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Review

Gardenia herbal active constituents: applicable separation procedures

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Abstract

Gardenia herb has been used as alternative drug for thousand years. They may provide therapeutic or cause toxic effect. Recently, large scale of biological screen, phytochemical separation, isolation, and identification were widely performed. Quality control of the active ingredients should be concern for the application of Gardenia herbs. Many systems have been developed for the determination of herbal ingredients. This article reviews some of the plants and their active constituents that have been used for medicinal applications. The sample preparation, separation, and determination of Gardenia herbal ingredients were discussed. Based on the separation, the method of gas chromatography, liquid chromatography, and capillary electrophoresis were also discussed.

Keywords: Gardenia herbal ingredients; Traditional Chinese medicines

Contents

Abbreviations: ESI, electrospray ionization; GC, gas chromatography; GC–MS, gas chromatography mass spectrometry; HPLC, high-performance liquid chromatography; LC–MS–MS, liquid chromatography and tandem mass spectrometry; MEKC, micellar electrokinetic chromatography; UV, ultraviolet

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1. Introduction

Gardenia is a popular ornamental shrub found worldwide. The fruits of *Gardenia jasminoides* Ellis (Rubiaceae) (Chinese herbal name is Zhi Zi) has been used for the remedy for hepatic pain due to cirrhosis, abdominal pain due to dysentery, anti-phlogistics, diuretic, laxative, choleretic, and homeostatic purposes in the treatment of trauma by external application [1]. In the theory of traditional Chinese medicine, Gardenia is belong to bitter and cold and enters the heart, lung and triple burner meridians [2]. Recent reports indicate that Gardenia herb also has mild antiangiogenic properties [3] and sedative effect [4].

The major constituents of Gardenia fruits are iridoid glycosides such as geniposide, gardenoside, gardoside, shanzhiside, scandoside methyl ester, methyl deacetyl asperulosidate geniposidic acid, 1-*O*-acetylgeniposide and genipin-1-β-gentiobioside. The chemical formulae of these major ingredients are list in Table 1. Geniposide is a major iridoid component, which has been recognized to have choleretic effects [5]. Some of the iridoid constituents are produced through enzymatic formation (Fig. 1). When the fruit of Gardenia was given orally, genipin may be produced from geniposide by human intestinal bacteria [6]. However, genipin was advised a genotoxic risk [7].

Table 1
Chemical formula of major ingredients in Gardenia herbs

Compound	Chemical formula
Genipin gentiobioside	$C_{17}H_{24}O_{10}$
Geniposide	$C_{17}H_{24}O_{11}$
Gardenoside	$C_{17}H_{24}O_{10}$
Gardoside	$C_{16}H_{24}O_{11}$
Shanziside	$C_{17}H_{24}O_{11}$
Scandoside methyl ester	$C_{17}H_{24}O_{11}$
Methyl deacetylaspelurosidate	$C_{16}H_{22}O_{10}$
Geniposidic acid	$C_{19}H_{26}O_{11}$
Acetylgeniposide	$C_{23}H_{34}O_{15}$

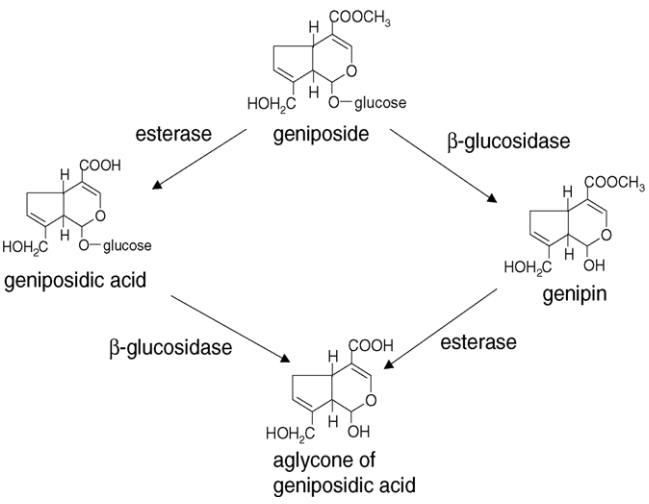

Fig. 1. Enzymatic reactions of geniposide.

G. jasminoides is also used for the source of blue pigment in the application of food colorants in which the fruit ingredient geniposide is hydrolyzed by β-glucosidase to produce genipin. Then, genipin react with amino acids to generate blue pigment [8].

Several reports warn the herbal medicine should be carefully used which may be a double-edge sword for the health care. To avoid the side effect of herbal remedy, additional assay should be required.

2. Isolation and purification

This section reviews the preparative scale methods to isolate and purify novel ingredients from the following Gardenia herbs, Gardenia Fructus (*G. jasminoides* Ellis fruits) and *G. jasminoides* cv. *Fortuneana* Hara (Table 2). Isolation of *Gardenia sootepensis* Hutch and aroma precursor from flower buds of *G. jasminoides* will also be reviewed. In addition, the development of industrial scale two-phase extraction processes to purify geniposide from herb medicine powders was included.

2.1. Preparative scale isolation and purification using extraction and subsequent chromatographic separations

Kikuchi's group at Tohoku Pharmaceutical University has reported the protocols to isolate nine new monoterpenoids from Gardenia Fructus [9], along with a known safranol-type monoterpene. These nine new monoterpenoids are as follows: gardenamide A, 6α-*n*-butoxygeniposide, 6β-*n*-butoxygeniposide, 6″-*O*-*p*-*cis*-coumaroylgenipin gentiobioside, jasminoside A, jasminoside B pentaacetate, jasminoside C, jasminoside D, and jasminoside E tetraacetate. The isolation procedures started from extracting *G. jasminoides* Ellis fruits with methanol. The concentrated residue of this extract, which was suspended in water, were successively extracted with several solvents, such as chloroform, diethyl ether, ethyl acetate, *n*-butanol, and water. Chloroform soluble fraction was chromatographed into several fractions using silica-gel column and one of the fractions was separated with C_{18} open column. Gardenamide A was found in one of the fractions collected from C_{18} open column elutes, having been purified with preparative HPLC. The other eight ingredients were in *n*-butanol soluble fraction. The isolation steps of 6α-*n*-butoxygeniposide, 6β-*n*-butoxygeniposide, jasminoside C, jasminoside D, and jasminoside E tetraacetate, were similar to the previous ones, except, prior to C_{18} open column separation, one more step using hydroxylated-dextran coated Sephadex LH-20 column was performed to separate the chosen fraction from the elutes out off silica-gel column. These five compounds were obtained when the chosen fraction from C_{18} chromatography elute were subjected to preparative HPLC. Jasminoside E tetraacetate was converted to its acetate to allow identification. The rest of novel components, 6″-*O*-*p*-*cis*-coumaroylgenipin gentiobioside, jasminoside A,

Table 2
Protocols to isolate and purify Gardenia Fructus and *Gardenia jasminoides* cv. *Fortuneana* Hara

Herbs	Isolated and purified compounds	Extraction solvents	Chromatographic columns	Reference
Gardenia Fructus	Monoterpenoids	Methanol; chloroform; *n*-butanol	Silica-gel column LH-20 column (optional) C_{18} open column Preparative HPLC	[9]
Gardenia Fructus	Terpenoids	Methanol; chloroform	Silica-gel column LH-20 column (optional) C_{18} open column Preparative HPLC	[10]
Gardenia jasminoides cv. *Fortuneana* Hara	Iridoid glycosides	Methanol; chloroform; water	LH-20 absorption column Silica-gel column LH-20 column Preparative HPLC	[11]

and jasminoside B pentaacetate, were isolated via running another fraction, collected from gel-silica chromatography elute, through a preparative HPLC column. Unlike the other components purified with prep. HPLC, the crude jasminoside B pentaacetate was acetylated in puridine to accomplish its purification. Optical rotation data of the above compounds were obtained with CD polarimeter. Their molecular structures were elucidated based on the spectral data acquired with ^{1}H and ^{13}C NMR spectroscopy and fast atom bombardment (FAB) mass spectrometry.

Machida et al. [10], in the same group as the above, have used similar protocols to isolate and purify four new terpenoid compounds, gardenate A, 2-hydroxyl gardenamide A, (1*R*, 7*R*, 8*S*, 10*R*)-7,8,11-trihydroxyguai-4-en-3-one 8-*O*-β-D-glucopyranoside, and jasminoside F pentaacetate, from Gardenia Fructus. Gardenia fruits were firstly extracted by methanol and then chloroform, diethyl ether, ethyl acetate, *n*-butanol, and water. The concentrated residues were eluted through silica-gel column, C_{18} open column and preparative C_{18} HPLC column successively to isolate gardenate A and 2-hydroxyl gardenamide A. The other two compounds, (1*R*, 7*R*, 8*S*, 10*R*)-7,8,11-trihydroxyguai-4-en-3-one 8-*O*-β-D-glucopyranoside and jasminoside F pentaacetate, were isolated and purified via successive separation steps with silica-gel column, hydroxylated-dextran coated Sephadex LH-20 column, and preparative HPLC column. The chemical structures of the above compounds were deciphered using spectral chemical data acquired with polarimetry, NMR spectroscopy, and FAB mass spectrometry.

Machida et al. [11] also published isolation and purification methods to obtain two new iridoid glycosides, 7β,8β-epoxy-8-dihydrogeniposide and 8-epiapodantheroside from the leaves of *G. jasminoides* cv. *Fortuneana* Hara along with other six known components, monotropein methyl ester, gardenoside, deacetylasperulosidic acid methyl ester, sandoside methyl ester, geniposide, and ixoroside, and three artifacts. This plant does not bear any medicinal fruits and its leave components were reported for the first time. Plant leaves were firstly extracted with methanol. The concentrated extract residues were partitioned between chloroform and water. The aqueous fraction was reduced. The residue was passed through hydroxylated-dextran coated HP-20 column. The absorbed materials were eluted out with methanol and then concentrated. This residue was separated with silica-gel column into several fractions. One of them was re-chromatographed using hydroxylated-dextran coated Sephadex HP-20 column. One of the collected fractions from the eluent was subjected to preparative C_{18} HPLC. All components, including two new iridoid compounds and other known and artifact ingredients, were separated with preparative HPLC. The structures of all compounds were determined with polarimetry, NMR spectroscopy, and FAB mass spectrometry data.

Wang et al. have published their study to identify ten compounds from *G. sootepensis* Hutch [12]. These 10 compounds including D-mannitol, beta-sitosterol, deacetylasperulosidic acid methyl ester, geniposidic acid, geniposide, scandoside methyl ester, and quinide dimers were isolated from *G. sootepensis* fruits with organic solvent extraction and chromatographic separation steps similar to other papers in the above. Their structures were also determined with spectral chemical data.

To study antioxidant properties of crocins from *G. jasminoides* Ellis fruits, similar isolation protocols using organic solvent extraction followed with a series of chromatographic separations have been reported [13]. Gardenia fruits were extracted with acetone. The extracts were concentrated and then frozen at −40 °C under vacuum. This dried crude extract washed with ether, passed through a cation-exchange column, and immediately through an anion-exchange one. The extract was further purified by preparative C_{18} HPLC to obtain crocin derivatives. Absorbance detector at 440 nm was sit at the outlet of HPLC to record separation results.

To identify aroma precursors of linalool and borneol, 1-linalyl 6-*O*-α-L-arabinopyranosyl-β-D-glucopyranoside and bornyl 6-*O*-β-D-xylopyranosyl-β-D-glucopyranoside were isolated from flowers buds just harvested before the opening

Table 3
Assays using separation procedures to determine Gardenia ingredient and related compounds

Procedures	Herb materials	Determined compounds	References
C_{18} HPLC with absorbance detection	*Gardenia jasminoides* Ellis, *Gardenia jasminoides* Ellis var. *grandiflora* Nakai	Geniposide	[16]
C_{18} HPLC with absorbance detection		Geniposide (in mice blood)	[17]
C_{18} HPLC with photo-diode array	*Gardenia jasminoides* Ellis, *Gardenia jasminoides* Ellis var. *grandiflora* Nakai	Geniposide, genepin, gardenoside, geniposidic acid	[18]
C_{18} HPLC with absorbance detection	*Gardenia jasminoides* Ellis	Geniposidic acid, gentibioside, gardenoside, genipin, geniposide, chlorogenic acid, crocin, crocetin	[19]
C_{18} HPLC with absorbance detection	*Gardenia sootepensis*	Geniposidic acid, geniposide, scandoside methyl ether	[20]
Silica-gel HPLC with absorbance detection	*Gardenia jasminoides* Ellis	Geniposide, gardenoside, geniposide, and geniposide acid, genipin-1-β-gentiobioside	[21]
C_{18} HPLC with mass detection	*Gardenia jasminoides* Ellis	Linoleic acid–crocin adduct, monohydroperoxides, dihydroperoxides	[13]
C_{18} HPLC with mass detection		Geniposide	This paper
Micellar electrokinetic chromatography (MEKC) with absorbance detection	*Gardenia jasminoides* Ellis	Crocin, geniposide, gardenoside	[27]
Gas chromatography–mass spectrometry (GC–MS)	*Gardenia jasminoides* Ellis (flower)	Farnesene, *cis*-ocimene, linalool, *cis*-3-hexenyl tiglate, methyl benzoate	[29]
Chiral gas chromatography–mass spectrometry (GC–MS)	*Gardenia jasminoides* Ellis (flower bud)	(*R*)-Linalyl 6-*O*-α-L-arabinopyranosyl-β-D-glucopyranoside, bornyl 6-*O*-β-D-xylopyranosyl-β-D-glucopyranoside	[14]

[14]. Methanol was used to extract plucked flower buds under ice cooling. Subsequent chromatographic separations used Amberlite XAD-2 adsorption column, hydroxylated-dextran coated Sephadex HP-20 column, and preparative Develosil ODS-10 reversed phase HPLC column. The final purification to obtain these two precursors was achieved using preparative YMC-Pack ODS-AM reversed phase HPLC column. The structure determinations were deciphered using polarimetry, NMR spectroscopy, and FAB mass spectrometry data. The chirality determinations of these two precursors are discussed below in Section 3.

2.2. Large scale preparation using two-phase aqueous extraction

Exploratory industrial scale preparation of geniposide isolated from Gardenia fruit has been investigated Pan et al. [15]. Unlike conventional purification processes of natural products, which employ organic solvent extraction and many subsequent chromatographic separations, it has been reported by these researchers an aqueous two-phase extraction process combined with a simple ethanol treatment to eliminate excess inorganic salts to obtain geniposide from herb medicine powders. This extraction system was compromised of PE62, a random copolymer composed of 20% ethylene oxide and 80% propylene oxide, KH_2PO_4, and ethanol. The authors started with a small scale system (4.8 g powders in 100 ml water) to look for optimal extraction conditions. They investigated a variety of inorganic salts, such as $MgSO_4$, $(NH_4)_2SO_4$, NaH_2PO_4, Na_2HPO_4, $CaCl_2$, NaCl, sodium acetate, sodium propionate, and glycine to examine the partition in a system with PE62 and a salt. They also studied the influence of sample loading effect, salt and PE62 concentration effect, and the addition amount of ethanol. They finally used the system containing 5% PE62, 7.5% KH_2PO_4, and 10% ethanol, to perform large scale extraction (500 g herb powders in a 10 l reactor). A 39 g aliquot of final product (in powder form) with 77% purity of geniposide using this extraction process has been reported. The purification product quality was assured via injecting filtered sample solution into reverse phase HPLC. The solution was separated with gradient elution conditions using mobile phase solution composed of 10% methanol in acetonitrile and 0.1% phosphoric acid. UV absorbance detection at 260 nm was employed to acquire chromatograms.

3. Analytical procedures

This section reviews analytical scale liquid chromatography separation procedures to determine Gardenia herbal ingredients and their derivatives with UV–vis absorbance and mass spectrometry detection techniques. In addition, other analytical scales separation methods including thin layer chromatography and gas chromatography are also discussed in the below (Table 3).

3.1. HPLC separation with UV and visible absorbance detection

Reverse phase HPLC has been the most popular method to determine Gardenia constituents. In addition to the above examples using preparative HPLC with UV or visible absorbance to record chromatographic separation results [9–13], there have been other several methods developed to assay Gardenia herbs in high accuracy and precision. Tsai et al. [16] developed an assay using C_{18} reverse phase HPLC to determine the geniposide concentrations in *G. jasminoides* and two preparations of mixed Chinese medicines, Huang-Lian-Jiee-Dwu-Tang and In-Chern-Hau-Tang. The mobile phase system used in this assay was acetonitrile–methanol–5 mM monosodium phosphate (pH 4.6) (5:15:80, v/v/v). The absorbance detector at the HPLC outlet was set at 240 nm to acquire chromatograms. Intra- and inter-assay of the analytes were equal or less than 10% in the range 0.1–50 μg/ml. The presence of geniposide in the herb and its preparations was assured by retention time, spiking with an authentic standard, the consistent ratio of signal intensities at 240 and 255 nm found in the standard, herbs, and preparations samples, and almost the same retention time shift when mobile phase composition was varied consistently from the above condition to 5 mM monosodium phosphate–methanol (80:20, v/v). This report has concluded the concentration of geniposide in the fruit of *G. jasminoides* Ellis var. *grandiflora* Nakai is higher than that in *G. jasminoides* Ellis. The concentration of geniposide in the traditional Chinese herbal medicine preparations, Huang-Lian-Jiee-Dwu-Tang and In-Chern-Hau-Tang was less than in the herb *G. jasminoides* Ellis itself.

In order to determine the protein-unbound geniposide in blood of anesthetized rats, a microdialysis probe was inserted into jugular vein for free form geniposide sampling from blood. Geniposide was separated using a Nova-Pak reversed-phase column (RP-C_{18}, 150 mm × 3.9 mm i.d.; particle size, 5 μm, Waters, Milford, MA, USA) maintained at ambient temperature (24 ± 1 °C). The mobile phase was comprised of acetonitrile–methanol–5 mM monosodium phosphate (pH 4.6) (5:15:80, v/v/v), and the flow rate of the mobile phase was 1 ml/min. The maximum UV absorbance for geniposide was set at a wavelength of 240 nm (Fig. 2). Fig. 3A shows a chromatogram of a standard of geniposide (5 μg/ml). Fig. 3B shows a chromatogram of a drug-free blood extract illustrates a clean, stable baseline with no interfering endogenous peaks. Run-time was set at 10 min, and no carry-over peaks were detected in subsequent chromatograms of plasma samples. Fig. 3C shows the chromatogram of a blood dialysate sample containing geniposide (5.25 μg/ml) collected from rat blood 10 min after geniposide administration (10 mg/kg, i.v) [17].

Tsai et al. [18] have developed an assay to determine geniposide, genipin, gardenoside, and geniposidic acid from *G. jasminoides* Ellis and *G. jasminoides* Ellis var. *grandiflora* Nakai using HPLC separation detected by photo-diode arrays. An isocratic system consisting of a reverse phase phenyl

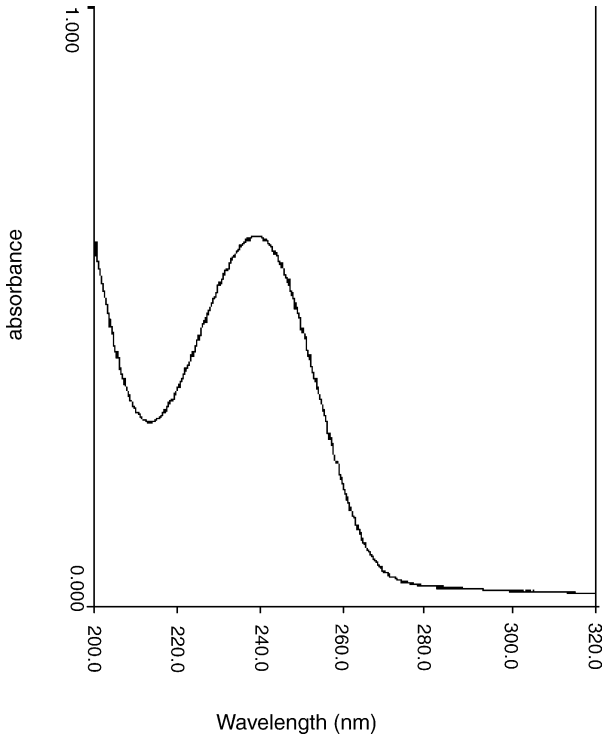

Fig. 2. The UV absorbance spectrum of geniposide. The absorbance maximum is at 240 nm.

column with a mobile phase of acetonitrile–water–perchloric acid (6:94:0.1, v/v/v, pH 4.0) was used to elute the active ingredients. Variations in extraction methods found that 0.1 M HCl is the best extraction solvent for geniposide and genipin. NaOH (0.1 M) for geniposidic acid and water for gardenoside. It was found that water extracts of *G. jasminoides* Ellis contained 56.03 ± 0.62, 1.72 ± 0.01, 2.16 ± 0.04, and 1.79 ± 0.01 mg/g of geniposide, genipin, gardenoside, and geniposidic acid, respectively. *G. jasminoides* Ellis var. *grandiflora* Nakai, however, contained 79.76 ± 0.62, 1.88 ± 0.04, 3.37 ± 0.21, and 6.38 ± 0.13 mg/g [18].

Xu et al. [19] have developed an assay using C_{18} HPLC method detected by UV and visible absorbance to determine nine compounds in Gardenia. Geniposidic acid, gentibioside, gardenoside, and genipin geniposide were determined at 240 nm. Chlorogenic acid was at 330 nm. The absorbance at 440 nm recorded the chromatographic peaks of crocin 1, crocin 2, crocin 3, and crocetin in this assay. The linear gradient mobile phase composition was methanol–acetonitrile (9:1) and 0.3% aqueous formic acid. Wang et al. have developed a reverse phase HPLC method to assay iridoid compounds in *G. sootepensis* [20]. The linear ranges of geniposidic acid, geniposide, and scandoside methyl ether determined using this method were 0.097–0.606, 0.0638–3.990, and 0.198–1.239 μg, respectively. The average recoveries of these three compounds were 100.0%, 99.9%, and 100.1%, and R.S.D. 1.23%, 1.38%, and 1.53% (n = 5), respectively.

In addition to reverse phase HPLC column, silica-gel column was also used to determine geniposide, gardenoside, and

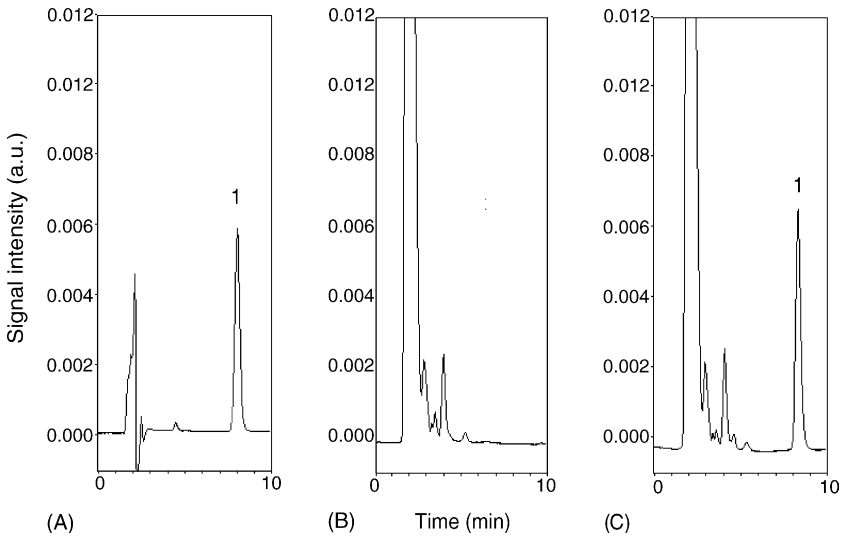

Fig. 3. The chromatogram of: (A) a standard of geniposide (5 μg/ml); (B) a drug-free blood extract; (C) a blood dialysate sample containing geniposide (5.25 μg/ml) collected from rat blood 10 min after geniposide administration (10 mg/kg, i.v.). These chromatograms were acquired with UV absorbance detector at 240 nm. 1: geniposide.

geniposidic acid, and genipin-1-β-gentiobioside in Gardenia Fructus [21]. Dry fruit powder (0.2 g) of *G. jasminoides* was extracted with water–methanol (1:1) under a hot water bath (85 °C). Diluted extracts with water and methanol were injected into silica-gel HPLC column to separate. Absorbance detection wavelength was 238 nm. Various parts Gardenia fruits, such as the whole fruits, peels, and seeds, collected from several Chinese provinces were assayed in this work.

3.2. HPLC separations with mass spectrometry detection

Electrospray ionization (ESI) mass spectrometry is a standard detection technique to record separation results of reverse phase liquid chromatography to assay natural products and drugs [22,23]. ESI is operated under atmospheric pressure, which provides operational convenience to couple with HPLC elution outlet without high vacuum interfaces. To achieve highly sensitive and selective mass spectrometric detection, typically molecular ion, generated in ESI chamber, and one of well-abundant fragment ions, produced in a cell between two mass analyzers via the collision, are selected to set up the molecular gating positions of the first and the second quadrupoles mass analyzer. The reaction takes place in the cell is collision between molecular ions and inert gases such as nitrogen or helium. This arrangement assures that only specific ion fragment generated from molecular ion will pass the gates. This highly selective detection tandem mass spectrometry (MS–MS) technique is known as selected ion monitoring, which provides pg sensitivity because of low level noises. Monitoring molecular ion or other specific ions, known as selected ion monitoring technique, with only one mass analyzer is often selective and sensitive enough to assay natural products in many applications.

However, the ionization of iridoid glycosides via electrospray is more complicated beyond its basic mechanism (Fig. 4) [24]. The *m*/*z* values of their molecular ions are solvent dependent. The fragmentation to lose glucose and other functionalities usually takes place as iridoid glycosides ionize. These findings clearly indicate an analytical challenging to develop LC–MS or LC–MS–MS assays to determine Gardenia extracts. It is, therefore, not surprising to find no such methods have been published.

We used liquid chromatography–electrospray mass spectrometry to verify the presence of geniposide in herb standards. LC–MS–MS analysis was performed using a Waters 2690 module with an automatic liquid chromatographic sampler and an autoinjection system connected to a Micromass Quattro Ultima tandem quadrupole mass spectrometry (Mi-

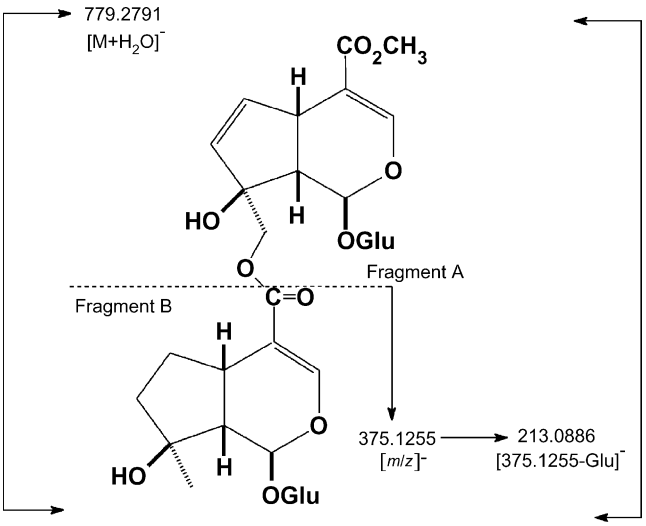

Fig. 4. The illustration of iridoid glycosides cleavage mechanisms[24].

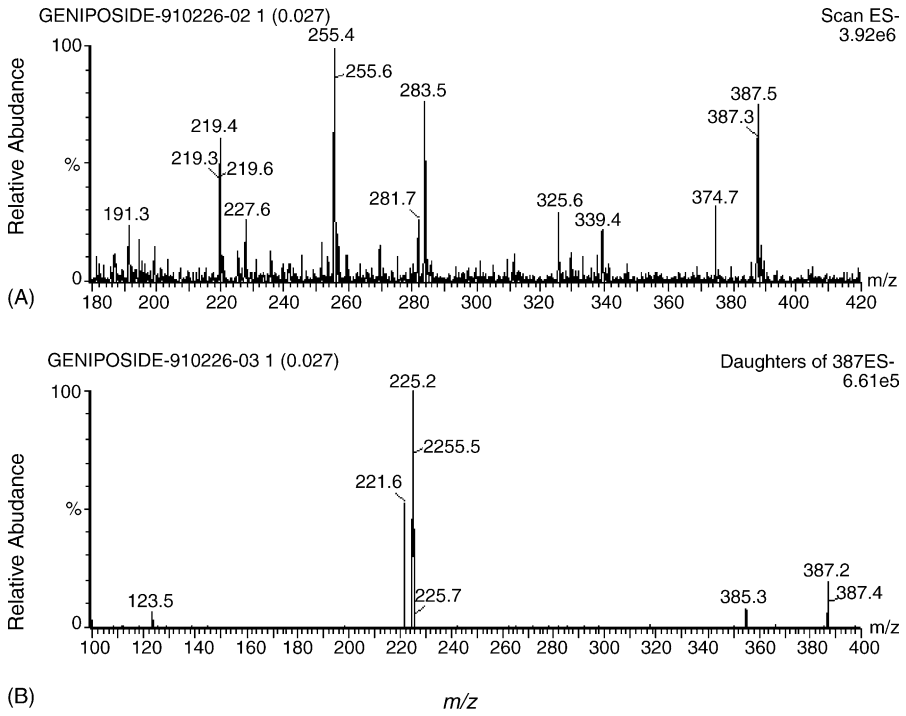

Fig. 5. (A) Negative electrospray spectrum of geniposide. Its molecular ion appears at 387 amu. (B) Mass spectrum of product ions generated from geniposide collision reactions. Molecular ions shown in the upper frame lost glucose functionality to form fragment ion at 225 amu.

cromass, Manchester, UK) equipped with one ESI source. The separation was achieved using a reversed-phase C_{18} column (150 mm × 4.6 mm i.d.) (Agilent, USA). The mobile phase consisted of methanol −20 mM ammonia solution (35:65, v/v) with a splitting flow-rate 0.2 ml/min. The volume of injection was 10 μl. For operation in MS–MS mode, a mass spectrometer with an orthogonal Z-spray electrospray interface was used. The infusion experiment was performed using a Mode 22 multiple syringe pump (Harvard, Holliston, MA, USA). During the analyses, the ESI parameters were set as follows: capillary voltage, 2.80 kV for negative ionization mode (ES−); source temperature, 80 °C; desolvation temperature, 300 °C; cone gas flow, 95 l/h; and desolvation gas flow, 440 l/h. The cone voltage and collision energy was 30 V and 8 eV, respectively. All LC–MS–MS data were processed by the MassLynx version 4.0 NT Quattro data acquisition software. Geniposide structure was derived using tandem mass techniques (Fig. 5). The product ion scan spectrum in the lower Fig. 5B shows ion fragment at 225 amu obtained from the collision reaction products of molecular ions (387 amu). This fragment was produced via the loss of glucose moiety, which is consistent with the typical fragmentation mechanisms of iridoid glycoside (Fig. 6). But, it is worth noting that in Fig. 5A, the negative molecular ion at 387 amu is hardly possible generated via the deprotonation of geniposide. The real ionization mechanism needs more investigation.

On the other hand, ESI mass spectrometry was employed to study antioxidant properties of crocin isolated from *G. jasminoides* Ellis [13]. The crocin oxidation reaction mixture consisted of 4 mg of crocin in 0.5 ml ethanol, 1.5 ml of 40 mM phosphate buffer (pH 6.0), and 20 μl of 20 mM $FeSO_4$, with or without 1.2–12 μl of linoleic acid. After a period of time, the incubated mixture at 37 °C in the dark was diluted with water and washed with 10 ml ether for three times to remove unreacted linoleic acid. The reaction mixture was then absorbed using C_{18} cartridge column and then eluted with methanol. The elute was evaporated under a nitrogen stream, lyophilized at −40 °C, and used for LC–MS determination. The C_{18} HPLC conditions were using methanol–water gradient elution. Negative mode electrospray was used to ionize adducts between linoleic acid and crocin, identified with ion traps mass analyzer. When crocin reacted with oxygen at the presence of $FeSO_4$, intermediates such as monohydroperoxides and dihydroperoxides of crocin were formed and detected with the other LC–MS method shown in Fig. 7. This method also employed C_{18} HPLC column. Its mobile phase was the mixture of acetonitrile–water (9:1) and 0.7% formic acid. Acetonitrile content was firstly set at 5% and gradually diminished to zero. Ionization of these intermediates was also via electrospray processes. These ions were recorded with quadrupole mass analyzer operated at either

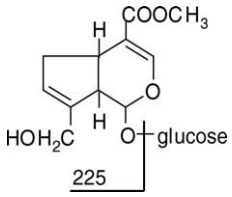

Fig. 6. The cleavage mechanism of geniposide.

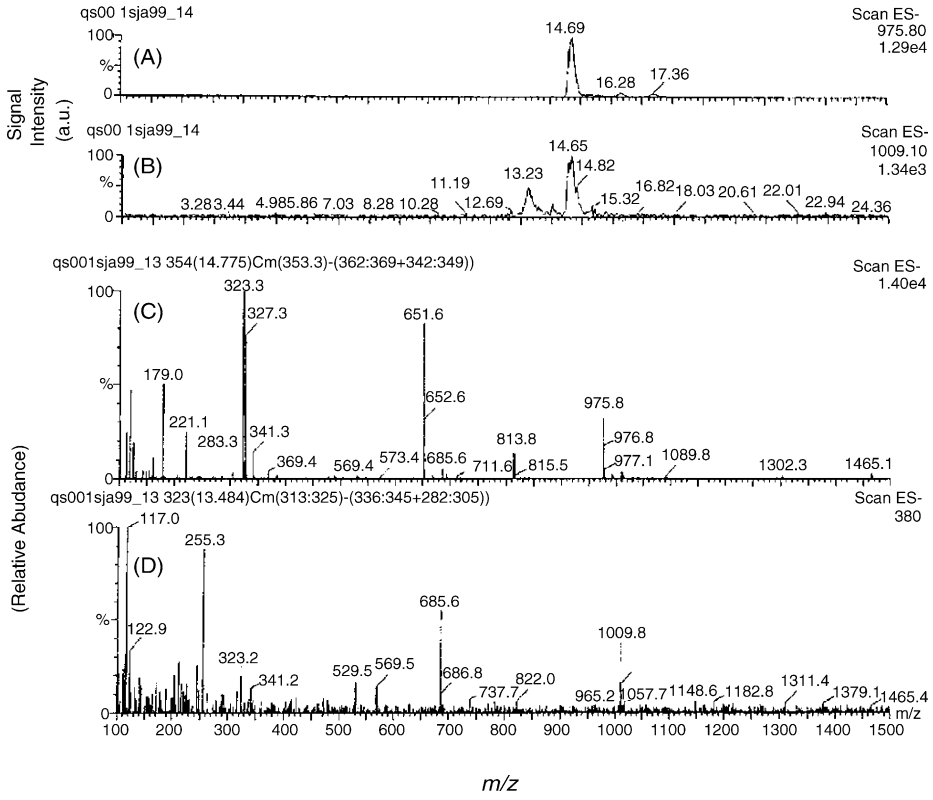

Fig. 7. LC–MS analysis of crocin oxidation intermediates at 37 °C at pH 6.0 after 3 days of incubation in the presence of $FeSO_4$: *m/z* 975 (A) monohydroperoxides and *m/z* 1009 (B) dihydroperoxides selected ion chromatograms; mass spectra at retention time of 14.775 min (C) and 13.484 min (D). The details of liquid chromatography elution gradients are in the content [13]. (Reprint permitted by American Chemical Society.)

full scan, selected ion monitoring, or selected reaction monitoring mode.

3.3. Thin layer chromatography methods

To develop the quality standards to assay Qingwei Huanglian Pills, Han et al. reported the thin layer chromatography (TLC) identification of *G. jasminoides*, *Scutellaria baicalensis* and *Anemarrhena asphodeloides* [25]. The quantitative method is simple, sensitive, reproducible and accurate. The recovery of berberine is 99.48%. The coefficient of variation is 1.42%.

3.4. Gas chromatography methods

Liu and Gao investigated the headspace constituents of fresh Gardenia flowers using gas chromatography–mass spectrometry (GC–MS) [26]. The headspace volatiles were trapped by solid-phase microextraction (SPME) and dynamic headspace sampling (DHS). SPME sampling was conducted with 100 μm PDMS fiber. In DHS sampling, purified nitrogen gas was purged through the trapping device. Mesh adsorbent was used and the volatiles were eluted by ether, and concentrated to 0.5 ml for GC/MS analysis. A wax-coated capillary column operated under programmed temperature gradient was employed in GC–MS determination. The authors published that the main compounds in headspace of fresh Gardenia flower included farnesene (64.86%), *cis*-ocimene (29.33%), linalool (2.74%), *cis*-3-hexenyl tiglate (1.34%), and methyl benzoate (0.25%). Comparison results obtained from SPME and DHS sampling indicated SPME afforded a simpler and more sensitive sampling method, while the direct determination of headspace provides more accurate information.

The chiralities of aroma precursors, 1-linalyl 6-*O*-α-L-arabinopyranosyl-β-D-glucopyranoside and bornyl 6-*O*-β-D-xylopyranosyl-β-D-glucopyranoside, isolated from flower buds of *G. jasminoides* were determined with GC–MS method [14]. The details isolation procedures are in Section 2. The isolated precursors and the crude enzyme preparation were mixed in pH 7.0 solution to incubate for days at the presence of sodium azide. Enzymatic hydrolysis took place over the incubation courses. The reaction mixture was added into ether solution of ethyl octonate prior to the injection into PEG-coated chiral capillary GC column. The gas chromatograms were recorded with mass spectrometric detection.

4. Electromigration procedures

This session reviews the procedures using electromigration mechanisms to separate and determine Gardenia

derivatives. Electromigration methods are also known as electrophoresis. Electrophoresis employs a capillary filled with elution buffers. The elution pumping is driven by external electric field. Separations of Gardenia derivatives using electrophoresis is performed adding surfactants into elution buffers to gauge the electromigration times of analytes due to the differential hydrophobic affinities between surfactants and analytes. The above method is called micellar electrokinetic chromatography (MEKC) which is a popular alternative method to HPLC. MEKC employs open capillaries filled with surfactant or polymer solutions. Low viscosity and versatile replacement of filling buffers provides operational convenience and flexibility to tailor separation performance [27,28]. Watanabe et al. [29] reported that MEKC with a surfactant (SDS) solution in a phosphate buffer at pH 7.0, containing acetonitrile successfully separated Gardenia yellow pigments, which mainly contain crocins. Separation results of the pigments using MEKC showed adequate reproducibility. No significant pattern difference appeared among 15 independent electrokinetic chromatograms acquired with UV–vis absorbance at 440 nm. Yellow pigments were extracted using solid-phase extraction (SPE) prior to MEKC separations. The MEKC separation results of Gardenia-derived pigments from extracted from dyed noodles and commercially available pigments are shown in Fig. 8. These separation procedures used capillaries (50 μm × 50 cm) filled with 20 mM SDS solution in 50 mM phosphate buffer at pH 7.0 containing 20% acetonitrile. The applied field strength was about 300 V/cm. These electropherograms were acquired with visible absorbance detection at 440 nm [29].

The authors also published the determination results using MEKC to separate geniposide and gardenoside detected at 440 nm [29]. These two ingredients were extracted from ground Gardenia fruits using distilled water. The buffer solutions of these MEKC separations were SDS solutions in borate buffer (pH 8.5). The contents of geniposide and gardenoside in Gardenia sample extracts from different places, including Korea, Taiwan, and several Chinese provinces, were determined.

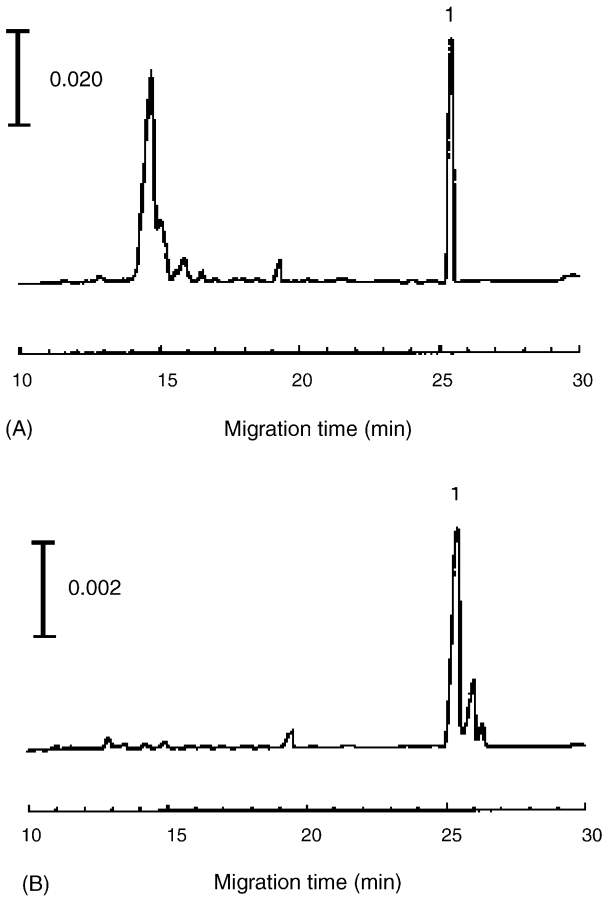

Fig. 8. Separation of yellow pigments extracted from commercial noodle (trace A) and commercial yellow Gardenia pigments (trace B) using micellar electrokinetic chromatography (MEKC) electromigration methods. The detail separation conditions are discussed in the content [29].

5. Conclusion

Organic solvent extractions followed with several subsequent liquid chromatographic separations have continued as the major isolation and purification protocols of Gardenia herbs medicines. Larger scale purification to meet the need of industrial applications using two-phase liquid–liquid extraction has been reported too.

HPLC assays have been the most popular methods to determine Gardenia herb ingredients. The detection schemes with HPLC include UV or visible absorbance detector at fixed wavelengths and photo-diode array to take absorbance spectrum. As for electrospray mass spectrometry, quantitative HPLC assay development is not straightforward. Semi-quantitative determination to verify the presence of geniposide in herb standards has been proved useful. Developed LC–MS assays have been published to study the anti-oxidation properties of crocins.

Alternative methods using micellar electrokinetic chromatography (MEKC) with optical absorbance detection have gained increasing attention to determine dye pigment components extracted from Gardenia materials. In addition, gas chromatography–mass spectrometry assays have also been used to determine the major constituents of Gardenia flower and the chiralities of aroma precursor identification in Gardenia flower buds.

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References

[1] W. Tang, G. Eisenbrand, Chinese Drug of Plant Origin, Springer–Verlag, Berlin, 1992, p. 539.
[2] Dictionary of Chinese Materia Medica (Zhong-Yao-Da-Ci-Dian), Juangsu New Medical College, Shanghai Scientific and Technological Publishers, 1985, pp. 1984–1987.
[3] E.H. Park, M.H. Joo, S.H. Kim, C.J. Lim, Phytother. Res. 17 (2003) 961.
[4] K. Ukita, T. Yamasaki, T. Ino, Y. Kawamoto, H. Saito, Jpn. Pharmacol. Ther. 22 (1994) 253.
[5] M. Harada, N. Tenmyo, M. Aburada, T. Endo, Yakugaku Zassi 94 (1974) 157.
[6] T. Akao, K. Kobayashi, M. Aburada, Biol. Pharm. Bull. 17 (1994) 1573.
[7] A. Ozaki, M. Kitano, N. Furusawa, H. Yamaguchi, K. Kuroda, G. Endo, Food Chem. Toxicol. 40 (2002) 1603.
[8] S.W. Lee, J.M. Lim, S.H. Bhoo, Y.S. Paik, T.R. Hahn, Anal. Chim. Acta 480 (2003) 267.
[9] K. Machida, R. Onodera, K. Furuta, M. Kikuchi, Chem. Pharm. Bull. 46 (1998) 1295.
[10] K. Machida, K. Oyama, M. Ishii, R. Kakuda, Y. Yaoita, M. Kikuchi, Chem. Pharm. Bull. 48 (2000) 746.
[11] K. Machida, E. Takehara, H. Kobayashi, M. Kikuchi, Chem. Pharm. Bull. 51 (2003) 1417.
[12] G. Wang, S. Zhao, D. Chen, Y. Lu, Q. Zheng, Zhongguo-Zhong-Yao-Za-Zhi 24 (1999) 38.
[13] T.Q. Pham, F. Cormier, E. Farnworth, V.H. Tong, M.R. Van Calsteren, J. Agric. Food Chem. 48 (2000) 1455.
[14] N. Watanabe, R. Nakajima, S. Watanabe, J.H. Moon, J. Inagaki, K. Sakata, A. Yagi, K. Ina, Phytochemistry 37 (1994) 457.
[15] I.H. Pan, H.H. Chiu, C.H. Lu, L.T. Lee, Y.K. Li, J. Chromatogr. A 977 (2002) 239.
[16] T.R. Tsai, T.Y. Tseng, C.F. Chen, T.H. Tsai, J. Chromatogr. A 961 (2002) 83.
[17] T.Y. Tseng, Pharmacokinetics of geniposide and its effect on the treatment of acupuncture as assessed by microdialysis, and its related herbal analysis. Master thesis. National Yang-Ming University, Taipei, Taiwan, 1999.
[18] T.H. Tsai, J. Westly, T.F. Lee, C.F. Chen, J. Liq. Chromatogr. 17 (1994) 2199.
[19] Y. Xu, J. Cao, Y.M. Wang, G.A. Luo, Yao-Xue-Xue-Bao 38 (2003) 543.
[20] G. Wang, S. Zhao, D. Chen, Zhongguo-Zhong-Yao-Za-Zhi 24 (1999) 616.
[21] T. Oshima, K. Sagara, T. Yoshida, Y.Y. Tong, G.D. Zhang, Y.H. Chen, J. Chromatogr. 455 (1988) 410.
[22] D.T. Rossi, M.W. Sinz, Mass Spectrometry in Drug Discovery, Marcel Dekker, New York, 2002.
[23] R. Willoughby, E. Sheehan, S. Mitrovich, A Global View of LC–MS, Global View Publishing, Pittsburg, 1998.
[24] L. Hamerskia, M. Furlana, D.H.S. Silvaa, A.J. Cavalheiroa, M.N. Eberlinb, D.M. Tomazelab, V.S. Bolzania, Phytochemistry 63 (2003) 397.
[25] G.R. Han, Y.D. Wang, L. Feng, Z.Q. Liu, H.B. Zhang, Zhongguo-Zhong-Yao-Za-Zhi 18 (1993) 93.
[26] B.Z. Liu, Y. Gao, Se-Pu 18 (2000) 452.
[27] J. Vindevogel, P. Sandra, Introduction to Micellar Electro-kinetic Chromatography, Huthig, Heidelberg, 1992.
[28] S. Terabe, N. Chen, K. Otsuka, Adv. Electrophor. 7 (1994) 87.
[29] T. Watanabe, A. Yamamoto, S. Nagai, S. Terabe, Food Sci. Technol. Int. Tokyo 4 (1998) 54;
T. Watanabe, S. Terabe, J. Chromatogr. A 880 (2000) 311.