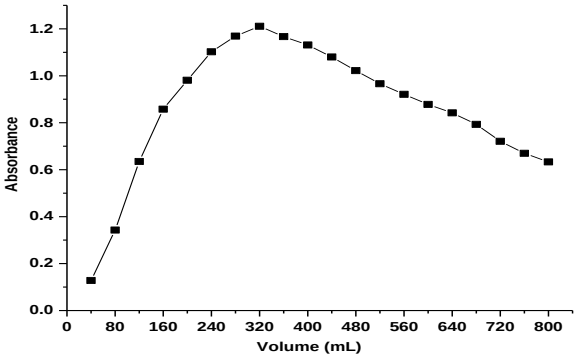

Fig. 3: Dynamic elution curve of hesperetin.

Compared with the solution coordination method [2, 22, 23], the synthesis of Cu(II)-hesperetin complex by ion exchange column method was simpler and lower cost. Furthermore, the Cu(II)-hesperetin complex could be synthesized and separated as soon as the reaction was finished. And most importantly, the ion exchange column method could be realized the continuous reaction of hesperetin and Cu^{2+} ions.

Effect of Cu^{2+} concentration on static elution

The concentration of Cu^{2+} in eluant has a great influence on the synthesis of the Cu(II)-hesperetin complex on the ion exchange column. It showed that the static regeneration ability of D296 resin was no longer significantly increased when the concentration of Cu^{2+} was up to 0.4mol/L (Fig. 4). If the ionic strength of the regenerant was too high, the macroporous resin would be shrunk and the adsorbate was intercepted in the pore. Furthermore, under the condition of high salt concentration, the surface tension of the solution was increased, which was not conducive to the migration of the hydrophobic hesperetin from the resin surface to the solution. But if ionic strength of the regenerator was too low, the chelating reaction may not be complete. Therefore, the appropriate concentration of Cu^{2+} was 0.4mol/L.

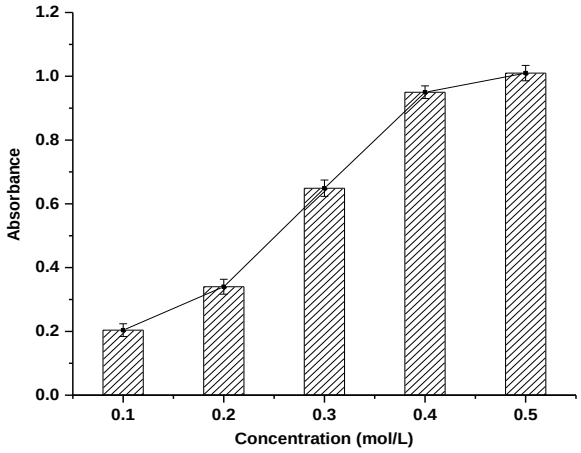

Fig. 4: Effect of Cu^{2+} concentration on the synthesis of the Cu(II)-complex.

Theoretical quantum chemistry calculation of hesperetin

The results presented in Fig. 5 showed that the negative charge of the oxygen atom in 4-carbonyl group and 5-hydroxyl group was the largest. The charge of the oxygen atom in the carbonyl group was 0.352 times of an electron and in hydroxyl group it was 0.318 times. Thus, the 4-carbonyl group and the 5-hydroxyl group were the tentative sites for coordination with copper ions. The stoichiometry ratio(metal:ligand=1:2) of the Cu(Ⅱ)-hesperetin complex has been reported [22, 23].

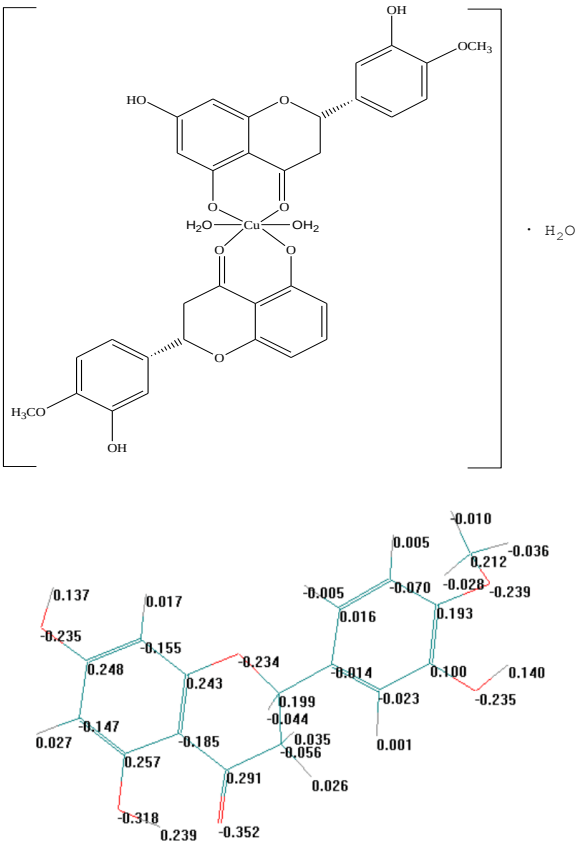

Fig. 5: The tentative structure of the Cu(α)-hesperetin complex and the charge of each atom in hesperetin optimized by the method of Complete neglect differential overlap. A red interconnection point stand for an oxygen atom.

Structure comparison of hesperetin and its copper complex

UV-Visible spectra analysis

The UV spectra of hesperetin and the Cu(II)-hesperetin complex were recorded. As shown in Fig. 6, a strong peak was observed in hesperetin spectra at 358 nm, corresponding to the B ring

portion (cinnamyl system) [24]. But after reacting with the Cu(Ⅱ) ions, the maximum absorption band was shifted to 381 nm, a slightly bathochromic-shift with respect to hesperetin, indicating the interaction of Cu(II) ions with the condensed ring in ligand. That was illustrated the formation of complex between hesperetin and copper(II) [25].

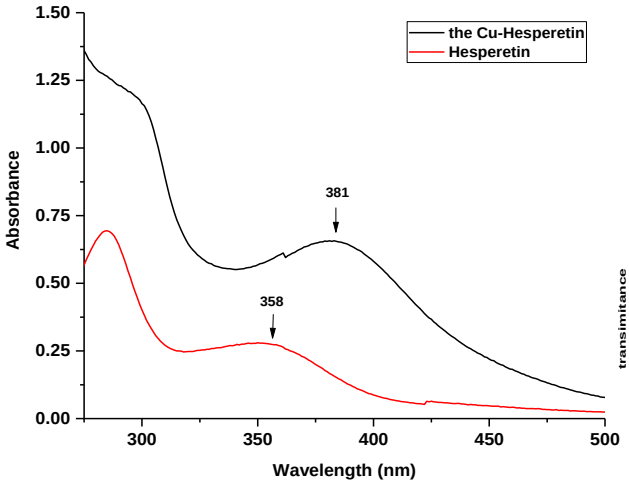

Fig. 6: UV–Vis spectra of hesperetin and its copper complex.

The chromophore and auxochrome in hesperetin were carbonyl group in ring C and hydroxyl group in ring A, respectively. The absorption of the Cu(II)-hesperetin complex was obviously higher than that of the ligand, indicating the carbonyl group and the hydroxyl group were involved in the complexation reaction [26].

FTIR Analysis of hesperetin and its complex

The IR spectra of hesperetin and the Cu(II)-hesperetin complex were presented in Fig. 7. The characteristic stretching *v*(C=O) of ligand was shifted from 1650 to 1614 cm^{-1}, indicating that the carbonyl group in hesperetin was involved in the coordination reaction and the formation of complex caused a red shift in absorption bands. This was due to the formation of coordination bonds between the oxygen atoms of the carbonyl group and the copper ions, resulting in the decrease of the electron cloud density of C–O [27]. The band of O–H stretching mode of hesperetin at 3480 cm^{-1} shifted to 3368 cm^{-1}, which could be explained by coordination of hydroxyl oxygen with copper ion [28]. The binding sites between metal ion and flavonoid were consistent with the previous report [2, 29-31]. After the coordination, a new peak at 605 cm^{-1} of the complex testified the formation of *v*(Cu–O) bands. The band related to the *v*(C–O–C) mode at 1276 cm^{-1} was not shifted by the copper complex formation, indicating that benzene rings didn't open after the coordination reaction, and the oxygen atom in the ligand C ring was not involved in the coordination.

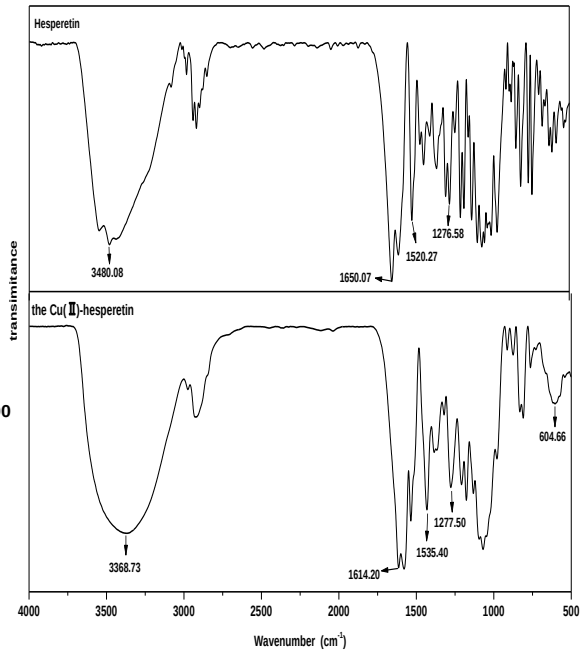

Fig. 7: Infrared spectra of hesperetin and Cu(II)-hesperetin complex.

XRD analysis of hesperetin and its complex

The X-ray diffraction patterns of three samples showed the various peaks with different intensities (Fig. 8), confirming the crystalline nature of hesperetin, copper chloride and its complex [32]. Obviously, $CuCl_2 \cdot 2H_2O$ belong to typical crystals with high degree of crystallinity, whose crystalline diffraction peaks were incisive. But the peak intensities of hesperetin and its copper complex were weaker than $CuCl_2 \cdot 2H_2O$, indicating the crystallinity of hesperetin and its complex were relatively low. Besides, the highest intensity peaks of $CuCl_2 \cdot 2H_2O$, hesperetin and their complex were found at 2θ values of 16.31°, 19.56° and 31.64°, respectively. The angle of the highest diffraction peak of the complex was larger than that of hesperetin and $CuCl_2 \cdot 2H_2O$, which indicated that the lattice constant of the newly formed complex became smaller. The diffraction patterns of

the complex were different from those of $CuCl_2 \cdot 2H_2O$ and hesperetin, illustrating that the exist of coordination reaction between hesperetin and Cu^{2+}.

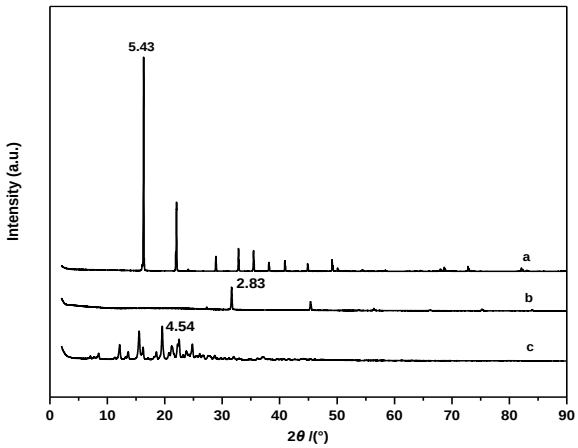

Fig. 8: The X-ray diffraction of (a) $CuCl_2 \cdot 2H_2O$, (b) the Cu(II)-hesperetin and (c) hesperetin. Interplanar crystal spacing (nm) has been marked in the diagram.

TG analysis of the Cu(II)-hesperetin complex

The analysis of TG and DTG curves (Fig. 9) made the molecular formula of this Cu(II)-hesperetin complex clearer. The results showed that the thermal decomposition of the complex displayed three stages from 30 °C to 500 °C. The first stage of decomposition from 30 °C to 90 °C was connected with the dehydration processes. The mass loss value of 2.89% was corresponded to the loss for a water molecule in the outer coordination sphere, which was consistent with the calculated mass loss value of a water molecule of 2.49%. The mass loss value of the next dehydration process in the temperature range of 100–230 °C was 4.96%, which was in close agreement with the calculated mass loss of 4.98% for the two combined water molecules. The final exothermic decomposition stage was considered to be the oxidative degradation of the organic part of the copper complex.

The solubility of hesperetin and its complex

Two calibration equation for hesperetin and its copper complex could be given by: $Y = 51.614x-0.0764$ ($R^2 = 0.9966$) and $Y = 43.855x-0.0412$ ($R^2 = 0.9987$) respectively, where x is the concentration of stock solutions. Then, the absorbance of these samples was substituted to the calibration equation, and the solubility of hesperetin and its complex were 18.6 and 260 μg/mL, respectively. Obviously, the complex was significantly more soluble (14 times) than hesperetin in 75% methanol, indicating that the chelation between hesperetin and copper ions could greatly improved its bioavailabilities and widen its application in food industry [33]. And the reason for the higher water solubility of the complex can be speculated as the "concentration effect" of Cu^{2+} ions.

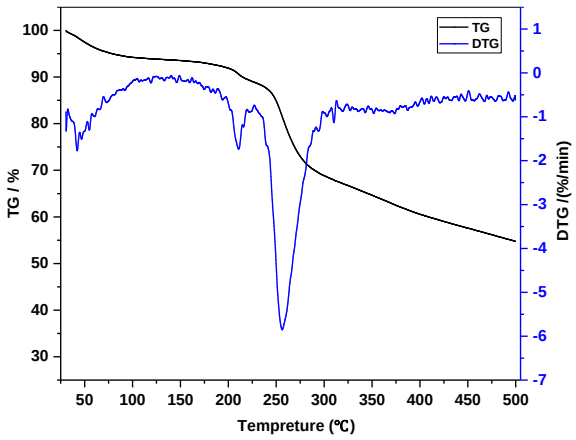

Fig. 9: TG-DTG curves of the Cu(II)-hesperetin complex.

Assay for α-amylase inhibitory activity in vitro

In this assay, the chelation reaction did not make any prominent improvement on free hesperetin behavior. Apparently, the inhibitory effect on α-amylase was concentration-dependent. Although hesperetin and its copper(II) complex were less potent than standard inhibitor acarbose (100 μg/mL), it also showed that hesperetin and its complex at the concentration range of 100–500 μg/mL could inhibit the activity of α-amylase to some extent (Table I).

Table-I: α-amylase inhibition activity of hesperetin and its complex.

Sample concentration (μg/mL)	Inhibition (%)	
	hesperetin	Cu(Ⅱ)-hesperetin complex
100	3.63 ± 0.83	4.10 ± 1.19
200	4.27 ± 0.92	4.92 ± 0.76
300	5.14 ± 1.18	5.42 ± 1.07
400	5.98 ± 1.09	6.37 ± 1.25
500	6.72 ± 1.63	7.26 ± 1.23

Positive control: Acarbose (100 μg/mL), inhibition (%)=40.71 ± 1.21. values are the means ± SD (n = 3).

Antioxidant activity of hesperetin and its complex by DPPH radicals scavenging method

Hesperetin has been proved to possess the properties of antioxidation and free radical scavenging [34], the scavenging effect of hesperetin and its complex were estimated by the DPPH method in this work (Fig. 10). The result of DPPH assay indicated that there was a increase in the antioxidant activity of Cu(II)-hesperetin complex (150.4 ± 1.9 μg/mL) compared to that of free hesperetin (180.1 ± 2.3 μg/mL). Although the scavenging ability of DPPH· radicals by hesperetin and its complex was lower than that of vitamin C (6.96 ± 0.09 μg/mL), the coordination reaction could better the antioxidation of scavenging DPPH· radicals by hesperetin, which may ascribe to hydrophobic compound was accessible in water for the reduction of DPPH· radicals [35]. Therefore, the better ability in scavenging DPPH· radicals by Cu(II)-hesperetin complex was probably related to its own chemical structure and the improvement on its solubility [36]. In general, result of the DPPH assay may be explained as the solubility and structural transformation in the coordination process.

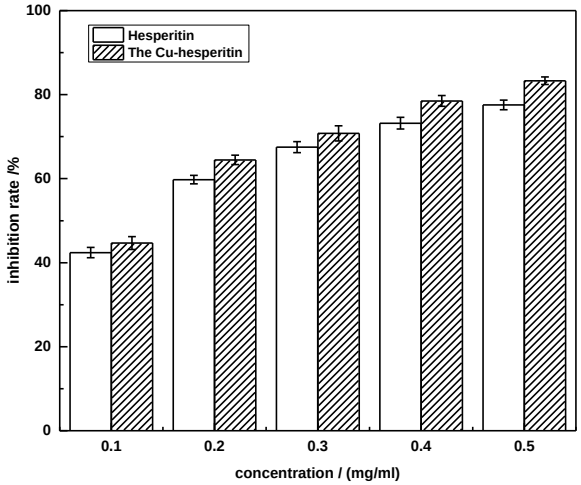

Fig. 10: Effects of hesperetin and its metal complex in different concentration on the scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. Values are expressed as the mean ± standard error of the mean (SEM) of three independent experiments.

Conclusion

In this paper, the integration of ligand hesperetin separation and coordination reaction was realized and a copper(II)-hesperetin complex was synthesized by a newly ion exchange column method. TG analysis made the molecular formula of the complex clearer. According to the UV-Vis, FTIR and XRD spectrum, the formation of the Cu(II)-hesperetin complex was confirmed and the carbonyl group and the hydroxyl group in hesperetin were involved in the coordination reaction, which was consistent with the result of theoretical quantum chemical calculations. Besides, the water solubility of the Cu(II)-hesperetin complex was significantly higher than that of free hesperetin. The results from DPPH method and α-amylase inhibitory activity assay showed that both hesperetin and Cu(II)-hesperetin complex were capable of reacting with free radical and could inhibit α-amylase activity to some extent. The results of this study indicated that the chelation of copper ions by hesperetin increase the antioxidant activity and solubility of the Cu(II)-hesperetin complex.

Acknowledgments

The authors thank the Science and Technolo -gy Planning Project of Guangzhou(201903010066), the Science and Technology Planning Project of Dep -artment of agriculture of Guangdong Province(2019 KJ144),the Science and Technology Planning Project of the Xinjiang production and Construction Corps, China(2018AB009), the Natural Science Foundation of Xinjiang Uygur Autonomous Region,China(2018 D01A03),the Science and Technology Innovation tea m's construction Foundation in Xinjiang Uygur Auto nomous Region (2014751002) and the National 111 Project, China (B17018) .

References

1. V. Kuntic, J. Brboric, I. Holclajtner-Antunovic and S. Uskokovic-Markovic, Evaluating the bioactive effects of flavonoid hesperidin: A new literature data survey, *Vojnosanit. Pregl.*, **71**, 60 (2014).
2. S. Selvaraj, S. Krishnaswamy, V. Devashya, S. Sethuraman and U. M. Krishnan, Membrane fluidization and eryptotic properties of hesperidin–copper complex, *RSC Adv.*, **2**, 11138 (2012).
3. G. V. Slot, W. Mattern and S. Rzeppa, Complex Flavonoids in Cocoa: Synthesis and Degradation b Intestinal Microbiota, *J. Agr. Food Chem.*, **58**, 8879 (2010).
4. A. Tripathi, H. Awasthi, D. B. Rokaya, D. Srivastava and V. Srivastava, Antimicrobial and Wound Healing Potential of Dietary Flavonoid

Naringenin, *The Nat. Prod.*, **9**, 61 (2019).
5. M. A. M. Abdelgawad, M. A. M. Abdelaziz, M. E. M. Elsayed, E. E. E. Elwakil and E. A. E. Abdellateef, In vitro antioxidant, total phenolic and flavonoid contents of six allium species growing in egypt, *J. Microbiol. Biotech. Food Sci.*, **4**, 343 (2018).
6. D. Nakabo, Y. Okano, N. Kandori, T. Satahira, N. Kataoka, J. Akamatsu and Y. Okada, Convenient Synthesis and Physiological Activities of Flavonoids in Coreopsis lanceolata L. Petals and Their Related Compounds, *Molecules*, **23**, 1671 (2018).
7. V. A. Kostyuk, A. I. Potapovich, E. N. Vladykovskaya, L. G. Korkina and I. B. Afanas'Ev, Influence of metal ions on flavonoid protection against asbestos-induced cell injury, *Arch. Biochem. Biophys.*, **385**, 129 (2001).
8. B. C. Wang, J. Z. Qian, F. Ying and J. Tan, The QSAR study of flavonoid-metal complexes scavenging OH free radical, *J. Mol. Struct.*, **1075**, 204 (2014).
9. E. González-Molina, R. Domínguez-Perles, D. A. Moreno and C. García-Viguera, Natural bioactive compounds of Citrus limon for food and health, *J. Pharmaceut. Biomed.*, **51**, 327 (2010).
10. M. Ersoz, A. Erdemir, D. Duranoglu, D. Uzunoglu, T. Arasoglu, S. Derman and B. Mansuroglu, Comparative evaluation of hesperetin loaded nanoparticles for anticancer activity against C6 glioma cancer cells, *Artif. Cells Nanomed. Biotechnol.*, **47**, 319 (2019).
11. F. Federico, G. V. Christina and T. L. Francisco, Hesperetin: A marker of the floral origin of citrus honey, *J. Sci. Food Agr.*, **61**, 121 (2010).
12. P. Hamideh, R. Ali, S. Fatemeh, R. Ramin and I. Mehrdad, Antioxidant and anti-inflammatory properties of the citrus flavonoids hesperidin and hesperetin: an updated review of their molecular mechanisms and experimental models, *Phytother. Res.*, **29**, 323 (2015).
13. S. Chakraborty, S. Chaudhuri, B. Pahari, J. Taylor, P. K. Sengupta and B. Sengupta, A critical study on the interactions of hesperitin with human hemoglobin: Fluorescence spectroscopic and molecular modeling approach, *J. Lumin.*, **132**, 1522 (2012).
14. P. Ryan and M. J. Hynes, The kinetics and mechanisms of the reactions of iron(III) with naringenin, hesperetin, 3-hydroxychromone and 3-hydroxyflavone. A comparison of the coordination power of 3-hydroxychromone and 5-hydroxychromone, *J. Coord. Chem.*, **61**, 3711 (2008).
15. Y. Sameena and I. V. M. V. Enoch, The influence of β-cyclodextrin on the interaction of hesperetin and its bismuth (III) complex with calf thymus DNA, *J. Lumin.*, **138**, 105 (2013).
16. S. Roy, S. Banerjee and T. Chakraborty, Vanadium quercetin complex attenuates mammary cancer by regulating the P53, Akt/mTOR pathway and downregulates cellular proliferation correlated with increased apoptotic events, *Biometals*, **31**, 647 (2018).
17. A. D. P. Alexiou, C. C. Decandio, S. D. N. Almeida, M. J. P. Ferreira, P. Romoff and R. C. Rocha, Metal-ligand coordination and antiradical activity of a trichromium(III) complex with the flavonoid naringenin, *J. Coord. Chem.*, **70**, 1 (2017).
18. J. J. M. Medina, L. G. Naso, A. L. Pérez, A. Rizzi, N. B. Okulik, E. G. Ferrer and P. A. M. Williams, Apigenin oxidovanadium(IV) cation interactions. Synthesis, spectral, bovine serum albumin binding, antioxidant and anticancer studies, *J. Photoch. Photobio. A.*, **344**, 84 (2017).
19. S. J. Yu, S. M. Zhu and X. A. Zeng, Integration of separation and coordination reaction of hesperidin in Ion-Exchange Chromatography column, *J. South China Univ. Techno.*, **35**, 137 (2007).
20. W. Hui, Y. J. Du and H. C. Song, α-Glucosidase and α-amylase inhibitory activities of guava leaves, *Food Chem.*, **123**, 6 (2010).
21. K. Lalhminghlui and G. C. Jagetia, Evaluation of the free-radical scavenging and antioxidant activities of Chilauni, Schima wallichii Korth in vitro, *Future Sci. OA*, **4**, O272 (2018).
22. L. V. Tamayo, L. R. Gouvea, A. C. Sousa, R. M. Albuquerque, S. F. Teixeira, R. A. de Azevedo, S. R. W. Louro, A. K. Ferreira and H. Beraldo, Copper(II) complexes with naringenin and hesperetin: cytotoxic activity against A 549 human lung adenocarcinoma cells and investigation on the mode of action, *Biometals*, **29**, 39 (2016).
23. M. Tan, J. Zhu, Y. Pan, Z. Chen, H. Liang, H. Liu and H. Wang, Synthesis, Cytotoxic Activity, and DNA Binding Properties of Copper (II) Complexes with Hesperetin, Naringenin, and Apigenin, *Bioinorg. Chem. Appl.*, **2009**, 1 (2009).
24. N. E. A. Ikeda, E. M. Novak, D. A. Maria, A. S. Velosa and R. M. S. Pereira, Synthesis, characterization and biological evaluation of Rutin–zinc(II) flavonoid -metal complex,

Chem.-Biol. Interact., **239**, 184 (2015).

25. G. Dehghan, J. E. Dolatabadi, A. Jouyban, K. A. Zeynali, S. M. Ahmadi and S. Kashanian, Spectroscopic studies on the interaction of quercetin-terbium(III) complex with calf thymus DNA, *DNA Cell Biol.*, **30**, 195 (2011).
26. S. M. Ahmadi, G. Dehghan, M. A. Hosseinpourfeizi, J. E. Dolatabadi and S. Kashanian, Preparation, characterization, and DNA binding studies of water-soluble quercetin--molybdenum(VI) complex, *DNA Cell Biol.*, **30**, 517 (2011).
27. U. Valentina, B. Mihaela, O. Rodica, D. Constantin and B. Stefania Felicia, Synthesis and characterization of some new complexes of magnesium (II) and zinc (II) with the natural flavonoid primuletin, *Molecules*, **18**, 7631 (2013).
28. S. Roy, S. Majumdar, A. K. Singh, B. Ghosh, N. Ghosh, S. Manna, T. Chakraborty and S. Mallick, Synthesis, Characterization, Antioxidant Status, and Toxicity Study of Vanadium–Rutin Complex in Balb/c Mice, *Biol. Trace Elem. Res.*, **166**, 183 (2015).
29. S. Stalin, K. Sridharan, D. Venkappayya, S. Swaminathan and K. Uma Maheswari, Flavonoid-metal ion complexes: a novel class of therapeutic agents, *Med. Res. Rev.*, **34**, 677 (2014).
30. S. Selvaraj, S. Krishnaswamy, V. Devashya, S. Sethuraman and U. M. Krishnan, Synthesis, characterization and DNA binding properties of rutin–iron complex, *RSC Adv.*, **2**, 2797 (2012).
31. S. Stalin, K. Sridharan, D. Venkappayya, S. Swaminathan and K. Uma Maheswari, Investigations on membrane perturbation by chrysin and its copper complex using self-assembled lipid bilayers, *Langmuir*, **27**, 13374 (2011).
32. N. Sahu, D. Soni, B. Chandrashekhar, D. B. Satpute, S. Saravanadevi, B. K. Sarangi and R. A. Pandey, Synthesis of silver nanoparticles using flavonoids: hesperidin, naringin and diosmin, and their antibacterial effects and cytotoxicity, *Int. Nano Lett.*, **6**, 173 (2016).
33. K. Slámová, J. Kapešová and K. Valentová, "Sweet Flavonoids": Glycosidase-Catalyzed Modifications, *Int. J. Mol. Sci.*, **19**, 2126 (2018).
34. C. Meng, Z. Guo, D. Li, H. Li, J. He, D. Wen and B. Luo, Preventive effect of hesperidin modulates inflammatory responses and antioxidant status following acute myocardial infarction through the expression of PPAR-γ and Bcl-2 in model mice, *Mol. Med. Rep.*, **17**, 1261 (2017).
35. B. M. Lue, N. S. Nielsen, C. Jacobsen, L. Hellgren, Z. Guo and X. Xu, Antioxidant properties of modified rutin esters by DPPH, reducing power, iron chelation and human low density lipoprotein assays, *Food Chem.*, **123**, 221 (2010).
36. J. Chen, Z. Zhu, K. Hang and X. Yang, Relationship between Structure and Activity of Eight Natural Flavonoids Against Oxidation, *J. East China Normal Univ.*, **5240**, 90 (2002).