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ORIGINAL ARTICLE

Comparison of the Content of Flavonoids, Total Phenols, and Carotenoids and Antioxidant Activity in Guang *Citri reticulatae* Pericarpium during the Aging Time

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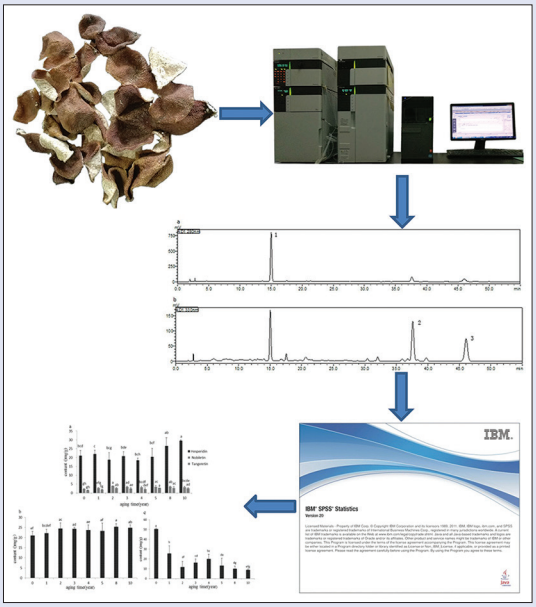
ABSTRACT

Background: Guang *Citri reticulatae* Pericarpium (GCRP) is a traditional Chinese medicine, widely used in respiratory and digestive diseases. GCRP must be aged by stored for several years before it can be used as medicine. In the aging process, content of composition, antioxidant activity, and color of GCRP have changed. **Objectives:** The objective of this study was to compare the content of flavonoids, total phenols, and carotenoids and antioxidant activity in GCRP during the aging time and analyze the correlations of the data. **Materials and Methods:** Forty batches of GCRP in different aging years were used for qualitative and quantitative analysis of flavonoids, total phenols, and carotenoids by ultra-high-performance liquid chromatography (HPLC)–mass spectrometry, HPLC–diode array detector, and ultraviolet methods. 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt and 1,1-diphenyl-2-trinitrophenylhydrazine methods were used to compare the antioxidant activity of GCRP. Colorimetric analysis method was used to determine the external color of GCRP objectively and quantitatively. SPSS software was used to analyze the correlation of all data. **Results:** With the increase of aging year, the content of three flavonoids and total phenols increased and the content of carotenoids decreased. The results showed that the antioxidant activity of ether extract increased significantly with aging year and methanol extract decreased. The correlation results showed that the content of carotenoids was more closely related to chromaticity values and antioxidant activity. **Conclusion:** The result provides more objective and detailed information to analyze the quality of GCRP during the aging time.

Key words: Aging time, antioxidant activity, carotenoids, flavonoids, Guang *Citri reticulatae* Pericarpium

SUMMARY

- The contents of hesperidin, nobiletin, and tangeretin, total phenols, carotenoids, and antioxidant activities reached a relative steady state at 3 years after aging. This study provided information on the parlance that aging time of 3 years can be used medically. The content of carotenoids was closely related to chromaticity values and antioxidant activity.



Abbreviations used: GCRP: Guang *Citri reticulatae* Pericarpium; UPLC-Q-TOF-MS: Ultra-high-performance liquid chromatography–quadrupole/time-of-flight mass spectrometry; DPPH: 1,1-diphenyl-2-trinitrophenylhydrazine; ABTS: 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt.

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INTRODUCTION

Guang *Citri reticulatae* Pericarpium (GCRP), the dried ripe pericarp of *Citrus reticulata* "Chachi" produced in Xinhui, China, is one of the most famous Chinese herbal medicines officially listed in the Chinese Pharmacopoeia.[1] GCRP is recognized as "a national geographical indication product" and its quality is superior to that of orange peel from other origins.[2] It is used as not only a common Chinese medicine for cough and abdominal distension with long history but also a traditional spice and condiment.[3-5] Phytochemical studies showed abundant compounds presented in GCRP, such as flavonoids, alkaloids, phenolic acids, essential oil, and carotenoids.[6-10] GCRP was reported

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to possess various pharmacological activities, involving anticancer, anti-inflammatory, antioxidant, and anti-leukemia.[11-17]

Since ancient times, GCRP has been considered to have better efficacy and lower side effects after aging by storage in shade and dry place.[18,19] Now, it is generally believed that new orange peel needs to be aged for more than 3 years before it can be used medicinally.[20,21] External color of GCRP is one of the important traditional methods to judge aging time of GCRP. When GCRP is aged, its color gradually deepens and finally becomes brownish-red to brownish-black. The reason is probably because internal pigment compositions change during the aging time. However, little is known about the change correlation of compositions and biological activities and external color in GCRP during the aging time. Previous studies showed that the main pigment components included flavonoids and carotenoids which possess strong antioxidant activity in GCRP.[22] Literature studies suggested that all the pharmacological effects may be associated with antioxidant ability.[11] To analyze the quality change of GCRP during the aging time, we qualitatively and quantitatively analyzed flavonoid, total phenol, and carotenoid compounds and compared their antioxidant activities *in vitro* in GCRP. The correlations of aging years, chromaticity values, pigment component contents, and antioxidant activities were statistically analyzed in this study.

MATERIALS AND METHODS

Samples collection

Samples were purchased from Xinbaotang Chenpi Co. Ltd. (Xinhui, Guangdong, China), which grow up in Xinhui District, Jiangmen city, Guangdong Province, China. Forty batches of dried samples of 0, 1, 2, 3, 4, 5, 8, and 10 aging years were collected. All samples were authenticated as dried and mature peel of *Citrus reticulata* "Chachi" by Professor Jizhu Liu (College of Traditional Chinese Medicine, Guangdong Pharmaceutical University). Voucher specimens were deposited in the Herbarium of Traditional Chinese Medicine of Guangdong Pharmaceutical University for specimen's identification and future reference.

Instruments and reagents

In the experiment, we used ultra-high-performance liquid chromatography–quadrupole/time-of-flight mass spectrometry (UHPLC-Q-TOF-MS, Waters, America) for qualitative analysis of components. LC-20A (Shimadzu, Japan) was used to quantitative analysis of components coupled with diode array detector (DAD). KQ-300E ultrasonic cleaner (Kunshan, China) was used to extract components. We used CR-400 color colorimeter (Japan Konica Minolta Co. Ltd.) and CR-A50 powder test kit (Japan Konica Minolta Co. Ltd) to measure the chromaticity values of the samples. UV2310 ultraviolet (UV)–visible spectrophotometer (Tianmei, China) was used to analyze the content of components and antioxidant activities. SPSS 22.0 statistical analysis software (IBM Company, USA) was used to analyze correlation. Hesperidin (MUST-1803250), nobiletin (MUST-18042205), tangeretin (MUST-18012910), and gallic acid (110722-201613) were purchased from Chengdu Must Biotechnology Co., Ltd. β-carotene (7235-40-7) was purchased from Henan Province Wanjia Shouhua Biotechnology Co., Ltd. The purities of all above standard references are more than 98%, which meets the requirements of quantitative analysis. Methanol of chromatographic grade was purchased from Merck, Germany. Acetic acid of chromatographic grade and Folin–Ciocalteu reagent were purchased from Shanghai McLean Biochemical Technology Co. Ltd.

1,1-diphenyl-2-trinitrophenylhydrazine (DPPH) and 2,2-diazo-bis (3-ethyl-benzothiazo-6-sulfonic acid) diammonium salt (ABTS) were purchased from McLean Chemical Reagent Co. Ltd. Other reagents are of analytical grade.

Qualitative analysis of flavonoids components

Forty batches of GCRP samples were cut into smaller pieces and grounded into powder (40 mesh). Sample powder was extracted with methanol (1:40) by ultrasonication for 30 min. The extracted solution was centrifuged at 2500 r/min for 20 min and then the supernatant was diluted to 10 ml with methanol.

Qualitative analysis was performed by HPLC coupled to mass spectrometry. Mobile phase consisted of methanol (A) and 0.2% acetic acid solution (B) with 0.3 ml/min flow velocity and 35°C column temperature. The gradient elution conditions were as follows: 0 min, 10% A; 8 min, 35% A; 20 min, 50% A; 32 min, 65% A; 36 min, 80% A; and 44 min, 10% A. Data were acquired using electrospray ionization in positive ion mode. The source parameters were set as: ion source temperature: 100°C; solvent gas temperature: 350°C; collision energy: 10V; taper hole voltage: 30V; capillary voltage: 3000V; desolvent gas: nitrogen; colliding gas: argon; flow rate of reverse blowing nitrogen in cone hole: 60 l/h; and desolvent nitrogen flow rate: 600 l/h. Real-time correction of experimental data collection was performed using lock mass pathway. Sample solutions were analyzed by UHPLC-Q-TOF-MS with 0.45 μm microporous membrane filtered in 330 nm detection wavelength.

Quantitative analysis of flavonoids components

Then, we used HPLC to determine the contents of hesperidin, nobiletin, and tangeretin components.[23,24] The extraction method of the sample is the same as above. Quantitative analysis was performed on an Agilent LC-20A system equipped with a diode array detector (DAD). Samples were separated on Inertsil ODS-3 C_{18} chromatographic column (4.6 mm × 250 mm, 5 μm) at 25°C. The mobile phase consisted of methanol (A) and 0.2% acetic acid solution (B) with 1.0 ml/min flow velocity. The optimized gradient elution was as follows: 0 min, 35% A; 10 min, 50% A; 23 min, 65% A; and 55 min, 70% A. Sample solutions were filtered with 0.45 μm microporous membrane and analyzed at 330 nm and 280 nm detection wavelength, respectively. The chromatographic peaks of three flavones were confirmed by comparing their retention time with those of standard references. The linearity, precision, stability, repeatability, and recovery of the method were validated. The detection limit and the quantification limit were set a signal-to-noise ratio of 3 and 10, respectively

Quantitative analysis of total phenols

The content of total phenols was measured using Folin–Ciocalteu colorimetric method.[25] Samples were extracted twice with methanol (1:20) by ultrasonication for 30 min. Then, after centrifugation for 20 min (2500 r/min), the supernatant was diluted to 10 ml with methanol. 0.1 ml aliquot of sample solution was mixed with 2 ml Folin–Ciocalteu reagent and 4 ml 10% sodium carbonate solution. The sample solutions were placed in a dark place for 5 min. Then, sample solutions were diluted to 10 ml with deionized water and incubated in 45°C water bath for 30 min in the dark. The absorbance of sample solutions was determined by UV spectrophotometer at 750 nm in triplicate. Gallic acid was used as the reference standard material to calculate total phenol content. The linearity, precision, stability, repeatability, and recovery of the method were validated.

Quantitative analysis of carotenoids

Samples were extracted twice with anhydrous ethanol: ethyl acetate: petroleum ether (1:2:7) 1:20 by ultrasonication for 30 min. Then, filtrate was dried by a rotary evaporative membrane apparatus. The dried samples were dissolved with petroleum ether and saponified with 20 ml 10% potassium hydroxide methanol solution for 12 h.

The lower aqueous phase was extracted with 40 ml petroleum ether; then, petroleum ether phases were combined and dried with anhydrous sodium sulfate for 3 h. Sample solution was concentrated and centrifuged for 20 min (2500 r/min), and supernatant was diluted with petroleum ether to 25 ml. The saponified sample solutions were determined absorbance by UV spectrophotometer determination at 445 nm with parallel measurement three times. β-carotene was used as the reference standard material to calculate the total carotenoid content. Moreover, the linearity, precision, stability, repeatability, and recovery of the method were validated.

2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt- free radical scavenging capacity

The ABTS-free radical scavenging capacity was measured as described with some modifications.[26] The ABTS-free radical was produced by mixing ABTS diammonium salt (7.4 mmol/l) with potassium persulfate (2.6 mmol/l) in 1:1 ratio. The mixture was kept in the dark at room temperature for 12 h to allow the completion of radical generation and then diluted with methanol (1:50).

The sample solutions were prepared according to total phenol and carotenoid methods, respectively. 8 ml aliquot of ABTS reagent was mixed with 0.05–0.7 ml methanol extraction solutions and 0.10–1.20 ml ether extraction solutions, respectively. After incubation for 10 min, the absorbance of sample solutions was determined by UV spectrophotometer at 734 nm with methanol and petroleum ether as blank control. Sample solutions were measured three times in parallel. The percentage inhibition was calculation as:

$$\text{Inhibition\%} = (A_0\text{-}A)/A_0 \times 100\%$$

Here, A_0 is absorption of blank control and A is absorption of sample solution.

1,1-diphenyl-2-trinitrophenylhydrazine-free radical scavenging capacity

The DPPH-free radical scavenging activity of samples was assayed according to literature with minor modification.[27,28] The sample solutions were prepared according to total phenol and carotenoid methods, respectively. The sample solutions of 0.4–1.2 ml methanol extraction and 1.00–5.00 ml ether extraction were oscillated for 10 s and incubated for 30 min after adding 3 ml DPPH working fluid (2×10^{-4} mol/l). Sample solutions were determined absorbance by UV spectrophotometer at 517 nm with methanol and petroleum ether as blank control. Measurements were performed in triplicate. The percentage inhibition formula is shown above in ABTS.

Determination of chromaticity values of external color

Appearance color of different aging year samples was measured by color colorimeter, which were expressed as L (luminosity), a (red-green), and b (yellow-blue). The condition included illuminant D65, 2° standard observation angle, lighting caliber ϕ 50 mm, the instrument error ΔEab <0.8, and standard deviation (SD) repeatability ΔEab <0.07 at room temperature. The instrument was calibrated by black and white tile standards equipped with the instrument before measurement. We measured chromaticity values of 40 batches of samples in triplicate at 10 a. m. in laboratory 410 Chinese medicine building. Moreover, the precision, stability, and repeatability of the method were validated.

Statistical analysis

All measurement results were reported as means and SD calculations (means ± SD). The antioxidant capacity IC_{50} values (defined as the concentration of sample at which the inhibition percentage reached 50%) were calculated by linear regression analysis. The data were subjected to analysis of variance, and significance of difference between means was determined by Duncan's multiple range test ($P < 0.05$) by SPSS 22.0 software. Bivariate linear correlation analysis methods, such as Pearson, Kendall's tau-b, and Spearman's rho, were used to analyze the correlation of aging year, component contents, antioxidant IC_{50}, and chromaticity values of samples by SPSS 22.0 statistical analysis software.

RESULTS AND DISCUSSION

Qualitative analysis of flavonoids

Under the detection wavelength of 330 nm, there were three characteristics of ion flow. We analyzed the mass spectrometry information of three characteristic peaks and compared with mass spectrometry information base. Briefly, in positive ion mode of ionization, hesperidin, nobiletin, and tangeretin gave protonated adducts at m/z 611.9, 403.6, and 373.6, respectively. Three compounds were confirmed as hesperidin, nobiletin, and tangeretin by comparing $[M+H]^+$ of peaks in the information base and retention time (the retention time was 8.5 min, 21 min, and 23 min, respectively) with previous literature.[29,30] By comparing chromatograms of samples, there was little change in peak patterns in different aging year samples.

Quantitative analysis of flavonoids

Methodological study showed that the linear regression equation, linear range, detection limit, and quantitative limit of hesperidin, nobiletin, and tangeretin are shown in Table 1. The intraday and interday precision, stability, repeatability, and sample recovery were all in accordance with the requirements, and the relative SDs (RSDs) were all <3%. The results showed that the developed method was applied to determine three flavonoid contents in GCRP samples. The contents of three compounds were calculated by external standard method based on the respective calibration curves with good separation degree of each detection peak in Figure 1.

The contents of three flavonoids in different aging year samples presented as means ± SD are shown in Figure 2a. In the 1st few aging years, the content of hesperidin decreased from 20.995 mg/g to 18.774 mg/g and then peaked at 29.614 mg/g in the 10th aging year with an increase of about 45%. The content of nobiletin increased from 2.298 mg/g in new peel to 3.916 mg/g in the 2nd aging year, then decreased to 3.4 mg/g in the 3rd year, and stabilized. The content of tangeretin increased from 1.491 mg/g in the new peel to 2.761 mg/g in the 2nd year, then decreased

Table 1: Calibration curve data, limit of detection, and LQD of hesperidin, nobiletin, and tangeretin by high-performance liquid chromatography

Components	Regression equation	R^2	Linearity range (mg/ml)	LOD (μg/ml)	Limit of quantitation (μg/ml)
Hesperidin	y=27055768x+291602	0.9996	0.010-0.790	1.095	1.406
Nobiletin	y=59434518x+284286	0.9994	0.004-0.400	0.642	2.243
Tangeretin	y=68812915x+266312	0.9997	0.004-0.400	0.821	1.380

LOD: Limit of detection; HPLC: High-performance liquid chromatography; LQD: Limit of quantitation

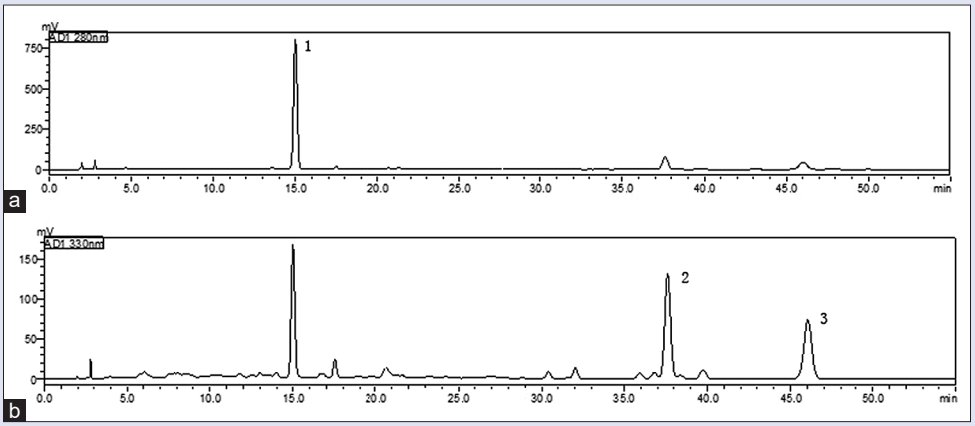

Figure 1: High-performance liquid chromatogram of Guang *Citri reticulatae* Pericarpium in different wavelengths (a: 280 nm, b: 330 nm). The peak 1 is hesperidin, peak 2 is nobiletin, and peak 3 is tangeretin, respectively

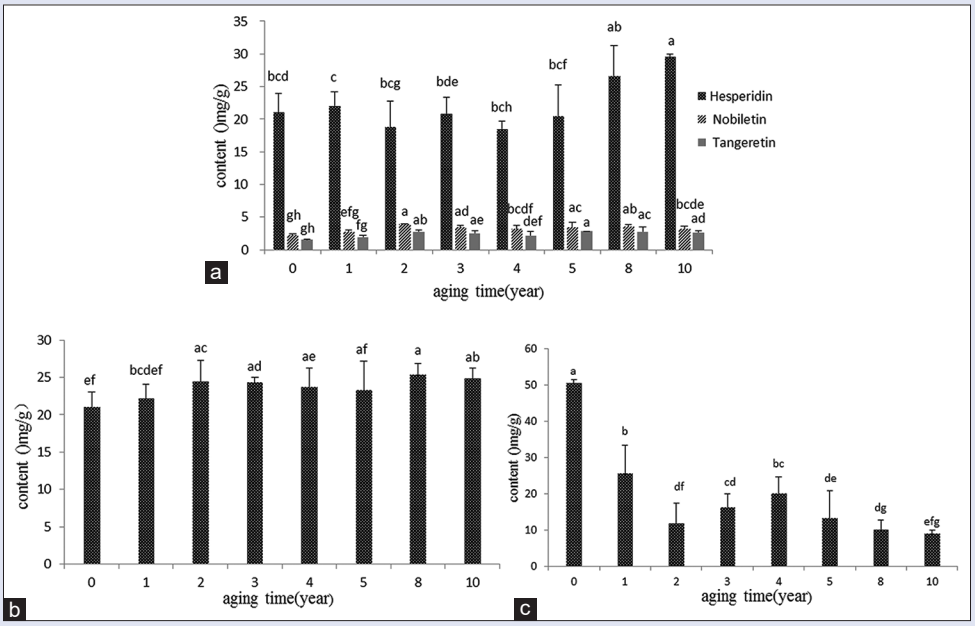

Figure 2: Comparison of compounds and contents of Guang *Citri reticulatae* Pericarpium in different aging years. (a: Hesperidin, nobiletin and tangeretin contents. b: Total phenols content. c: Carotenoids con tent) Different letters represent significant differences ($P < 0.05$)

to 2.5 mg/g in the 3rd year, and stabilized. On the whole, the content of hesperidin decreased first and then increased with aging time and reached the peak in the 10th aging year, whereas the content of nobiletin and tangeretin increased first and reached a stable state from the 3rd aging year.

Quantitative analysis of total phenols

The calibration curve of gallic acid exhibited good linearity ($r = 0.9987$) in linear regression equation (y = 0.1481x + 0.0774) within test range of 1.1–11 μg/ml. The precision, stability, repeatability, and recovery of the method were all in accordance with the requirements. Moreover, RSDs were all <3%. The contents of total phenols in different aging year samples presented as means ± SD are shown in Figure 2b. With the increase of aging year, the content of total phenols increased from 20.978 mg/g in the new peel to 24.330 mg/g in the 3rd year and then tended to be stable.

Quantitative analysis of carotenoids

The calibration curve of β-carotene exhibited good linearity ($r = 0.9997$) in linear regression equation (y = 0.1393x + 0.0158) within test range of 0.6–7.2 μg/ml. The precision, stability, repeatability, and recovery were all in accordance with the requirements. Moreover, RSDs were all <3%. The contents of carotenoids in different aging year samples presented as means ± SD are shown in Figure 2c. With the increase of aging year, the content of carotenoids decreased from 50.320 μg/g in the new peel to 25.490 μg/g in the 2nd year and 8.920 μg/g in the 10th year. The content of carotenoids decreased obviously, especially in the aging 1st year.

Antioxidant activity of methanol extraction

Antioxidant activity was expressed in sample concentrations at 50% inhibition (IC_{50}) which was calculated according to the linear equation between different concentrations of samples and the inhibition rate.

The lower IC_{50}, the stronger antioxidant activity and the higher IC_{50}, the lower antioxidant activity. The antioxidant activity of ABTS IC_{50} increased from 2.11 μg/ml in the new peel to 3.55 μg/ml in the 2nd year and then stabilized in 2.5–2.8 μg/ml from the 3rd aging year. It reached a high of 3.25 μg/ml in the 5th year. The antioxidant activity of DPPH IC_{50} increased from 54.93 μg/ml in the new peel to 78.56 μg/ml in the 3rd year and peaked at 87.76 μg/ml in the 8th aging year. The results revealed that the antioxidant activity of methanol extract showed a decreasing trend and tended to be stable after the 3rd aging year in ABTS and DPPH. The data are shown in Figure 3a and b.

Antioxidant activity of ether extraction

The antioxidant activity of ether extraction was expressed by ABTS IC_{50} and DPPH IC_{50}. ABTS IC_{50} decreased from 0.263 μg/ml in new peel to 0.128 μg/ml in the 3rd aging year and reached the lowest value of 0.059 μg/ml in the 10th aging year. The DPPH IC_{50} decreased from 1.683 μg/ml in the new peel to 1.088 μg/ml in the 3rd aging year and reached the lowest value of 0.899 μg/ml in the 10th aging year. The results revealed that the antioxidant activity of ether extract increased significantly with aging year. The enhancement trend of ABTS was more obvious than DPPH and tended to be stable after the 3rd aging year. The data are shown in Figure 3c and d.

Determination of chromaticity values

The precision, stability, and repeatability of the method were all in accordance with the requirements, and the RSDs were all <3%. The results showed that the developed method was applied to determine external color in GCRP. The L, a, and b values revealed an obvious decreasing trend with the increase of aging year, as shown in Table 2. The L value decreased from 30.40 of new orange peel to 24.41 in the 3rd aging year and 22.72 in the 10th aging year. The a value decreased from 7.05 of new orange peel to 2.44 in the 3rd aging year and 0.63 in the 10th aging year. The b value decreased from 8.06 of new orange peel to 1.43 in the 3rd aging year and − 0.30 in the 10th aging year. The change of chromaticity value is consistent with change trend of external color by naked eye. With the aging year increased, the color gradually becomes from orange red to deepen and finally dark brown. The color becomes dark and deep rapidly in the 1st aging year. From the 2nd aging year, the trend of color changes gradually slowed down. These changes are in accordance with those of the literature research results.[31]

Correlation analysis

The results of statistical analysis showed that there were significant differences in the correlation between aging year and chromaticity values, content of carotenoids, content of total phenols, and antioxidant activity, as shown in Tables 3-5.

All the three correlation analysis methods showed that aging year was negatively correlated with chromaticity values and carotenoid content, with a higher than 0.5, whereas aging year was positively correlated with correlation coefficient content of total phenols, with a correlation coefficient between 0.3 and 0.4. There was a positive correlation between total phenols content and antioxidant activity (IC_{50}), with a correlation coefficient between 0.3 and 0.4. The content of total phenols was negatively correlated with chromaticity values, with a correlation coefficient of 0.3–0.4. There was a significant difference in the correlation between carotenoid content and chromaticity values, with a positive correlation coefficient higher than 0.5, and the content of carotenoid was the same as that of antioxidant activity (IC_{50}). The above results showed that there is a certain correlation between aging year and chromaticity values, content of total phenols, content of carotenoid, and antioxidant activity.

Table 2: Chromaticity values of Guang *Citri reticulatae* Pericarpium in different aging years (*n*=5)

Aging year	L	a	b
0	30.40±0.44	7.05±0.45	8.06±0.27
1	24.96±0.18	3.00±0.41	1.90±0.38
2	24.33±0.13	2.03±0.40	1.05±0.33
3	24.41±0.22	2.44±0.42	1.43±0.38
4	24.22±0.31	2.43±0.28	1.24±0.31
5	23.63±0.21	1.50±0.28	0.51±0.18
8	23.34±0.10	1.01±0.08	−0.21±0.07
10	22.72±0.16	0.63±0.08	−0.30±0.11

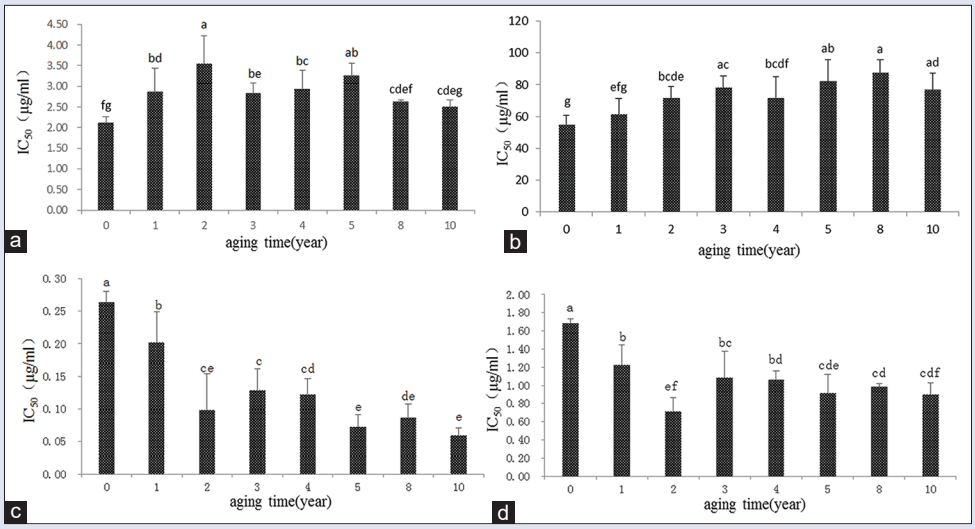

Figure 3: Comparison of antioxidant activity of Guang *Citri reticulatae* Pericarpium extraction in different aging years (a: 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt IC_{50} of methanol extraction. b: 1,1-diphenyl-2-trinitrophenylhydrazine IC_{50} of methanol extraction. c: 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt IC_{50} of ether extraction; d: 1,1-diphenyl-2-trinitrophenylhydrazine IC_{50} of ether extraction). Different letters represent significant differences ($P < 0.05$)

Table 3: Correlation coefficients of aging year and chromaticity values and contents of carotenoids and total phenols

Correlation test methods Aging year	L	a	b	Content of carotenoids	Content of total phenols
Pearson	−0.709**	−0.754**	−0.699**	−0.662**	0.417**
Kendall's tau-b	−0.840**	−0.787**	−0.781**	−0.522**	0.357**
Spearman's rho	−0.943**	−0.900**	−0.906**	−0.697**	0.485**

**Significant differences (P<0.01)

Table 4: Correlation coefficients of content of total phenols and antioxidant activity and chromaticity values

Correlation test methods Content of total phenols	ABTS IC_{50}	DPPH IC_{50}	L	a	b
Pearson	0.313*	0.438**	−0.470**	−0.462**	−0.481**
Kendall's tau-b	0.244*	0.326**	−0.340**	−0.284*	−0.322**
Spearman's rho	0.349*	0.453**	−0.491**	−0.404**	−0.470**

*Differences (P<0.05); **Significant differences (P<0.01). ABTS: 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; DPPH: 1,1-diphenyl-2-trinitrophenylhydrazine; IC_{50}: Half maximal inhibitory concentration

Table 5: Correlation coefficients of content of carotenoids and antioxidant activity and chromaticity values

Correlation test method Content of carotenoids	ABTS IC_{50}	DPPH IC_{50}	L	a	b
Pearson	0.916**	0.849**	0.906**	0.905**	0.907**
Kendall's tau-b	0.726**	0.521**	0.532**	0.550**	0.533**
Spearman's rho	0.882**	0.678**	0.713**	0.747**	0.724**

**Significant differences (P<0.01). ABTS: 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; DPPH: 1,1-diphenyl-2-trinitrophenylhydrazine; IC_{50}: Half maximal inhibitory concentration

CONCLUSION

In this study, three characteristic flavonoids such as hesperidin, tangeretin, and nobiletin in GCRP of different aging years were identified and compared the contents by ultra-HPLC mass spectrometry and HPLC-DAD. The contents of total phenols and carotenoids in samples of different aging years were established and compared. The antioxidant activities of GCRP in different aging years were compared by ABTS and DPPH *in vitro*. The chromaticity values of the exterior color of samples in different aging years were compared. The correlation among aging years, contents of total phenols and carotenoids, chromaticity values, and antioxidant activity was analyzed. The results showed that the contents of flavonoids, total phenols, and carotenoids and antioxidant activities reached a relative steady state at 3 years after aging. This study provided information on the parlance that aging time of 3 years can be used medically.

The correlation results showed that carotenoids are more closely related to chromaticity values and antioxidant activity, which were positively correlated with chromaticity values and negatively correlated with antioxidant activity. The results revealed that the content of carotenoids decreased with the increase of aging year, whereas the antioxidant activity increased, which suggested that the antioxidant activity might be related to the changes of the composition type and content ratio of carotenoids.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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