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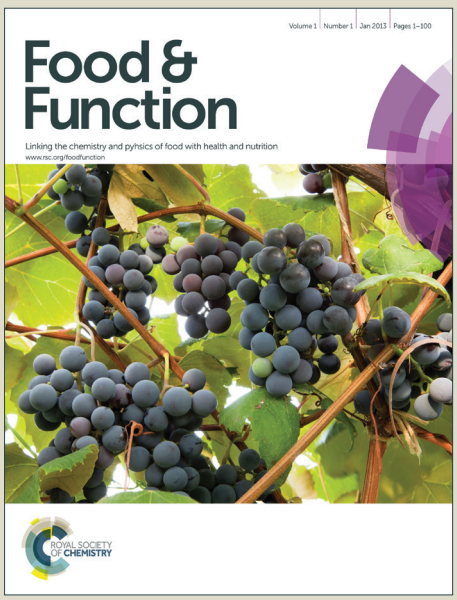

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Optimization of Ultrasound-Assisted Extraction of Gardenia Fruit Oil with Bioactive Components, and their Identification and Quantification by HPLC-DAD/ESI-MS^2

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ABSTRACT: Compounds in *Fructus Gardeniae* have been shown to possess a wide array of biological activities. However, Gardenia oil extracted from its fruit is less reported, and its composition remains uncertain. To fully characterize lipophilic compounds in Gardenia oil, three conventional extraction (CE) and ultrasound-assisted extraction (UAE) methods were investigated. The oil extraction yield obtained by UAE was 51.8% higher than that of cold-pressed extraction (CPE) acquired. The fatty acid profile in UAE oil with different solvents was characterized by GC-MS. Petroleum ether was observed to be an ideal solvent with 8.59% extraction yield and 78.88% recovery rate, and with 3.11 ratios of unsaturated fatty acids to saturated fatty acids. Response surface methodology (RSM) with Box–Behnken Design (BBD) was applied to optimize conditions in UEA of oil to maximize extraction yield. Furthermore, the bioactive components in oil extracted by UAE were qualitatively identified and quantified by HPLC-DAD/ESI-MS^2 and HPLC-DAD analysis. The eight compounds in Gardenia oil including geniposide, *trans*/*cis*-crocin-1, crocin-2, crocin-3, crocin-4, and *trans*/*cis*-crocetin were structurally revealed. The corresponding transfer rates of the bioactive components showed that the lipophilic *trans*/*Cis*-crocetin could be completely transferred from fruit to oil, with the highest concentration of 11.38 μg/g oil among all compounds quantified. These findings could deliver the potential application and large-scale production of functional Gardenia oil with bioactive components possessed health benefits.

Key words: Gardenia oil; ultrasound-assisted extraction (UAE); fatty acids, bioactive components; response surface methodology (RSM)

1. Introduction

The fruits of Fructus Gardeniae (Rubiaceae) (*Gardenia jasminoides* Ellis, Chinese name Zhizi) are traditionally utilized as herbal medicine for the therapeutic effects including detoxification, liver-protection, calming irritability, cooling blood, cholagogue, homeostatic antipyretic, antitumor, treatment of jaundice, fever, hypertension and ulcers of the skin, as well as cerebrovascular disease treatment.[1,2] Those health functions in Gardenia fruits (GFs) are majorly ascribed to the existence of bioactive ingredients responsible for protective functions against oxidative damage, exhibiting sedative, analgesic, antipyretic, antipyretic, diuretic, fibrolytic, homeostatic, and anti-inflammatory effects.[3,4] The bioactive components (as shown in **Figure 1**) identified in GFs were principally categorized into iridoid, carotenoids (crocins), monoterpenoids and their glycosides, flavonoid, organic acid esters (quinic acid and vanillic acid derivatives), and sterols.[5] Iridoid glycosides include geniposide, gardenoside, gardoside and scandoside methyl ester.[6] GFs with excellent natural resources of bioactive compounds possess a wide array of biological activities, exhibiting as antioxidant, antitumor, antihyperlipidemic and neuroprotective effects.[7,8].

Consumers are becoming more and more concerns over the safety of synthetic colorants. Many naturally occurred colorants with bioactives offer health benefits. Therefore, replacement of artificial food dyes with natural colorants is a current marketing trend. Natural colorants derived from GFs are particularly of interest in contributing blue, yellow, green, and red colorants in food systems.[9,10] In Japan, gardenia blue iridoid pigment extracted from Gardenia jasminoides is approved for food use. Geniposide, a major active ingredient in GFs, is applied as a yellow dye, particularly for food coloring, and also used as a functional food and traditional medicine in Asian countries.

Traditional extraction methods for bioactives and colorants are being substituted by new technologies (such as UAE and supercritical fluid extraction) with environmentally friendly solvents and higher yields of extracts. The conventional extraction (CE) methods of oil from GF display some limitations with respect to the high solvent consumption, long extraction time, high energy input, and requests of improvement of yield and quality of the extracts. In addition, the disadvantages of CE may be the slow process of solvent diffusion into a solid matrix, the loss of biological activities due to oxidation, hydrolysis and ionization.[11] UAE has

been used to extract food components such as antioxidants, aromas, pigments, and other organic and mineral components from a variety of matrices.[12] Ultrasound with 20-100 kHz frequencies is an effective and useful extraction technique, which is often used for extracting plant compounds to overcome drawbacks in CE,[13] and shows possible benefits of effective mixing, rapid energy and enhancement of mass transfer, reduced temperature, selective extraction, fast response to process extraction control, increased production, improved solvent penetration into the plant tissue via the ultrasonic jet and capillary effects, and cell disruption through the collapse of cavitation bubbles near the cell walls.[14] Ultrasound can boost existing extraction processes and enable new commercial extraction opportunities. The efforts with UAE with bioactives (polyphenols, anthocyanins, tartaric acid) were comprehensively and critically reviewed,[13] which includes bioactives from plant and animal resources with increased yield of extracted components, increased rate of extraction, achieving reduction in extraction time and higher processing throughput. UAE has been applied in the edible oil industry to improve efficiency and reduce extraction time.[15] The effects of various parameters including particle size, extraction solvent, solid/solvent ratio, temperature, extraction time, electrical acoustic intensity, liquid height and duty cycle of ultrasound exposure on the extraction yield of all-trans-β-carotene from citrus peels by UAE were assessed.[16] Ethanol exhibited a higher extraction yield during UAE in comparison with CE. In UAE, many parameters including energy input, time, temperature, solvent composition, and liquid-solid ratio can influence the extraction process, while there may be interactions between/among parameters. Additively, CE for optimizing a multivariable system are usually one variable at a time, which are time-consuming, and ignorance of interactions existed between/among the variables. RSM can be effectively used to evaluate the effects of multiple parameters and their interaction on one or more response variables, reduce the number of experiments, and provide a mathematical model.[17]

Previous studies have revealed that GFs are rich in oil, containing plenty of unsaturated fatty acid, especially palmitic acid and linoleic acid, which exhibit pharmacological effects on regulating blood pressure, body fat metabolism, and reducing serum cholesterol as well as adjusting plant nerve.[18,19] However, little data are available in literatures about the optimization of UAE for Gardenia oil, and limited information is available regarding oil

transfer rate from GFs to oil, as well as few studies are conducted in focusing on elucidation and identification of bioactive components in the oil. In our work, a RSM was applied to optimize the variables affecting the UAE in oil extraction. Therefore, the objectives were to: 1) compare oil extraction yield between CE and UAE; 2) characterize fatty acid profile affected by different organic solvents in UAE; 3) apply RSM to optimize UAE conditions for obtaining high extraction yield; and 4) identify and quantitate bioactive components using HPLC-DAD/ESI-MS^2 analysis in oil.

2. Materials and methods

2.1. Samples and chemicals

Mature Gardenia fruits (*Gardenia jasminoides* Ellis), procured from Xiao Gan area in Hubei, China, were dehydrated at 50 °C for 8 h with around 10 % final moisture content. The dried samples were ground in a universal high-speed smashing FW100 (Tianjin Taisite Instruments Co., Ltd., Tianjin, China) in the size range between 150-250 μm. Finally, the dried powder was kept at -18 °C until use. All the chemicals and organic solvents used were of analytical and chromatographic grade. Crocins (> 90%) were purchased from Chengdu MUST Bio-technology co., Ltd., China. Crocin-1 (> 98%) and Crocin-2 (> 98%) were obtained from Chengdu Biopurify Phytochemicals Ltd., China. Geniposide (> 95%) was from Wuhan Jiu Chen Biological Technology co., Ltd., China. Crocetin (> 90%) was purchased from MP Biomedicals, LLC, France. The other chemicals used were purchased from Sinopharm Chemical Reagent Co., Ltd., (Shanghai, China).

2.2. Traditional extraction (TE)

The oil extraction from GFs was previously reported[20] with some modifications as follows. Briefly, 5.00 g of Gardenia powder was weighted out, and blended with 30 mL petroleum ether in an Erlenmeyer flask of 100 mL. Then the mixture was heated at 80 ± 1 °C for 4 h, and stirred at 400 rpm by a magnetic stirrer (RCT Basic Safety Control, IKA-Werke, Staufen, Germany). The mixture was centrifuged at 10,000 rpm for 30 min. The supernatant was collected, and then vacuum-evaporated at 45 °C to remove solvent until reaching the constant

weight. The Gardenia oil was collected and maintained at 4 °C in the dark. Extraction yield (%) was expressed as the percent the weight of extracted oil over the weight of sample, as shown in Eq. (1).

$$\text{Extraction yield}\,(\%) = \frac{\text{The weight of extracted oil}}{\text{The weight of sample}} \times 100 \quad (1)$$

2.3. Soxhlet extraction (SE)

The Gardenia oil was extracted in Soxhlet method as previously described[21] with some modifications. Generally, 5.00 g of Gardenia powder was extracted by petroleum ether with Soxhlet apparatus at 45 °C for 12 h. After removing the solvent and weighing the extracted oil, the yield of extracted oil was calculated. The SE method was observed to have the highest extraction yield among 4 methods. The recovery rate (%) was calculated as Eq. (2).

$$\text{Recovery rate}\,(\%) = \frac{\text{Extraction yield by different method}}{\text{Extraction yield by SE method}} \times 100 \quad (2)$$

2.4. Cold-pressed extraction (CPE)

The CPE of Gardenia oil was performed as previously reported[22] with some modifications. Briefly, GF powders were squeezed in a cold pressing machine CA59G (German Monforts Group, Moenchengladbach, Germany). The process was carried out at room temperature with three cold pressed series. Then, the cold-pressed oil was centrifuged at 5,000 rpm for 10 min to get rid of impurities. The oil was collected and stored at 4 °C in the dark until analysis. Since the oil composition from cold pressure is relatively complete and without organic solvent, Gardenia oil extracted by this method was selected to perform GC-MS to characterize fatty acid profile.

2.5. Ultrasound-assisted extraction (UAE)

The UAE of Gardenia oil was carried out as previously described[23] with some modifications. Four organic solvents (n-hexane, cyclohexane, petroleum ether, and ethyl acetate) were examined. Briefly, the dried powders were well mixed with the solvent in a 100 mL of Erlenmeyer flask. A 250 W, 50 Hz ultrasonic cleaning bath E31-SB-5200 (Ningbo SCIENTZ biotechnology Co., Ltd., Zhejiang, China) with intensity of 1 W/cm^2 was applied

for UAE and performed under the designed conditions (solvent/solid ratio: 3～11 mL/g; extraction time 30～60 min). Then the liquid part was subjected to vacuum filtration on a Buchner funnel (D: 7.5 cm) fitted with a quantitative cellulose filter paper (pore size 80～120 μm). Afterwards, the extracts were evaporated using a rotary evaporator (Hei-VAP Advantage ML/HB/G3, Heidolph, Germany) at 40 °C. Finally, the solvents were removed through flushing nitrogen gas. All these extracted oil were stored at 4 °C in the dark, and used to make comparisons in efficiency with different conditions of UAE. The UAE conditions were optimized according to RSM, which targeted at obtaining maximal extraction yield of Gardenia oil.

2.6. Experiment design and statistical analysis

The variables affecting extraction yield were investigated for solvent/solid ratio (3~11 mL/g), extraction time (30~60 min), and extraction temperature (25~55 °C). RSM was employed to examine the performance of UAE on oil production, and to determine the optimum levels of the processing parameters in order to acquire the maximum extraction of Gardenia oil. The effects were discussed at 3 levels (-1, 0, 1) through a Box-Behnken Design (BBD) for the three independent variables SSr (X_1), ETi (X_2), ETe (X_3). The oil extraction yield as a response value for different experimental combinations was given in **Table 1**. This design was to evaluate the simple and quadratic effects and interactions of the operational parameters including extraction temperature, time, and solvent/solid ratio. A multiple nonlinear regression mathematical model was proposed through the fitting of multiple quadratic regression models for the purpose of predicting the response over certain experimental region. The regression coefficients were obtained through the RSM analysis.

The statistical analysis was performed by the Design-Expert 8.0.6 software program (Stat-Ease, Inc., Minneapolis MN, USA). Based on the experimental data, the fitting model was built up, and the statistical significance of the model terms was examined by regression analysis and analysis of variance (ANOVA). The practical yield was obtained under the optimal conditions. The mean values were compared using Dunnett's Two-Tailed t Test at the 95% significant level. $P < 0.05$ was considered as statistically significant. All data were reported as the mean ± SD of three replications.

2.7. Fatty acid Profile analysis by GC-MS in Gardenia oil

Oil methyl ester treatment was performed according to the reference[24] with some modifications. Generally, 0.03 g extracted Gardenia oil was weighed out, and put into a 10 mL centrifuge tube. Then, 2.5 mL n-hexane and 100 μL 0.5 $mol \cdot L^{-1}$ sodium methoxide solution were added into the tube. The mixture was blended for 5 min, then separated by employing centrifugation at 4 °C with 5,000 rpm for 10 min. Finally, the supernatant was gathered, and further diluted into 5 mL with n-hexane for analysis. For determining the compositions of fatty acid in Gardenia oil, fatty acid methyl esters (FAME) were analyzed by GC-MS (Agilent 7890A/5975C, USA) equipped with an elastic quartz capillary column (SP-2560, 100 m×250 μm i.d., 0.25 μm film thickness). Helium (99.99%) was used as carrier gas with a flow rate of 1.0 mL/min. The temperature of front injector and flame ionization detector (FID) were set at 220 and 250 °C, respectively. The oven temperature was programmed as 100 °C for 1 min duration, then increase to 230 °C at 3 °C /min heating rate, and maintaining 20 min. The injection volume was 1 μL and the split mode was 10:1. In MS, electron ionization (EI) was the selected ion source with the ionization energy of 70 eV. Ion source temperature was 230 °C. Scan ranged from 50 to 550 amu at 2.84 scans/s. Solvent delay time was 14 min. The detection mass spectra were matched with standard mass spectral library (NIST11) after retrieving the compounds through data processing system (MSD ChemStation). Compounds with matching rate over 90% were taken as the target compounds.

2.8. HPLC-DAD/ESI-MS^2 analysis

10.00 g of Gardenia powders were weighed out, and mixed with 50 mL of 70% methyl alcohol. The mixture was processed for 1 hour with 1 W/cm^2 UEA at 40 °C, and further made up to 100 mL with 70% methyl alcohol. 1.00 g of Gardenia oil was weighed out, and mixed with 20 mL of n-hexane in a 50 mL colorimetric tube, and then 15 mL 70% methyl alcohol was added with vigorously mixing. The aqueous layer was taken after separation, and evaporated to make up to 10 mL with deionized water. The sample was filtered through a 0.45μm polytetrafluoroethylene membrane, and kept at -18 °C until analysis.

The HPLC-DAD/ESI-MS^2 analysis was performed on an Accela series HPLC instrument

coupled with an Accela LTQ XL mass spectrometer (Thermo Fisher, San Jose, CA, USA) equipped with Electro spray ionization (ESI) interface. The HPLC chromatographic separation was carried out on a Luna C18 (Phenomenex, San Francisco, CA, USA). The separation conditions were as follows: column temperature was set at 30 ℃; UV-Vis spectra were recorded in the range from 220 to 780 nm; Chromatograms were acquired with channel A (238 nm) for geniposide, channel B (325 nm) for chlorogenic acid, and channel C (440 nm) for crocins and crocetin. Injection volume was 20 μL. 0.3% formic acid aqueous solution (A) and 100% Acetonitrile (B) were made as mobile phase. The elution gradients were as the following: initially 90% of mobile phase A, 10% B; followed from 90% to 75% A, 10% to 25% B in 10 min; 70% A and 30% B at 11 min, 60% A and 40% B at 30 min, 0% A and 100% B at 35 min, and then returned to 90% A and 10% B at 40 min. The flow rate was 0.8 mL/min. The MS conditions were set as sheath gas (N_2) flow rate of 50 arb, aux gas (N_2) flow rate of 2 arb, spray voltage of 4.5 kV, capillary temperature of 350 ℃, capillary voltage of 12 V, and collision energy of 25～35V. All data acquisition were performed by the software X-caliber (2.1), and analyzed in both positive and negative electro spray ionization mode (mainly for crocetin) to provide abundant structural information. The mass spectrometer was programmed to do a series of two scans: a full mass and a MS-MS of the most intense ion by relative collision energy of 15 and 20. The HPLC-DAD (1500 system, Scientific System Inc., USA) was used to quantitate bioactive compounds. The sample preparation and HPLC chromatographic separation conditions were the same as HPLC-DAD/ESI-MS^2 analysis. All data acquisition and analysis were processed using CSChromTM Plus Chromatographic System (version 3.6).

3. Results and discussion

3.1. Yield and recovery rate of Gardenia oils extracted by different methods

The extraction yield (%) and recovery rate (%) of Gardenia oil extracted by SE, TE, CPU, and UAE, respectively, were examined. SE was monitored to have the highest extraction yield ($P < 0.05$) at 10.89 ± 0.03%, followed by UAE (8.59 ± 0.06%), TE (7.40 ± 0.03%), and CPE (5.66 ± 0.15%). A comparison showed oil extraction yield obtained by UAE was 51.8% higher than the content acquired by CPE. Significant differences were found in extraction

yield in four different extraction methods ($P < 0.05$). The data clearly indicated that UAE was more effective than those of TE and CPE, by which the recovery rate of Gardenia oil was 78.88%, 68.00% and 51.92%, respectively, if considering the SE extraction yield for Gardenia oil as 100%. Ultrasound offers advantages of productivity, yield and selectivity. Extraction performed under the action of ultrasound is believed to be affected by cavitation phenomena and mass transfer enhancement. The ultrasonic waves cause cavitation effect accompanied by lots of bubbles on the verge of burst when large amplitude of ultrasonic wave is traveling through the liquid medium during the ultrasonic process. Under above circumstance, plant tissues are disrupted, consequently enhancing solvent contact with available extractable compositions; and further mass transfer is increased by shear force created for the implosion of cavitation bubbles, thus making solvent extraction fully performed and improving the extraction yield.[20,25]

3.2. RSM analysis

RSM method is an effective technique for analyzing interactions among factors and optimizing the processes when multiple variables may influence the outputs. In our work, Box-Behnken design for optimization of UAE was presented in **Table 1**. The fitting effect of model, individual factors and interactions were discussed through analysis of variance. Meanwhile, the obtained experimental values of Gardenia oil were evaluated by multiple regression method. The results from variance analysis showed that the response surface model was suitable for the following reasons: (1) The F-value in total model is 8.45, suggesting a significant model; (2) The "lack of fit" is not significant with F-value of 2.02. 0.2536 of *P*-value indicates the model has a good fitting effect; (3) 10.935 of Adeq Precision is greater than 4, indicating a high predictable degree in the model; and (4) The R^2 with 0.9157 is sufficiently high, suggesting a good correlation. *P*-values of the model factors less than 0.05 are significant. According to the results, factor ETi, the interaction between ETi and ETe as well as the square of ETe have highly significant effects on the extraction yield, while other interactions have minor significance. The regression model could be expressed as Eq. (3):

$$Y = 15.46 - 0.22X_1 - 0.28X_2 + 0.042X_3 + 0.002X_1X_2 + 0.008X_1X_3 + 0.003X_2X_3 - 0.022X_1^2 + 0.001X_2^2 - 0.003X_3^2 \quad (3)$$

The Xi, Xj are independent variables, which stand for the experimental factor levels. Y is a dependent variable, which stands for the predicted response (extraction yield, %).

3.3. Verification experiment

Through RSM analysis, the optimum process conditions in extracting Gardenia oil were acquired. Verification experiment was applied as: solvent/solid ratio of 4/1 ($mL \cdot g^{-1}$), extraction time of 40 min; and extraction temperature of 37 °C. The suitability of the model equation for predicting the optimum response values was verified using the optimal conditions. The experimental value (**Table 1**) of extraction yield was 8.57%, which was approaching to values of 8.49% predicted by the regression models. Results suggest that the slightly modified parameters are reliable, thus can be used to extract oil in Gardenia fruit powder.

3.4. Fatty acid profile analysis in Gardenia oil

The fatty acid profile in Gardenia oil extracted by UAE with various organic solvents and CPE process through methyl ester treatment were directly identified by GC-MS, as shown in **Figure 2** (A) and (B). The chromatograms suggested that 13 major fatty acids were present in Gardenia oil extracted by both CPE and UAE. In **Table 2**, it clearly shows that, among saturated fatty acids, palmitic acid was dominant, followed by stearic acid in both CPE and UAE Gardenia oils. Among unsaturated fatty acids, Octadecadienoic acid was the most abundant fatty acid in Gardenia oil, composing over 50% of total methyl fatty acid composition.

3.5. Effect of organic solvents on fatty acid composition

Organic solvent plays a key role in determining the extraction yield of oil, as well as the types and content of fatty acids. Organic solvents are selective to the oil composition in the extraction process for the polarity differences.[21] In our study, four organic solvents with different polarity have been applied to extract oil from GF powder in order to obtain the optimal solvent. Extraction yield by different organic solvents in UAE method was more

effective than that of CPE method, as indicated by the former could acquire more than 10% extraction yield in comparison to the latter from (**Table 2**). Area normalization method was employed to calculate the relative content of different fatty acids. The ratio of unsaturated fatty acids and saturated fatty acids (P/S) is one of the most important indicators to evaluate the quality of the oil. The larger the P/S is, the better the quality of the oil could be. Petroleum ether is more efficient than other solvents in extraction of Gardenia oil by using UAE method. Interestingly, P/S of oil extracted by ethyl acetate was the maximum among four organic solvents, followed by n-hexane (3.35%), petroleum ether (3.11%), and cyclohexane (2.87%). Fatty acids in Gardenia oil were more selective to petroleum ether with the highest extraction yield, thus petroleum ether would be considered as an ideal solvent for further study.

3.6. Identification and quantitation of bioactive components in Gardenia oil

HPLC-DAD/ESI-MS^2 was used to elucidate and identify various bioactive components in Gardenia oil. The chromatograms (total ions scan and channel A, B, and C at 238 nm, 325 nm and 440 nm, respectively) were shown in **Figure 3**. Structural analysis of major bioactive compounds was performed by a series of mass spectra scans. The mass spectra of the molecular ions (MS) and their fragments ions (MS^2) of the bioactive components identified in oil were shown in **Figure 4**. The peak of a maximum absorbance at 247 nm with retention time at 9.85 min revealed an ion peak at m/z 411 recorded at positive ion mode, which could be attributed to the molecular ion $[M+Na]^+$ of geniposide (**Figure 4A**). The MS^2 spectra showed characteristic fragments at 379, 249, 231, 203 as previously reported for geniposide,[26,27] and the loss of 162 amu was ascribed to the cleavage of the glucosyl moiety. Both the peak with retention time at 15.94 showing an absorbance maximum at 465 and 442 nm and the peak at 24.41 min displaying an absorbance maximum at 460 and 435 nm exhibited the same ion peaks at m/z 999 and the same MS^2 spectra fragments at m/z 675, 513, 347 (**Figure 4B**), which were ascribed to *trans*/*cis*-crocin-1. The peak with retention time at 18.14 min showing an absorbance maximum at 462 and 443 nm revealed an ion peak at m/z 837 $[M+Na]^+$ recorded at positive ion mode and MS^2 fragments at m/z 675, 513, 347, the same fragmentation pattern as crocin-1, which could be ascribed to crocin-2, an homologue of crocin-1 and crocin-3 (**Figure 4C**). The peak with retention time at 28.96 min revealing an

absorbance maximum at 460 and 435 nm showed an ion peak at m/z 513 $[M+Na]^+$ and MS^2 fragments at m/z 347 and 329 recorded at positive ion mode (**Figure 4D**) and was ascribed to crocin-4. Both the peak with retention time at 36.85 min showing an absorbance maximum at 452 and 427 nm and the peak at 37.36 min displaying an absorbance maximum at 469 and 443 nm displayed the same ion peaks at m/z 327 $[M-H]^-$ recorded at negative ion mode and the same MS^2 spectra fragments at m/z 283 and 239 (**Figure 4E**) as previously reported as *trans*/*cis*-crocetin.[28,29]

Based on the maximal UV-Vis spectrum wavelength, the molecular ion and fragment ions, eight bioactive compounds in Gardenia oil were identified and further confirmed by comparison with reference standards (**Table 3**). The concentrations of the major bioactive components in Gardenia oil were then quantified through HPLC-DAD analysis, and their corresponding transfer rates from Gardenia fruit into oil by UAE were calculated and shown in **Table 4**. The content of crocin-3, crocin-4 in oil was calculated according to the Eq. (4):

$$m_2 = \frac{M_2}{M_1} \times \frac{S_2}{S_1} \times m_1 \quad (4)$$

M_1, M_2 represent the molar masses of crocin-1 and crocin-3 (or crocin-4), respectively; S_1, S_2 represent the peak areas in liquid chromatography of crocin-1 and crocin-3 (or crocin-4), respectively; m_1, m_2 represent the content of crocin-1 and crocin-3 (or crocin-4) in Gardenia oil, respectively.

The transfer rate of bioactive components from Gardenia fruit to oil could be calculated according to Eq. (5):

$$\text{Transfer rate}\,(\%) = \frac{W_1}{W_2} \times 100 \quad (5)$$

W_1, W_2 represent the content of bioactive components in Gardenia oil and Gardenia fruit, respectively.

As listed in **Table 4**, geniposide was dominant in Gardenia fruit powder with 1391.15 μg/g, while crocin-4 had the lowest content of 5.48 μg/g among the bioactive compounds identified. In UAE Gardenia oil, the content of geniposide extracted by petroleum ether is the highest with 53.41 μg/g, while the content of *trans*/*cis*-crocetin extracted by ethyl acetate was the

highest with 11.38 μg/g among the bioactive compounds quantified. The small quantities of bioactive components including crocin-1, crocin-2, crocin-3, crocin-4, and geniposide have been transferred into the oil from the Gardenia fruit powder. It was reported that all crocin derivatives in saffron occur as pairs of *cis*-*trans* isomers except crocin-1.[30] Crocin analogues including crocins 1-4 are almost glycosides of *trans*-crocetin in saffron, among which *trans*-crocin 3 and 4 are the most abundant.[7] Interestingly, here it was indicated that the lipophilic *trans*/*cis*-crocetin could be completely transferred from GF powder into oil extracted by UAE with ethyl acetate solvent.

Biological and pharmacological activities in extracts of Gardeniae Fructus, including scavenging activity again oxygen free radicals, inhibiting apoptosis of NIH/3T3cells, anti-inflammatory, protective activity against oxidative damage, MMP-inhibition and cell morphology, have been demonstrated.[31] Geniposide shows many biological activities including anti-thrombotic, anti-inflammatory, antitumor, immunosuppression, neuroprotection, and hypo-glycemic effect.[32] In particular, geniposide, as a promising anti-inflammatory drug, have been tested in animals and human.[33] Through 1D and 2D NMR techniques and mass spectrometry, three new iridoid glycosides were isolated and identified from the fruit of Gardenia jasminoides.[34] Compounds 8 and 18 exhibited strong inhibitory activity on nitric oxide production with IC_{50} values of 11.14 ± 0.67 and 5.99 ± 0.54 μM, respectively, in lipopolysaccharide-activated macrophages. Geniposide is hydrolyzed to form aglycone genipin by β-D-glucosidase in the intestine and liver. Besides being applied as blue colorant in food industry, genipin is also an effective cross-linking reagent for biological tissue fixation, and stimulates glucose transport in C2C12 myotubes via an IRS-1 and calcium-dependent mechanism.[35] Lot of studies have been conducted with respect to the biological and pharmacological properties of crocin, which exhibits beneficial effects on many organs including the nervous system, gastrointestinal, cardiovascular, genital, endocrine, immune systems.[7] Recently, it was shown that crocin improved locomotor function and mechanical behavior in rat model of contused spinal cord injury through decreasing calcitonin gene related peptide.[36] Additively, glycosylation of crocetin is crucial, since it confers hydrosolubility to the pigment.

4. Conclusions

Gardenia oil extracted from its fruit is less reported, and its composition remains uncertain. There is a strong interest in investigating extraction optimization of natural bioactive compounds to acquire higher quality products with health benefits. In this study, Gardenia oil extracted from GFs has been examined. In comparison with CE (TE, SE, CPE), UAE has been observed to demonstrate relatively high extraction yield. UAE with different solvents was applied to evaluate the effects on oil extraction yield and fatty acid profile. Petroleum ether in UAE was proved to be preferable for a better extraction yield, while a higher proportion of unsaturated fatty acids to saturated fatty acids were achieved when ethyl acetate in UAE was applied. The UAE conditions were explored via RSM, and the extraction parameters were optimized to improve extraction yield. The mathematical model with the prediction of experimental data of the extraction could be helpful in the extraction process of the natural products. The maximal Gardenia oil extraction yield was acquired at the optimal conditions under 4/1 (mL/g) of petroleum ether to solid ratio, 37 ℃ for 40 min extraction. Through qualitative and quantitative analysis of bioactive components in Gardenia oil, eight compounds including geniposide, *trans*/*cis*-crocin-1, crocin-2, crocin-3, crocin-4, and *trans*/*cis*-crocetin were elucidated, identified, and further calculated for their corresponding transfer rates. Those bioactive compounds may be attributed to the health benefits of Gardenia oil, and show the potential applications. Identification of other special components transferred from Gardenia fruit into oil during UAE process and the nutritional evaluation could be carried out in future research. In addition, the effects of ultrasound intensity and solvent concentration on extraction of bioactive compounds are worth to be investigated.

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