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Isolation of the sweet components from *Siraitia grosvenorii*

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Abstract

Powdered concentrate from dried Luo Han Guo fruit was subjected to liquid extraction by Soxhlet (hexane:ethanol, 1/4 v/v) or with subcritical water (scH_2O) or with supercritical ethanol carbon dioxide ($scCO_2$). Whereas exhaustive Soxhlet extraction of the crude dried fruit powder (CDFP) was inefficient, pressurized water extraction in the presence of chromatographic support (Alumina, Celite or Silica gel) was beneficial to the scH_2O recovery of mogrosides as determined by colourimetry. With a flow rate of 0.7 mL min^{-1} scH_2O and a back pressure of 11.7 MPa, a maximum recovery was obtained at 150 °C; yet increases in recovery for extractions beyond 10 min were marginal. The recovery of target compounds were very inefficient for $scCO_2$ alone but was improved with the addition of 0.3 mL min^{-1} ethanol as co-solvent to the mobile phase and by adding chromatographic support to the substrate. Increased pressure during the $scCO_2$ extractions were beneficial to the recoveries that were maximized at 60 °C. However, increases in the recoveries of mogrosides for extractions beyond 90 min for the dried fruit powder or beyond 30 min for the partially purified concentrate were very modest. Of the three extraction techniques, Soxhlet, $scCO_2$ or scH_2O, the latter technique, in tandem with ultra-sonication of the dried fruit powder proved to be very efficient so that there was little value to partially purifying this substrate prior to pressurized fluid extraction.

Keywords: Mogrosides; Extraction; Luo Han Guo fruit; Subcritical water; Supercritical carbon dioxide; Ultra-sonication

1. Introduction

Several novel compounds are "high-intensity" sweeteners that may be worthy targets for chemical synthesis or for semi-synthetic modification to produce substances with enhanced sweetness properties. The high-intensity sweeteners that are approved in North America are synthetic substances (acesulfame-potassium, aspartame, neotame, saccharin and sucralose). Additionally, there are some 80 sweet compounds (exclusive of monosaccharides, disaccharides, and polyols) that have been isolated from vascular plants. These plant-derived compounds belong mainly to three major structural classes, namely, the terpenoids, flavonoids, and proteins.

Siraitia grosvenorii SWINGLE (also known as Luo Han Guo), a perennial vine of the Cucurbitaceae family (cucumber and melon) is a traditional medicinal herb that is cultivated principally in the Guangxi autonomous region of China. The fruits of Luo Han Guo have been used for hundreds of years in China as a natural sweetener and as a folk medicine for the treatment of lung congestion, colds and sore throats. The non-caloric sweet taste of *S. grosvenorii* fruit results primarily from the content of mogrosides, a group of cucurbitane-type triterpene glycosides that are present at about 1% in the flesh of the fruit (Kinghorn & Soejarto, 1986). Whereas the sweetness of high-intensity natural sweeteners in plants is frequently associated with an elevated content of sugars and polyols (greater than 5% w/w, Hussain et al., 1990) the yield of saccharides and polyols from *S. grosvenorii* dried fruit is only 2.4% (w/w). The major sweetening components are triterpene glycosides and include mogroside IV, mogroside V, mogroside VI, siamenoside I and 11-oxo-mogroside V (Fig. 1).

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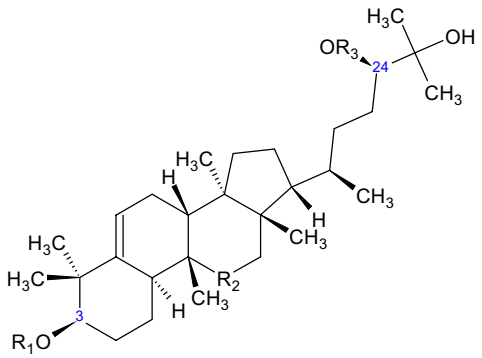

Common Name	-R_1	-R_2	-R_3
Mogroside IV	-Glc–Glc	HO, H	-Glc–Glc
Mogroside V	-Glc–Glc	HO, H	-Glc(Glc)Glc
Mogrol	H	HO, H	H
11-oxo-mogrol	H	=O	H
11-oxo-mogroside V	-Glc–Glc	=O	-Glc(Glc)Glc
Siaminoside-1	-Glc	HO, H	-Glc(Glc)Glc

Glc = β-D-glucopyranosyl

Fig. 1. Structures of selected triterpene glycosides and related compounds from dried Luo Han Guo fruit.

The mixed mogrosides have been estimated to be about 300 times as sweet as sucrose so that an 80% extract was nearly 250 times sweeter than sugar (Kasai et al., 1989). In studies of structure taste relationships, the number of glucose units and the oxygen functionality at the 11-position of the aglycone moiety, seemingly, are responsible for the perception of taste; glycosides of 11α-hydroxy compounds taste sweet whereas glycosides of 11β-hydroxy compounds are tasteless (Kaiser, Matsumoto, Nie, Zhou, & Tanaka, 1988). Glycosides of 11α-hydroxy-aglycone and five glucosyl units in mogroside V confer a sweet taste (Matsumoto, Kasai, Ohtani, & Tanaka, 1990). The purified, sweet principle, mogroside V, has been approved as a high-intensity sweetening agent in Japan (Jakinovich, Moon, Choi, & Kinghorn, 1990) and the extract has gained generally recognized as safe (GRAS) status in the USA as a non-nutritive sweetener and flavor enhancer.

1.1. Extraction techniques

Subcritical water extraction (SWE, also called pressurized hot water extraction, PHWE, or superheated water extraction) is based on the unique solvent properties of water, namely its disproportionately high boiling point for its mass, a high dielectric constant and high polarity (Smith, 2006). The method involves heating water above its boiling point but below its critical point (374 °C) under elevated pressure so that the water remains in a liquid state. As the temperature rises, there is a marked and systematic decrease in permittivity, an increase in the diffusion rate and a decrease in the viscosity and surface tension. In consequence, more polar target materials with high solubility in water at ambient conditions are extracted most efficiently at lower temperatures whereas moderately polar and non-polar targets require a less polar medium induced by elevated temperature (Miller & Hawthorne, 2000). SWE has been found to be an efficient extraction method and a viable alternative to steam distillation and solvent extraction in the extraction of essential oils from plant materials. Satisfactory results have been reported for SWE of essential oils from marjoram (*Thymus mastichina*), clove (*Syzygium aromaticum*), fennel (*Foeniculum vulgare*) and sage (*Salvia officinalis*) (Jimenez-Carmona, Ubera, & Luque de Castro, 1999; Ong & Len, 2004). Besides essential oils, the technique has also been applied to the extraction of lactones from kava root (*Piper methysticum,* Kubatova, Miller, & Hawthorne, 2001) or from *Ginkgo biloba* (Lang & Wai, 2003), and iridoid glycosides from *Veronica longifolia* leaves (Suomi, Siren, Hartonen, & Riekkola, 2000).

An alternate extraction technique that has received increased attention for the isolation of natural products is supercritical fluid extraction (SFE). SFE has several advantages over conventional liquid–liquid and solid–liquid extraction techniques that include the elimination of most of the organic solvents that can pose a safety risk during extraction, elimination of carry-over of solvent residues to the final extracts, and the possibility of avoiding the detrimental effects of these solvents on the environment (Jarvis & Morgan, 1997; Lang & Wai, 2001). It is generally accepted that the solvent power of a supercritical fluid is related mainly to its density in the region of its critical point. The critical temperature (T_C) of non-associating fluids such as CO_2 or C_2H_6 are generally less than 50 °C whereas for associating fluids such as water (T_C, 374 °C) or ethanol (243 °C), this parameter is much higher. Carbon dioxide is the most common solvent for supercritical applications in food-related industries because of its lack of toxicity and readily achieved critical parameters (T_C, 31.1 °C; P_C, 73.8 bar). Moreover, it is non-flammable, non-reactive, non-corrosive and abundant. It is the second least expensive solvent after water, environmentally benign and does not leave any solvent residue after extraction.

The disadvantages of SFE include the low polarity of the most commonly used fluid, (carbon dioxide), possible problems caused by the presence of water, unpredictability of the matrix effect and the need for specialized/expensive equipment (Venkat & Kothandaraman, 1998). A number of compounds have been tested as supercritical fluids, including pentane, nitrous oxide, ammonia and Freon® fluorocarbons but, on the grounds of cost and safety, CO_2 either alone or modified with ethanol or some other polar solvent is by far the most widely used supercritical extrac-

tion solvent. The practical aspects of SFE and its applications have been reviewed recently (Lang & Wai, 2001). SFE has been successfully applied to the extraction of essential oil from Angelica *archangelica* L. roots, as well as from Matricaria *chamomilla* flowerheads, and the extraction of lycopene from tomato skins (Donenau & Anitescu, 1998; Ollanketo, Hartonen, Riekkola, Holm, & Hilturen, 2001). Supercritical carbon dioxide ($scCO_2$) is a relatively good solvent for hydrocarbons and other non-polar materials. Additionally, owing to an appreciable quadrupole moment, small polar molecules such as ethanol or dimethyl sulfoxide display appreciable solubility in this medium. As a result, adding small amounts of a polar modifier such as ethanol can increase the solubility of moderately polar non volatile materials in $scCO_2$ such as flavanones and xanthones from Maclura *pomifera*, flavonoids from Scutellaria *baicalensis* roots and apigenin from Matricaria *chamomilla* (da Costa, Margolis, Benner, & Horton, 1999). A thorough review on the application of SFE to the isolation of plant products has been published by Jarvis and Morgan (1997).

Nowadays, soft drinks incorporating extracts of *S. grosvenorii* fruit containing sweet cucurbitane-type triterpene glycosides such as mogroside V, are available commercially (Kinghorn & Soejarto, 2002). The objectives of the current studies were to assess the efficacy of automated extraction techniques to isolate/concentrate fractions rich in mogrosides from crude *S. grosvenorii* powder.

2. Materials

Powdered concentrate from dried *S. grosvenorii* fruit was obtained from China Natural Products Group Inc., Cincinnati, OH. Amberlite XAD-2 (20–60 mesh), IRA-400 (20–50 mesh), Aluminum oxide (neutral, 150 mesh) and (basic, 150 mesh), Celite 545, Silica gel 60 (150 mesh) and Vanillin (99% purity) were purchased from Sigma Aldrich, Oakville, ON. Perchloric acid (70%, v/v), acetic acid (glacial) and ethanol were purchased from Fisher Scientific, Napean, ON. Carbon dioxide and nitrogen were purchased from MEGS, St-Laurent, QC. All reagents were ACS Reagent grade or better.

3. Methods

3.1. Purification of mogroside V

Powdered dried fruit concentrate of *S. grosvenorii* in water (8 mg mL^{-1}, pH 7.0) was added to the head of a column of Amberlite XAD-2 resin (25 × 1 cm) and eluted (~2 mL min^{-1}) with water until the eluate was no longer coloured. The adsorbed mogrosides were displaced by elution with 5 resin bed volumes of alkaline (pH 9.5) 50% aqueous ethanol. The combined aqueous ethanol extract was then evaporated under reduced pressure at 45 °C to obtain a brownish residue. Elution from a second column (25 × 1 cm) of Amberlite IRA-410 resin with water furnished a decolourized extract. The concentrated eluate was separated further by preparative thin layer chromatography on (20 × 20 cm) silica gel GF glass plates. Recovery of the fraction with R_f, 0.23 following development of the plate with *n*-butanol–acetic acid–water (4:1:1), furnished a crystalline analytical sample. Negative and positive electrospray ionization (ESI) mass spectra and ^{13}C nuclear magnetic resonance (NMR) spectra were in accord with published spectra for authentic mogroside V.

3.2. Calibration curve for mogroside V

Mogroside V analytical standard (120–420 μg) was combined with 0.2 mL of freshly prepared vanillin – glacial acetic acid (5% w/v) solution and 0.8 mL of perchloric acid. After thorough mixing, the solution was heated at 60 °C for 15 min then cooled immediately in ice bath. Glacial acetic acid, 5 mL, was added and the absorbance of the solution at 590 nm was recorded (after 10 min of reaction) with an Ultrospec-100 spectrophotometer (Biochrom Ltd., Cambridge, England). Control solution, that contained no analyte, was treated analogously.

3.3. Subcritical water extraction

The laboratory assembled extractor consisted of a high performance liquid chromatography (HPLC) pump, a transfer line of stainless steel (ss) tubing {1 m × 1.6 mm inner diameter (i.d.)} fashioned into a coil (that served to heat the influent solvent) and an extraction cell (1 mL capacity, 75 × 4 mm i.d., Supelco Canada, Mississauga, ON) and a short section of ss transfer line. The coiled transfer line – extraction cell assembly was mounted within a gas chromatography oven to provide repeatable extraction temperatures. The effluent transfer line that exited the oven was terminated with a capillary silica restrictor (250 × 0.050 mm i.d.) that maintained an elevated pressure within the system. High pressure needle valves (SSI model 02-0120, Supelco, Oakville, ON) were placed before the heating coil and before the silica pressure regulator.

In operation, crude powdered fruit concentrate (50 mg), admixed with chromatographic support (1:1, v/v), was added to a layer of sand contained within the extraction cell and then capped with a second layer of sand sufficient to fill the vessel. The vessel was sealed, mounted within the oven and once equilibrated to the desired operating temperature, the exit valve was opened and sequential 6 mL fractions of eluate were collected, diluted to 10 mL with water and stored to await analysis.

3.4. Ultra-sonication

Equilibration was achieved by sonicating a suspension of crude powder in distilled water (1:3 w/w). Ten successive treatments with an ultrasonic homogenizer (XL 2020 Sonic dismembrator, Misonix Inc. NY) were performed in a 5 mL plastic tube jacketed with an ice bath. An extended horn of 25 cm L × 1.2 cm W, tuned at 20 kHz frequency,

delivered ultrasonic energy (240 W) in a pulsed mode with a fixed vibration amplitude setting of 6–7. The equilibration consisted of pulsed surges of power delivered for 4 s followed by a 5 s cooling phase. The resulting slurry was transferred to the extraction cell.

3.5. Supercritical CO_2 extraction

The extractor assembly consisted of source of pressurized CO_2, a mixing device and an extraction cell. A diaphragm compressor (Newport Scientific, Jessup, MD) served to further compress the effluent from a K-type cylinder of CO_2. Ethanol, delivered with an HPLC pump (Varian 9010 pump, Walnut Creek, CA) was added to the CO_2 mobile phase ($\sim$1 mL min^{-1}) via a mixing tee and transferred to a ss equilibration coil (1 m × 1.6 mm i.d.) and then to the extraction cell (1 mL capacity, 75 × 4 mm i.d., Supelco Canada, Mississauga, ON). The temperature equilibration coil and the extraction cell were mounted within a gas chromatography oven to permit repeatable temperature control. Pressure within the extractor assembly was maintained with a capillary silica restrictor (250 × 0.050 mm) that terminated the assembly. High pressure needle valves (SSI, model 02-0120, Supelco, Oakville, ON) were mounted in series at the entrance and exit of the oven. A pressure release valve (Tyco Valves and Controls, Montreal, QC) incorporating a rupture disk (rated to 27 MPa) was positioned upstream of the extraction cell and configured to vent the system if the pressure within the system became excessive.

After the extraction cell had been equilibrated to the desired operating temperature (40–80 °C), the cell {loaded with plant material (50 mg) as above} was washed with $scCO_2$ (at 13.8–20.7 MPa) for 2 min to purge air from the system. The extraction was commenced by introducing ethanol (0.1–0.5 mL min^{-1}) via the HPLC pump. Successive 10-min fractions of extractor eluate were collected during 2 or 3 h.

4. Results and discussion

Crystalline analytical mogroside V standard was isolated from the powdered plant concentrate by elution from a macroporous resin (Dowex XAD-2) with 50% aqueous ethanol followed by filtration through a strongly basic Amberlite IR-120 H column. The concentrated eluate from the ion exchange material was further purified by preparative thin layer chromatography on silica gel. The fraction corresponding to R_f 0.23 following elution with butanol–acetic acid–water (4:1:1) was crystallized from ethanol. Spectroscopic characterization of the crystalline isolate {positive and negative electrospray ionization mass spectrometry (ESI–MS), proton and carbon-13 nuclear magnetic resonance (^{1}H, ^{13}C NMR) spectra} provided results that were in accord with published data for authentic mogroside V (Chang, Chen, Si, & Shen, 1994; Kasai et al., 1989; Si, Chen, Chang, & Shen, 1996).

5. Extraction with subcritical water or ethanol

In preliminary trials, the influence of extraction parameters on the recovery of mogrosides from the crude dried fruit powder was assessed in the presence of four different support materials (Alumina, Celite 545 and Silica gel) admixed with the substrate powder, at five temperatures (70–170 °C), and time of extraction (7–30 min). The Luo Han Guo powder was pre-mixed (1:1 w/w) with either of two grades of Alumina, with Celite 545 or with Silica prior to extraction. The recovery of the mogrosides fraction, as determined colourimetrically, was compared with colourimetry performed directly on the powder. The extraction temperature (70–170 °C) was also varied (Table 1, experiments 1–5). There was a continued increase in recovery of mogrosides up to 150 °C. However, at 170 °C, the recovery of mogrosides had decreased to 39.5% that seemed to have resulted from degradation of the target fraction. At this temperature, the extract had darkened visibly and the smell had become increasingly acrid. The presence of filtration aid seemed to be beneficial to the recovery of the target compounds yet differences in recovery, after 10 min of extraction, were evident. The neutral alumina slightly improved recovery relative to the Celite 545 and appreciably improved recovery relative to the basic alumina or the silica gel. (Table 1, experiments 6–9). Moreover, the extractions were repeatable with standard deviations of the order of 1%. In consequence, subsequent trials were performed by mixing the substrate powder with neutral alumina (1:1, w/w). Trials were performed at 150 °C and the time of extraction was varied from 5 to 30 min (Table 1, experiments 10–15). The recovery with extended extraction time (10 min vs. 30 min) was increased but only modestly (58 vs. 62%).

Table 1
Influence of the support material, extraction temperature and time on the recovery (% ± 1 SD[a]) of mogrosides from the powdered Luo Han Guo dried fruit by extraction with subcritical water

Experiment	Stationary support	Temperature (°C)	Time (min)	Recovery (%)
1	Al_2O_3, neutral	70	10	22.9
2	Al_2O_3, neutral	100	10	43.7
3	Al_2O_3, neutral	120	10	43.9
4	Al_2O_3, neutral	150	10	47.1 ± 0.96
5	Al_2O_3, neutral	170	10	39.5
6	Al_2O_3, basic	150	10	45.9
7	Al_2O_3, neutral	150	10	55.9 ± 1.0
8	Celite 545	150	10	53.9 ± 1.4
9	Silica Gel	150	10	34.2
10	Al_2O_3, neutral	150	5	49.8 ± 2.0
11	Al_2O_3, neutral	150	7	55.7 ± 1.9
12	Al_2O_3, neutral	150	10	58.3 ± 0.97
13	Al_2O_3, neutral	150	15	59.7 ± 0.48
14	Al_2O_3, neutral	150	20	61.1 ± 0.50
15	Al_2O_3, neutral	150	30	62.4 ± 0.47

[a] Mean recovery ± one standard deviation based on three replicate trials.

Ethanol was also evaluated as a near critical mobile phase for temperatures between 70 and 170 °C. However, in all cases the recovery of mogrosides was very modest. A maximum recovery of 11.4% was obtained at 100 °C and the extraction procedure seemed to be repeatable (mean percent recovery of 4.6 ± 0.52 at 170 °C).

6. Extraction with supercritical carbon dioxide ($scCO_2$)

Extractions with $scCO_2$ were performed on two substrates, the crude dried fruit powder (CDFP) and the partially purified concentrate (PPC) after passage through the two columns of resin (Amberlite XAD-2 and Amberlite IR 410). The recovery of mogrosides with neat $scCO_2$ was very low but was increased by the addition of ethanol modifier.

As anticipated, the recovery of mogrosides from the CDFP increased with increasing pressure (20.9–27.6%, Table 2A) as did the recovery from the PPC (37.8–66.8%, Table 2B) for extractions at 60 °C. In contrast, the influence of temperature was less predictable, with extractions anticipated to be accelerated with increased temperature but density (and therefore solvating power) being decreased. For the temperature range of 40–80 °C, a maximum recovery was obtained at 60 °C.

Increased time of extraction also increased the recovery of target compounds from both the CDFP and from the PPC substrates (Table 3). However, the recoveries for extraction times beyond 30 min in the case of the PPC (20.7 for 30 min of extraction vs. 22.8 for 90 min) or beyond 90 min for the CDFC substrate (18.9 for 90 min vs. 22.1 for 180 min of extraction) were very modest. By contrast, both the identity of the support material and the addition rate of modifier influenced recoveries appreciably. For both substrates, the recovery in the presence of washed sand was appreciably greater than from neutral alumina (Table 4A) and the recovery in the presence of ethanol was greater at 0.3 mL min^{-1} than at 0.1 mL min^{-1} but not different from 0.5 mL min^{-1} (Table 4B). Conventional Soxhlet extraction of the crude powder was also evaluated with a mixture of ethanol:hexane (1:1 v/v). However, after 24 h of continued extraction, the recovery amounted to 5.1% and once cooled, the light orange extract was somewhat turbid (Table 5).

Finally, another means of increasing the contact between the mobile phase and the substrate was explored.

Table 2
Influence of the extraction temperature, pressure, modifier addition rate and time on the recovery (% ± 1 SD[a]) of mogrosides from A the crude dried fruit powder or B partially purified concentrate by extraction with supercritical carbon dioxide

Temperature (°C)	Pressure (MPa)	Time (min)	EtOH modifier addition rate (mL min^{-1})	Recovery (%)
A				
60	13.8	60	0.5	20.9 ± 4.4
60	17.2	60	0.5	23.6 ± 3.4
60	20.7	60	0.5	27.6 ± 1.5
40	20.7	15	0.5	18.9 ± 3.2
60	20.7	15	0.5	27.6 ± 1.5
80	20.7	15	0.5	21.7 ± 4.6
B				
60	13.8	90	0.3	37.8 ± 1.4
60	17.2	90	0.3	59.6 ± 4.7
60	20.7	90	0.3	66.8 ± 2.4
40	20.7	40	0.1	0.91 ± 0.62
60	20.7	60	0.1	22.8 ± 1.6
80	20.7	80	0.1	18.4 ± 1.0

[a] Mean recovery ± one standard deviation based on three replicate trials.

Table 3
Influence of the cumulative extraction time on the recovery[a] (% ± 1 SD[b]) of mogrosides from A, crude dried fruit powder or B, partially purified extract from dried Luo Han Guo fruit by extraction with supercritical carbon dioxide

Extraction time (min)	Cumulative recovery (%)
A	
30	0.9 ± 1.1
60	13.1 ± 2.6
90	18.0
120	20.9
150	22.3
180	22.1
B	
30	20.7 ± 1.3
60	21.0 ± 1.5
90	22.8 ± 1.6
120	22.8 ± 1.7

[a] Trials were performed with substrate admixed 1:1 with neutral Al_2O_3 at 80 °C and 17.2 MPa in the presence of 0.5 mL min^{-1} EtOH.
[b] Mean recovery ± one standard deviation based on three replicate trials.

Table 4
Influence of the support material or modifier addition rate on the recovery[a](% ± 1 SD[b]) of mogrosides from A, crude dried fruit powder (CDFP) or B, partially purified extract (PPE) from dried Luo Han Guo fruit by extraction with supercritical carbon dioxide

Substrate	Support material	Cumulative recovery (%)
A		
CDFP	Al_2O_3, neutral	4.2 ± 0.98
CDFP	Sand	37.0 ± 3.2
CDFP	Celite 545	25.5 ± 2.0
PPC	Al_2O_3, neutral	0.46
PPC	Sand	9.3
Substrate	EtOH modifier addition rate (mL min^{-1})	Recovery (%)
B		
PPC	0	0.63 ± 0.27
PPC	0.1	22.8 ± 1.6
PPC	0.3	66.8 ± 2.4
PPC	0.5	67.3 ± 1.2

[a] Trials were performed for 60 min (A) or 90 min (B) with substrate admixed 1:1 with support material at 60 °C and 20.7 MPa in (A) the presence of 0.5 mL min^{-1} or (B) 0.1–05 mL min^{-1} EtOH.
[b] Mean recovery ± one standard deviation based on three replicate trials.

Table 5
Optimized extraction parameters for the recovery[a] of mogrosides from Luo Han Guo dried fruit powder (CDFP) or partially purified concentrate (PPC)

Substrate	$scCO_2$		scH_2O	Soxhlet
	CDFP	PPC	CDFP	CDFP
Mobile phase flow rate (mL min^{-1})	1	1	0.7	–
Pressure (MPa)	20.7	20.7	1.17	ambient
Temperature (°C)	60	60	150	85
Support material	sand	sand	Al_2O_3 neutral	–
Extraction time (min)	30	90	30	1440
EtOH addition rate (mL min^{-1})	0.5	0.5	0.0	–
Recovery (%)	37.0 ± 3.2	67.3 ± 1.2	62.4 ± 0.50	5.1

[a] Mean recovery ± one standard deviation based on three replicate trials.

Whereas it was not practical to perform the subcritical water extraction in the presence of sonication, a sequential treatment was evaluated. The crude dried fruit powder was wetted with water (~3:1, w/w) and subjected to sonication within an ice jacket to minimize heating. The sonicated CDFP suspension was then transferred to the extraction cell and extracted with scH_2O. For the combined process a recovery of 83.6% was achieved.

7. Conclusions

These studies demonstrated that a fraction rich in mogrosides could be isolated from the crude dried powder of Luo Han Guo fruit. Of three extraction procedures, Soxhlet, supercritical carbon dioxide supplemented with ethanol or subcritical water, the latter provided the highest recovery. It was unnecessary to partially purify the extract prior to extraction. Sonication of an aqueous suspension for 40s followed by extraction at 150 °C under sufficient pressure to maintain the liquid state mobilized more than 83% of mogrosides fraction as determined colourimetrically.

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