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Identification and determination of geniposide contained in *Gardenia jasminoides* and in two preparations of mixed traditional Chinese medicines

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Abstract

A high-performance liquid chromatographic method was applied to the determination of the geniposide concentration in *Gardenia fruit* and preparations of traditional Chinese medicine using a mobile phase of acetonitrile–methanol–5 m*M* monosodium phosphate (pH 4.6) (5:15:80, v/v/v). Intra-assay and inter-assay accuracy and precision of the analyses were ≤10% in the range of 0.1 through 50 μg/ml. The presence of geniposide in the medicinal herb and its preparations was ascertained by retention time, spiking with an authentic standard, change of detection wavelength and change of the composition of the mobile phase. The concentration of geniposide in the fruit of *Gardenia jasminoides* Ellis var. *grandiflora* Nakai is higher than that in *Gardenia jasminoides* Ellis. The concentration of geniposide in the traditional Chinese herbal medicine preparations, Huang-Lian-Jiee-Dwu-Tang (66.27±1.98 mg/g) and In-Chern-Hau-Tang (68.54±2.62 mg/g) was less than in the herb *Gardenia jasminoides* Ellis (73.44±2.62 mg/g) itself.

Keywords: *Gardenia jasminoides*; *Gardenia fruit*; Pharmaceutical analysis; Geniposide; Glycosides; Iridoid glycosides

1. Introduction

Geniposide (Fig. 1) is one of the major iridoid glycosides in the fruit of *Gardenia jasminoides* Ellis (Rubiaceae) (Chinese herbal name: Zhi-Zi) and *Gardenia jasminoides* Ellis var. *grandiflora* Nakai (Chinese herbal name: Shui-Zhi-Zi) [1]. The crude extract of *Gardenia fruit* has been used in traditional Chinese medicine to treat irritability in febrile diseases, jaundice, acute conjunctivitis, epistaxis, hematemesis, hematuria, pyogenic infections and ulcers of the skin, and also, externally, sprains and painful swellings due to blood stasis [2]. Since any substance considered for its therapeutic value must be evaluated for consistency, a proper identification of the crude herbs is an obvious requirement. Concentrated pharmaceutical herbal products and the decoction herbal products have been widely adopted for clinical use in Taiwan, Japan, China, Korea and other Asian countries, and even in certain European and North American countries.

Herbal medicines offer an alternative to Western medicines and are often considered to be non-toxic

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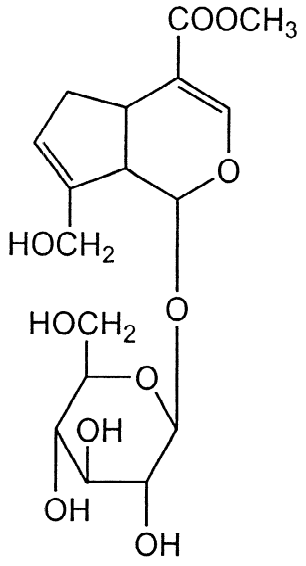

Fig. 1. Chemical structure of geniposide.

by the general public. A major criticism of herbal products is that they fail to meet modern quality assurance standards established for conventional medicines. Thus, quantitative determination of the active or marker components in medicinal herbs is definitely necessary for quality control. Several high-performance liquid chromatographic (HPLC) methods have been developed for the determination of geniposide in herbs using both a phenyl silica column [3] and a reversed-phase C_{18} column [4–7]. A capillary electrophoretic method was also used for the separation of geniposide from various iridoids [8]. The aim of this current study was to develop a simple, reliable routine analytical method for the quality control of herbal medicine preparations by determining the geniposide concentration in the fruit of *Gardenia jasminoides* Ellis and two traditional herbal preparations, Huang-Lian-Jiee-Dwu-Tang and In-Chern-Hau-Tang.

2. Experimental

2.1. Chemicals and reagents

Geniposide was isolated from the fruit of *Gardenia jasminoides* according to the method of Inouye et al. [1] and tentatively identified by ^{13}C-NMR spectroscopy (Bruker Model AC300 p, Karlsruhe, Germany) and UV spectrophotometry (Hitachi U-3200, Tokyo, Japan). Authentic geniposide was purchased from Nacalai Tesque (Kyoto, Japan). The fruit of both *Gardenia jasminoides* Ellis, *Gardenia jasminoides* Ellis var. *grandiflora* Nakai and other herbal drugs were purchased from traditional herbal drug stores in Taipei, Taiwan. HPLC grade solvents and reagents were obtained from E. Merck (Darmstadt, Germany). Triple deionized water (Millipore, Bedford, MA, USA) was used for all preparations. The formulation of the traditional Chinese herbal preparation Huang-Lian-Jiee-Dwu-Tang contained the herbs of *Coptidis rhizoma* (6 g), *Scutellariae radix* (6 g), *Phellodenri cortex* (6 g) and *Gardeniae fructus* (6 g), while In-Chern-Hau-Tang contained the herbs of *Artemisiae capillaris* (12 g), *Gardeniae fructus* (8 g) and *Rhei rhizoma* (4 g).

2.2. Chromatography

The HPLC system consisted of a chromatographic pump (BAS PM-80, Bioanalytical System, West Lafayette, IN, USA), an injector (Rheodyne 9125, Cotati, CA, USA) equipped with a 20 μl sample loop and a Dynamax UV–Vis detector (Varian, Walnut Creek, CA, USA). Geniposide was separated using a Nova-Pak reversed-phase column (RP-C_{18}, 150×3.9 mm I.D.; particle size 4 μm, Waters, Milford, MA, USA) maintained at ambient temperature (24±1 °C). The mobile phase was comprised of acetonitrile–methanol–5 m*M* monosodium phosphate (pH 4.6) (5:15:80, v/v/v), its flow-rate was 1 ml/min. The mobile phase was filtered through a Millipore 0.45 μm filter and degassed prior to use. The detection wavelength was set at 240 nm. The output signal of the detector was recorded using an EZChrom chromatographic data system (Scientific Software, San Ramon, CA, USA).

2.3. Method validation

All calibration curves (peak-areas vs. concentrations) of geniposide (external standards method) were made prior to the experiments with correlation values of at least 0.995. The intra-day and inter-day variabilities for geniposide were assayed (six replicates) at concentrations of 0.1, 0.5, 1, 5, 10 and 50

μg/ml on the same day and on six sequential days, respectively. The accuracy (% bias) was calculated from the nominal concentration (C_{nom}) and the mean value of the observed concentration (C_{obs}) as follows: bias (%) = $[(C_{obs} - C_{nom})/(C_{nom})] \cdot 100$. The relative standard deviation (RSD) was calculated from the observed concentrations as follows: % RSD = [standard deviation (SD)/C_{obs}] · 100.

2.4. Standard decoction

Powders (1 g) of *Gardenia jasminoides* Ellis, *Gardenia jasminoides* Ellis var. *grandiflora* Nakai, Huang-Lian-Jiee-Dwu-Tang and In-Chern-Hau-Tang were individually boiled with 50 ml of water in two batches, one for 5 and one for 15 min. These filtrates were diluted to a total final volume of 50 ml.

2.5. Recovery

An appropriate amount of *Gardenia jasminoides* Ellis to be extracted was divided into four portions (one as control group) and each portion (except the control group) was spiked with standard geniposide at three concentrations (1, 2 and 4 μg/ml). All samples were filtered through a 0.45 μm Millipore filter and assayed by HPLC to calculate recoveries.

3. Results and discussion

The modified isocratic reversed-phase HPLC method [3] was more efficient for the determination of geniposide than a gradient HPLC system [4,6,7] and several solvent systems [5]. Fig. 2A shows the typical authentic chromatogram of geniposide (10 μg/ml), Fig. 2B and C show the chromatograms of the crude extract of *Gardenia jasminoides* Ellis and *Gardenia jasminoides* Ellis var. *grandiflora* Nakai, respectively. The retention time of geniposide (5.8 min) was consistent in the authentic standard and the herbal preparations.

The presence of geniposide in the crude extract of herbal medicines was identified by spiking the crude herbal extract with authentic geniposide. The absorbance ratio of geniposide at 240 and 255 nm was 1.7, consistent with the geniposide peak area ratio which was repeated both at 240 nm and 255 nm,

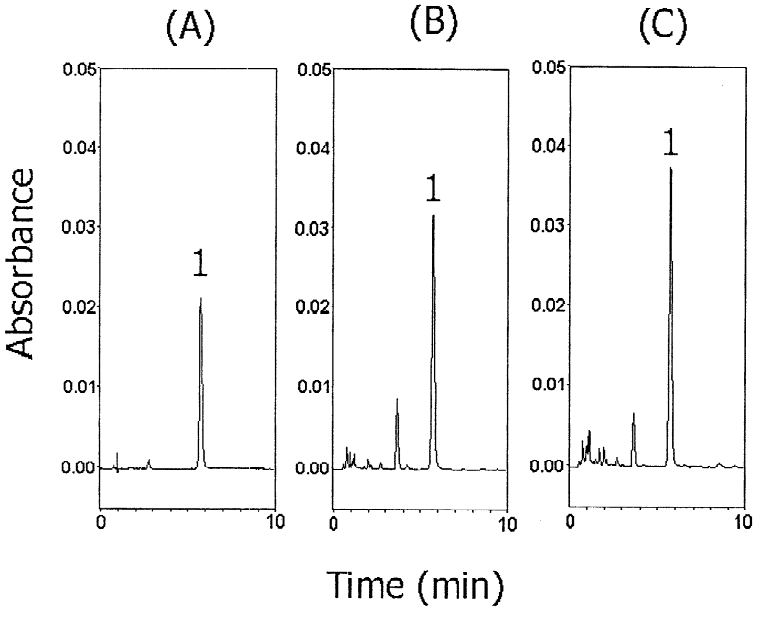

Fig. 2. Typical chromatograms of authentic geniposide and the crude extract of *Gardenia fruit*, recorded at 240 nm. (A) Authentic geniposide (10 μg/ml). (B) Crude extract of *Gardenia jasminoides* Ellis containing 14.7 μg/ml geniposide. (C) Crude extract of *Gardenia jasminoides* Ellis var. *grandiflora* Nakai containing 17.3 μg/ml geniposide. 1: geniposide.

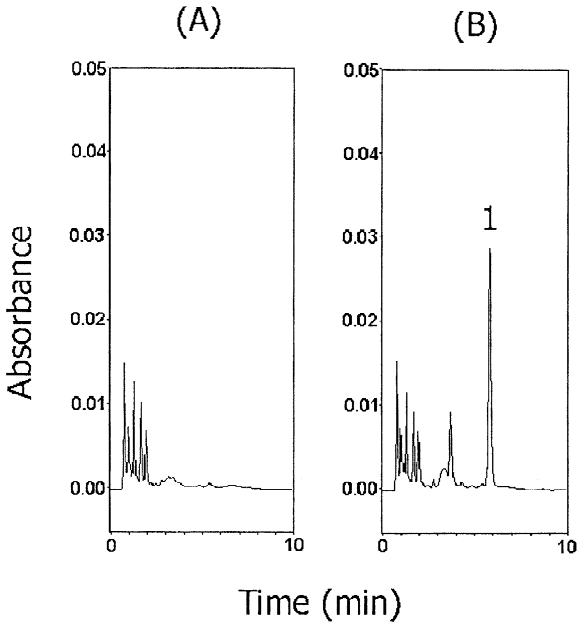

Fig. 3. Chromatogram of a blank In-Chern-Hau-Tang sample free of *Gardenia jasminoides* Ellis (A) and standard decoction of In-Chern-Hau-Tang (B) monitored at 240 nm. 1: geniposide.

respectively. If any ingredient interfered with the peak of geniposide in various *Gardenia fruit* samples, the peak area ratio at 240 nm and 255 nm would be altered.

The retention time of geniposide varied from 5.8 min to 8.5 min as the composition of the mobile phase was changed from 5 m*M* NaH_2PO_4–methanol–acetonitrile (80:15:5, v/v/v) to 5 m*M* NaH_2PO_4–methanol (80:20,v/v). No other plant ingredient interfered significantly with geniposide as the composition of the mobile phase was changed.

Intra-assay and inter-assay accuracy data for geniposide are listed in Table 1: all % bias and RSD values were within ±9%. The detection limit for geniposide (at a signal-to-noise ratio of 4) is 50 ng/ml. The average recovery of geniposide is 97.46±1.5%, as shown in Table 2: the method is sufficiently sensitive to allow measurement of geniposide in medicinal herbs and their preparations.

Traditional Chinese medicines are usually prepared by boiling in water. The boiling time may affect the concentration of geniposide in the crude extract of the herbal medicine. In order to improve

Table 1
Intra-day and inter-day accuracy and precision data for the HPLC method for the determination of geniposide

Nominal concentration (μg/ml)	Observed concentration (μg/ml)[a]	RSD (%)	Accuracy (% bias)
Intra-assay (n=6)			
0.10	0.093±0.005	5.4	−7.0
0.50	0.492±0.008	1.6	−1.6
1.00	0.995±0.005	0.5	−0.5
5.00	4.993±0.021	4.2	−0.1
10.00	10.015±0.029	0.3	0.2
50.00	50.023±0.084	0.2	0.1
Inter-assay (n=6)			
0.10	0.095±0.008	8.4	−5.0
0.50	0.498±0.008	1.6	−0.4
1.00	0.988±0.008	0.8	−1.2
5.00	5.007±0.015	0.3	0.1
10.00	9.995±0.049	0.5	0.05
50.00	50.032±0.064	0.1	0.1

[a] Observed concentration data are expressed as mean±SD, $n=6$.

the extraction rate of geniposide from *Gardenia jasminoides* Ellis and *Gardenia jasminoides* Ellis var. *grandiflora* Nakai, two decoction boiling times (5 and 15 min) were examined. The geniposide content of *Gardenia jasminoides* Ellis var. *grandiflora* Nakai was significantly higher than that in *Gardenia jasminoides* Ellis (Table 3), but boiling time made no difference.

In addition to single herb use, the mixed Chinese herbal preparations, Huang-Lian-Jiee-Dwu-Tang and In-Chern-Hau-Tang are also popular in the Orient. To test the specificity and selectivity of the proposed analytical method, a blank sample of In-Chern-Hau-

Table 2
Recovery (%) of geniposide in spiked *Gardenia jasminoides* Ellis (geniposide concentration 1.43 μg/ml)

Added geniposide (μg/ml)	Measured concentration (μg/ml)	Recovery (%)
1	2.41±0.03	98.26±2.6
2	3.35±0.05	95.71±2.6
4	5.37±0.16	98.42±4.2

Data expressed as mean±SD, $n=3$. Recovery=[(measured concentration−geniposide concentration)/added geniposide]×100.

Table 3
Effect of boiling time on the geniposide concentration (mg/g) in the *Gardenia fruit* extract

Boiling time (min)	Geniposide concentration (mg/g)	
	Gardenia jasminoides Ellis	*Gardenia jasminoides* Ellis var. *grandiflora* Nakai
5	73.44±2.62	87.87±1.72[a]
15	72.68±1.36	85.27±1.15[a]

Data expressed mean±SD, $n=5$.
[a] Significant difference ($P<0.05$ by Student's *t*-test) compared with the group of *Gardenia jasminoides* Ellis.

Tang and Huang-Lian-Jiee-Dwu-Tang, free of *Gardenia jasminoides* Ellis was examined for interference (Figs. 3A and 4A). No peak was found at the retention time of geniposide, compared to the chromatograms of regular samples (Figs. 3B and 4B). The geniposide concentrations of the single herb *Gardenia jasminoides* Ellis (73.44±2.62 mg/g) and herbal preparations In-Chern-Hau-Tang (68.54±6.62 mg/g) and Huang-Lian-Jiee-Dwu-Tang (66.27±1.98 mg/g) were significantly different.

The most common processing methods for traditional Chinese herbal medicines include: baking, decoction, fermenting, frying, lime processing, roasting, rolling, scraping, simmering, smoking and soaking. These processes are used in order to: change quality, decrease particle size, desiccate particles, enhance solubility, facilitate absorption, impart flavor and color, increase pharmacological action, reduce toxic side-effects, remove impurities, remove odors. In order to investigate whether the time of baking affected the concentration of geniposide in *Gardenia jasminoides* Ellis, baking times of 0 to 20 min were tested (Table 4).

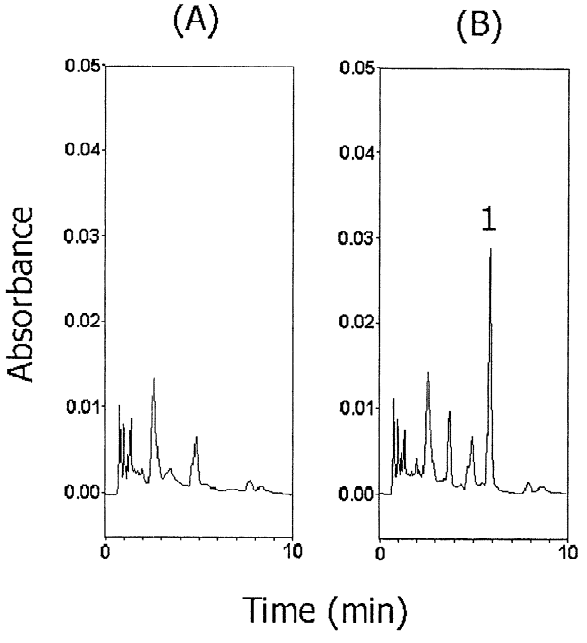

Fig. 4. Chromatogram of marker substance geniposide, that was separated from the traditional herbal preparation, a blank Huang-Lian-Jiee-Dwu-Tang sample free of *Gardenia jasminoides* Ellis (A) and standard decoction of Huang-Lian-Jiee-Dwu-Tang (B) monitored at 240 nm. 1: geniposide.

In summary, a low-cost HPLC method has been developed for the measurement of geniposide concentration in various species of *Gardenia fruit*, and the traditional herbal preparations, Huang-Lian-Jiee-Dwu-Tang and In-Chern-Hau-Tang. Geniposide remained stable after boiling for 15 min, but decomposed after 15 min of baking. This paper extends the list of marker compounds analyzed in botanical products, such as hypericin in St. John's wort [9–12], ginkgoterpenoids in *Ginkgo biloba* [13–15] and ginsenosides in ginseng, ginkgoflavone glycosides [16,17].

Table 4
Effect of baking time on the geniposide concentration *Gardenia jasminoides* Ellis

Baking time (min)	Geniposide concentration (mg/g)
0	73.44±2.62
10	72.18±1.22
15	67.37±0.84[a]
20	52.59±1.56[a]

Data expressed mean±SD, $n=6$.
[a] Significant difference ($P<0.05$ by Student's *t*-test) compared with the control group, in which the herb was not baked.

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