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Comparative study of chemical compositions and antioxidant activities of Zhizi fruit extracts from different regions

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ABSTRACT

The fruits of *Gardenia jasminoides* Ellis are folk medicines in China and their major components are geniposide and water soluble pigment crocins. This study compared the chemical profiles and free radical scavenging activities of two Zhizi species from five provinces of China, including Jiangxi, Anhui, Hunan, Sichuan and Henan. The results showed that Jiangxi Zhizi contained higher levels of volatiles (71.84%), crocins (20.38 mg/g), geniposide (31.36 mg/g) and flavonoids (84.42 μg quercetin/mg) than four other Zhizi fruits; whereas Hunan Zhizi provided higher total phenolics (33.81 μg catechin/mg) and ABTS/DPPH radical scavenging activities. These findings implied that Jiangxi Zhizi would be suitable for extraction of gardenia yellow and geniposide, as well as preparation of essential oil. This information may provide valuable guidance for application of Zhizi fruits to biomedicine industry in China.

1. Introduction

Gardenia jasminoides (Zhizi), an evergreen tree that belongs to the Rubiaceae family, includes two variations: *G. jasminoides* Ellis and G. *jaminoides* Eills *f. Longicarpa* Z.W. Xieet Okada in China [1]. Zhizi grows in many temperate regions and has fragrant white flowers [2]. The dried ripe fruits of Zhizi are famous herbal medicines and natural dyes in China. *G. jasminoides* Eills as Chinese traditional medicine which is commonly used to treat anxiety, depression, insomnia, psychosis and other mental disorders. Recently, it was also found to have antioxidative, anti-inflammatory, melanogenesis inhibitory and hepatoprotective activities [3, 4, 5, 6, 7, 8]. Among the two varieties, *G. jasminoides* Z.W. Xieet Okada provides more crocins and due to this specific characteristics, it is popular as a colorant [1].

The major constituents of Zhizi fruits are iridoid glycosides, crocins, ubiquitous quinic acid derivatives and volatiles [9, 10]. The major volatile compounds in essential oil of *G. jasminoides* are aliphatic acids, ketones, aldehydes, esters, alcohols, and aromatic derivatives, which showed antidepressant activity [7]. Numerous studies have reported that iridoids in Zhizi fruits exhibited many biological activities, including anti-inflammatory, antidiabetic properties, antithrombotic activies, as well as protection against lipopolysaccharide (LPS)-induced apoptotic liver damage [11, 12, 13]. In addition, crocins are considered as a natural colorant as well as the main components of the gardenia yellow. Moreover, it is known as the only water-soluble carotenoids present in plants. Crocins have been used as a natural food colourant for a long time, mainly in coloured juice, jelly, candy and noodles, therefore, crocins content in Zhizi fruits may play a key role in the evaluation of the herbs when it is used as a food colorant [14, 15].

As a traditional medicine, the chemical composition (e.g. geniposide, crocins and volatiles) of Zhizi fruits varied with their places of production, leading to the difference in their quality and application [16, 17, 18]. However, little information is available on the comparision of chemical composition of Zhizi fruits present in different provinces of China. Therefore, in this study, the chemical composition of two Zhizi species from five different provinces (Fig. 1) were studied. The Zhizi fruits were first extracted by subcritical fluid extraction (SFE) to obtain essential oil, and the remaining residues were then extracted with 50% ethanol to get active compounds. The chemical composition of oil was analyzed by GC-MS, and the contents of main components of residues

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Fig. 1. The Zhizi samples from different regions.

from different samples were quantified by HPLC with standard compounds. Furthermore, the antioxidant activities of the extracts were determined by using ABTS and DPPH radical assays.

2. Materials and methods

2.1. Plant materials

The Zhizi fruits were grown and harvested from provinces of Hunan (Z1), Henan (Z2), Anhui (Z3), Jiangxi (Z4), Sichuan (Z5) respectively in October 2017 (Fig. 1). All samples were identified by one of the authors, and later corresponding voucher specimens (No. HFUT-ZZ-001-005) were deposited in the herbarium.

2.2. Chemicals and reagnts

Standard compounds: crocin-1, crocin-2, 5-chlorogenic acid, geniposide were purchased from National Institutes for Food and Drug Ccontrol. The organic solvents and n-hexane used for extraction were purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). 2,2′-Azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), 1,1-Diphenyl-2-pic-rylhydrazyl (DPPH), potassium persulfate, sodium sulfate, Folin–Ciocalteu's phenol reagent (2 N) were purchased from Hefei Bomei Biotechnology Co., Ltd. (Hefei, China). Milli-Q water (obtained through a Millipore filter system, Millipore Co., USA) was used throughout, and acetonitrile for HPLC was purchased from Fisher Scientific (Pittsburgh, PA, USA).

2.3. Extraction of essential oil by SFE

SFE was performed using the apparatus CBE-100L (Henan Subcritical Extraction Biological Technology Co., Ltd, Henan province, China). Dried samples (1kg) were extracted with 1.5L subcritical fluid n-butane under 0.35MPa, 40°Cfor 40 min. The extractant fluid was condensed by reducing pressure to get crude essential oil. Then the fluid was compressed by compressor and was liquefied after condensation. This liquefied fractions can be recycled for future use. Extraction were perfomed two times and the crude essential oil was centrifuged by 4000 rpm for 3 min to remove insoluble components. The oils were stored in 4 °C refrigerator for further analysis.

2.4. GC-MS analysis

The essential oils were filtered by anhydrous sodium sulfate and diluted ten times by n-hexane. Then it was filtered using 0.45μm membrane (Ampel) for GC-MS analysis. GC-MS conditions: capillary column HP-5 (30 m × 0.25 mm×0.25μm) was used for separating and identifying the aroma compounds of the fruits. The oven temperature was set as according to the following order/sequence: initial temperature of 60 °C (held for 1min), up to 130 °C by the rate 10 °C/min (held for 10 min), then heated to 250 °C by the rate of 5 °C/min (held for 10min), following to the 280 °C by the rate 10 °C/min (held for 10min); injection port temperature was 250 °C. Helium was used as the carrier gas at a flow rate of 1.2 mL/min with the split ratio 50:1. The transmission line temperature was 280 °C. The injection volume was 1 μL. The MS fragmentation was performed by electronic impact (EI) mode with the ion source temperature of 230 °C. Solvent delay time was 4 min. The acquisition was on full-scan mode and mass acquisition range was 35–500 m/z.

2.5. Extraction of main compounds from residues

Zhizi residue powder (1 g) was extracted by 8 mL 50% ethanol for 3 h at 50 °C. All extract solvents were condensed by evaporator and then dried by lyophilizer (SIM International Group, USA) with -70 °C and pressure <100Pa. The extracts were stored in refrigerator until for analysis.

2.6. HPLC analysis for main components

The extracts from the residues were diluted to the concentration 2 mg/mL and filered by 0.45 μm membrane and injected for HPLC system (iChrom 5100, Dalian, China) to analyze the contents of chlorogenic acid, geniposide, crocin-1 and crocin-2. Acetonitrile (A) and water mixed with 0.8% formic acid (B) were used for separation on a YMC-C18 column (hydrosphere, 150 × 4.6mm, 3μm particles) at a flow rate of 0.8 mL/min under gradient elution as follows: 0–3 min, 5% A; 3–5 min, 5% A-15% A; 5–10 min, 15% A-25% A; 10–26 min, 25% A-100% A; 26–37 min, 100% A, and injection volume of 3 μL for all samples. The standard compounds including chlorogenic acid, geniposide, crocin-1 and crocin-2 were analyzed by gradient dilution for standard curve. The R^2 for standard curve was >0.99, and linearity was determined on five levels of concentration with three injections for each level with different concentration range (Table 1). The detection limit (LOD) and the quantitation limit (LOQ) of four compounds were determined by calculation of the signal-to-noise ratio. A signal-to-noise ratio 3:1 is generally considered acceptable for estimating the detection limit. The sample that produces a signal-to-noise ratio of approximately 10:1 corresponds to the concentration at which the analyte can be reliably quantified, and the recovery of pure compound was finished by adding the known authentic compound to Zhizi extract (Table 1).

2.7. Measurement of the in vitro antioxidant activity

2.7.1. Determination of total phenolics and flavonoids from Zhizi fruit residues

Zhizi fruit residues were analyzed for total phenolics and flavonoids content according to the method of Shang et al. [19]. Quercetin and catechin were used as the standards for flavonoids and phenolics, respectively. The results were expressed as mg quercetin/g sample for flavonoids and mg catechin/g sample for total phenolics.

Table 1
Regression data, precision, LODs and LOQs for five phenolic compounds obtained with the optimized HPLC ESI–TOF–MS method.

Compound	Equation[a]	R^2	LOD[b] (μg/mL)	LOQ[c] (μg/mL)	Linearity range (μg/mL)	Recovery (%)
Chlorogenic acid	y = 2339673.81x-88.96	1.00	0.05	0.16	1–200	102
Geniposide	y = 1237458.01x-9.17	1.00	0.60	1.63	1–200	123
Crocin-1	y = 3143125.13x+205.23	1.00	0.12	0.37	1–1000	95
Crocin-2	y = 1100960.61x+48.70	1.00	0.08	0.24	0.5–500	110

[a] y is the peak area in HPLC, x is the quanlity (mg) injected.
[b] LOD: limit of detection (S/N 3:1).
[c] LOQ: limit of quantification (S/N 10:1).

2.7.2. ABTS and DPPH assays for Zhizi residues

The modified ABTS and DPPH radical cation methods were used to evaluate the free radical scavenging ability of samples according to the method of Shang et al. [19]. Vitamin C was used as the standard compound to measure the antioxidative activity of samples. The antioxidant activity was defined as the concentration of the sample extracts necessary to scavenge 50% of the ABTS/DPPH radicals (SC_{50}) and expressed as mg/mL.

2.8. Statistical analysis

All data were analyzed in five independent biological replicates. The main compounds quantification and antioxidant activity values were expressed as mean values ± standard deviation and subjected to one-way ANOVA analysis, followed by Tukey-HSD (HonestlySignificant Difference) post hoc test ($p < 0.05$).

3. Results and discussion

3.1. Volatile components in Zhizi

The volatile componets of Zhizi from supercritical fluid extraction were potential sources for the development of novel antidepressant food supplements and medicines [20]. In this study, SFE was employed for essential oil extraction. Z5 Zhizi provided the highest essential oil extraction efficiency (7.64%), followed by Z4 (7.22%), Z2 (5.38%), Z3 (4.49%), and Z1 (4.35%). Relative content was calculated by integrated peak area in the data analysis program. Table 1 shows the essential oil compositions of Zhizi. In all samples studied, the main volatile components are fatty acids including palmitic acid, linoleic acid, cis-13-octadedienoic acid, octadedienoic acid, squalene, and vitamin E. Linoleic acid was the major component, being present in the range of 5.33–33.70% by the normalization method of peak area, followed by squalene (16.29–27.08%), erucic acid (7.45–23.61), palmitic acid (7.30–13.23%), VE (3.82–4.80%). However, erucic acid was undetectable in Z5, VE was not found in Z2 and Z5, other minor fatty acids also were different in five samples (Table 2). The main components from SFE were similar to those of supercritical fluid extraction that palmitic acid was the main components reported by Tao et al. [20]. The gene differences among different varieties would result in the differences in the content and kind of the lipid components present in each sample. Moreover it may be due to the diffrences of the geographical place as five samples were collected from different area of China. Furthermore, their compositional differnces may be effected by cultivation environment, weather condition, region and harvest season [21, 22, 23, 24]. Therefore, species and origins of Zhizi would affect the fatty acid composition [25].

3.2. Main components analysis

Gardenia yellow is a popular natural pigment in food processing and crocin is identified as the main component of gardenia yellow, which always coexists with geniposide and chlorogenic acid. The chlorogenic acid and geniposide make yellow colour easy to fade and turn it into green. In this study, crocin-1 and crocin-2, geniposide and chlorogenic

Table 2
Volatile composition (%) of Zhizi from different regions.

No	Compounds	Relative contents (%)				
		Z1	Z2	Z3	Z4	Z5
1	Estragole		0.0878			0.0872
2	3,3-Dimethyl-6-methylenecyclohexene		0.4839			
3	2,4-Decadienal, (E,E)-	0.3242	0.4736	0.3293		
4	2,4-Decadienal, (E,E)-	0.4448	0.5861			0.4362
5	Pentadecanoic acid, 14-metthyl-, methyl ester		0.3146			
6	n-Hexadecanoic acid	7.307	13.5359	3.6826	9.1674	13.2369
7	n-Hexadecanoic acid		0.1535		0.4936	0.6174
8	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	0.1343	0.3669	0.8879		0.2969
9	9-Octadecenoic acid (Z)-, methyl ester		0.1813		0.169	0.2585
10	9,12-Octadecadienoic acid (Z,Z)-	13.3261	16.6927	5.3258	16.2151	33.6987
11	Cis-13-Octadecenoic acid	12.8252	15.4846	7.4547	23.6072	
12	Octadecanoic acid	2.7792	4.1448			
13	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	27.0798	18.1513	16.2944	17.569	16.5017
14	Vitamin E	3.8178		4.8063	4.2465	
15	Ethanone, 1-(3,5-dimethylpyrazinyl)-				0.1765	
16	Hexadecanoic acid, ethyl ester				0.2003	
17	1,3-Cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl-					0.0513
18	Nonadecane					0.0682
19	2,4-Decadienal					0.2281
20	Hexadecanoic acid, methyl ester					0.2979
21	1,2-Benzenedicarboxylic acid, disoctyl ester					0.7428
22	2,4-Cycloheptadien-1-one, 2,6,6-trimethyl-			0.3767		
23	1(3H)-Isobenzofuranone, 3-butylidene-			0.4375		
24	Oleic Acid			1.2328		
25	Eicosane	0.1105				

acid contents of various samples were determined using HPLC analysis, and a typical HPLC chromatogram of the ethanol extracts from gardenia was recorded at 330 nm, 238 nm and 440 nm. Peaks were identified by comparison of their HPLC retention time with the authentic compounds. The contents of four active components in different samples are shown in Table 3. Z4 contains more crocin-1 (11.67 mg/g dry weight), crocin-2 (8.71 mg/g dry weight) and geniposide (31.36 mg/g dry weight) compounds than other samples, Z5 contain 0.91 mg/g dry weight chlorogenic acid. Z4 would be the best source for gardenia yellow extraction, followed by Z3, Z1, Z5 and Z2 ascrocin-1 and crocin-2 are the main components of gardenia yellow. These results were similar to previous studies that the content of geniposide in *G. jasminoides* Eills is higher than *G. jaminoides* Eills f. longicarpa Z.W. Xie et Okada, and crocin is more plenty in *G. jaminoides* Eills f. longicarpa Z.W. Xie et Okada [1, 17]. Different species and distribution would contribute to active components difference. Z1-Z3 distributed in eastern and central of China, and the temperatures in these regions were higher than the production place of Z4-Z5, therefore, the chemical composition of the Zhizi fruits maybe affected by the temperature of the production places. Additionally, the contents of active components from Zhizi fruit and Zhizi fruit residues were compared, and the results showed that the extraction efficiency for four compounds were increased after removing the essential oils from Zhizi fruits. SFE high pressure extraction process would destroy the structure of fruits matrix and facilitates the effectiveness of active components extraction procedures [26].

3.3. Measurement of the in vitro antioxidant activities

The antioxidant activity of the extracts was determined by ABTS and DPPH radical assays. Z1 showed the highest ABTS and DPPH radical scavenging ability (Table 4). The content of crocins in Z1 is lower than others. However, it shows a consistency with other studies related to higher radical scavenging ability. In constrast, crocins might not be playing a main role in antioxidant capacities of extracts partcularly in ABTS radical scavenging, DPPH radical scavenging and lipid peroxidation models [18]. The hydroxycinnamoylquinic acid derivatives (CQAs) were the major contributors to the free radical scavenging activity in Zhizi fruits [9]. In this study, we quantified the content of 5-chlorogenic acid and there was no relationship between chlorogenic acid and radical scavenging ability. This maybe due to other unidentified derivatives present in the samples. At the same time, the antioxidant activity in the ABTS assay was correlated with phenolic content to some extent ($r^2 = 0.75$) while other components were not correlated with ABTS and DPPH assays.

Table 3
Active components of Zhizi from different residues.

Region	Chlorogenic acid (mg/g DW[a])	Geniposide (mg/g DW)	Crocin-1 (mg/g DW)	Crocin-2 (mg/g DW)
Z1	0.81 ± 0.02	34.64 ± 0.45	8.76 ± 0.04	7.19 ± 0.01
Z2	0.76 ± 0.01	27.88 ± 0.37	9.85 ± 0.51	4.77 ± 0.02
Z3	0.69 ± 0.01	30.73 ± 0.41	11.18 ± 0.38	6.29 ± 0.01
Z4	0.35 ± 0.01	33.10 ± 0.36	12.21 ± 0.06	4.17 ± 0.01
Z5	0.98 ± 0.02	27.96 ± 0.45	8.91 ± 0.02	8.89 ± 0.02

[a] DW: Dry weight.

Table 4
Comparative analysis of the antioxidative activities of Zhizi fruit residues.

Region	Flavonoids (mg quercetin/g)	Total phenolics (mg catechin/g)	ABTS (SC_{50} mg/mL[a])	DPPH (SC50 mg/mL)
Z1	51.03 ± 0.46	44.06 ± 0.35	0.07 ± 0.01	0.93 ± 0.02
Z2	56.16 ± 0.51	20.72 ± 0.18	0.11 ± 0.01	1.16 ± 0.02
Z3	60.29 ± 0.55	22.54 ± 0.20	0.09 ± 0.02	1.20 ± 0.03
Z4	84.42 ± 0.64	33.81 ± 0.35	0.08 ± 0.02	1.01 ± 0.01
Z5	63.92 ± 0.48	29.03 ± 0.15	0.10 ± 0.01	1.50 ± 0.01

[a] SC_{50} mg/mL: the concentration for scavenge 50% of the ABTS/DPPH radicals.

4. Conclusions

Zhizi is widely distributed in various provinces of China. The qualities of essential oil and Zhizi yellow dye are different due to their different origins. The results of this study indicated that there is a difference of volatiles from different varities of Zhizi samples. n-Hexadecanoic acid, squalene, linoleic acid are the main volatiles present in five samples, and the sample from Henan province contains 30% more linoleic acid than others. At the same time, the content of crocins, the main components of gardenia yellow are different according to the origins. The sample of Jiangxi province contains the highest, followed by Anhui, Sichuan, Henan and Hunan provinces. Meanwhile, Zhizi from Jiangxi contains much more geniposide and flavonoids than others. Hunan Zhizi with high total phenolics showed the best antioxidative activities towards to the ABTS and DPPH radicals. In conclusion, the volatiles and active components of Zhizi may vary depending on its origin.

Declarations

Author contribution statement

Ya-Fang Shang: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.
Yi-Ge Zhang, Heng Cao: Performed the experiments.
Yi-Long Ma: Analyzed and interpreted the data.
Zhao-Jun Wei: Conceived and designed the experiments.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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