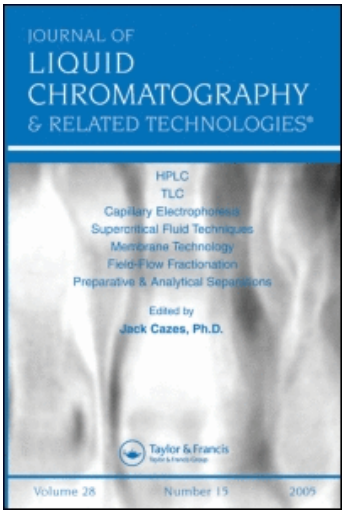

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Identification and Determination of Geniposide, Genipin, Gardenoside, and Geniposidic Acid from Herbs by HPLC/Photodiode-Array Detection

T. -H. Tsm[ab]; J. Westly[c]; T. -F. Lee[c]; C. -F. Chen[ab]

[a] National Research Institute of Chinese Medicine, Taipei, Taiwan [b] Institute of Pharmacology, National Yang-Ming Medical College, Taipei, Taiwan [c] Department of Zoology, University of Alberta Edmonton, Alberta, Canada

To cite this Article Tsm, T. -H. , Westly, J. , Lee, T. -F. and Chen, C. -F.(1994) 'Identification and Determination of Geniposide, Genipin, Gardenoside, and Geniposidic Acid from Herbs by HPLC/Photodiode-Array Detection', Journal of Liquid Chromatography & Related Technologies, 17: 10, 2199 — 2205

To link to this Article: DOI: 10.1080/10826079408013541

URL: http://dx.doi.org/10.1080/10826079408013541

JOURNAL OF LIQUID CHROMATOGRAPHY, 17(10), 2199-2205 (1994)

IDENTIFICATION AND DETERMINATION OF GENIPOSIDE, GENIPIN, GARDENOSIDE, AND GENIPOSIDIC ACID FROM HERBS BY HPLC/PHOTODIODE-ARRAY DETECTION

TUNG-HU TSAI[1,2], JEFFREY WESTLY[3], TZE-FUN LEE[3], AND CHICH-FU CHEN[1,2]

[1]*National Research Institute of Chinese Medicine*
Taipei, Taiwan
[2]*Institute of Pharmacology*
National Yang-Ming Medical College
Taipei, Taiwan
[3]*Department of Zoology*
University of Alberta
Edmonton, Alberta, Canada

ABSTRACT

An improved high-performance liquid chromatographic technique with photodiode-array detection was developed for the identification and determination of the active components geniposide, genipin, gardenoside and geniposidic acid from *Gardenia jasminoides* Ellis and *Gardenia jasminoides* Ellis var. *grandiflora* Nakai. An isocratic system consisting of a reverse-phase phenyl 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, 0.1 M NaOH for geniposidic acid and water for gardenoside. It was found that water extracts of *Gardenia 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. *Gardenia 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.

Correspondence to: C.F. Chen, Institute of Pharmacology, National Yang-Ming Medical College, Taipei 112, Taiwan.

INTRODUCTION

The fruit of both *Gardenia jasminoides* Ellis (Chinese name: Zhi-Zi; ZZ) and *Gardenia jasminoides* Ellis var. *grandiflora* Nakai (Chinese name: Shui-Zhi; SZ) have been used in the traditional Chinese medicine for the treatment of various inflammatory and hepatic-disease [1]. Kimura et al. [2] reported that geniposide, an iridoid glycoside from the fruit of both ZZ and SZ, reduced serum triglyceride, lipid peroxide and phospholipid in rats fed a high sugar diet. The suppressive effect of geniposide on the hepatotoxicity, hepatic DNA binding of aflatoxin B1 in rat [3] and in C3H10$T_{1/2}$ cells [4] have been examined. The crude extracts of ZZ and SZ [5-8] or purified geniposide [9] may also facilitate biliary extraction for treatment of icterus. As both species of *Gardenia jasminoides* are available in the herbal market, it is important to quantitate variations between these herbs. We developed a simple, rapid and sensitive method to identify and to quantify the contents of its major components, geniposide, genipin, gardenoside and geniposidic acid. The variations resulted by different extraction techniques were also examined in the present study.

MATERIAL AND METHOD

Reagent

ZZ and SZ were purchased from a traditional Chinese herbal drug store in Taipei. Authentic gardenoside, geniposide, genipin, and geniposidic acid were obtained from Nacalai Tesque (Kyoto, Japan) and acetonitrile, methanol, n-hexane, ethanol (99.5 %), ammonia solution (32 %) and perchloric acid (70 %) from E. Merck (Darmstadt, Germany).

Apparatus

The HPLC system consisted of an injector (Rheodyne 7125, Cotati, CA, USA) , a Waters Model 990 photodiode-array detector (Milford, MA, USA) and a chromatographic pump (Waters Model 510). Separation was achieved on a Nova-Pak reversed-phase phenyl column (Waters, 150 x 3.9 mm, particle size 5 μm) at room temperature. The mobile phase was acetonitrile-water-perchloric acid (6:94:0.1, v/v/v, pH 4, adjusted by ammonia solution) at a flow rate of 1.0 ml/min. The detection wavelength was 238 nm.

Extraction

ZZ or SZ powder (0.5 g) was boiled for 15 minutes with 50 ml of one of the following extraction solvents: water, methanol, 50 % ethanol, 0.1 M HCl, or 0.1 M NaOH. This procedure was repeated twice and the two filtrates were combined and diluted to a final volume of 100 ml.

Authenticity of samples

The compounds separated by the proposed HPLC method were identified by comparing their retention times and spectra with those of authentic samples of geniposide, genipin, gardenoside and geniposidic acid.

Determination of geniposide, genipin, gardenoside and geniposidic acid

Calibration curves for geniposide, genipin, gardenoside and geniposidic acid in methanol were constructed with various concentration of these compounds (0.1, 0.2, 0.5, 1 and 2 μg). The contents of these components in the crude extract of ZZ and SZ was determined from a regression equation for the area under the curve verses concentration of these four components.

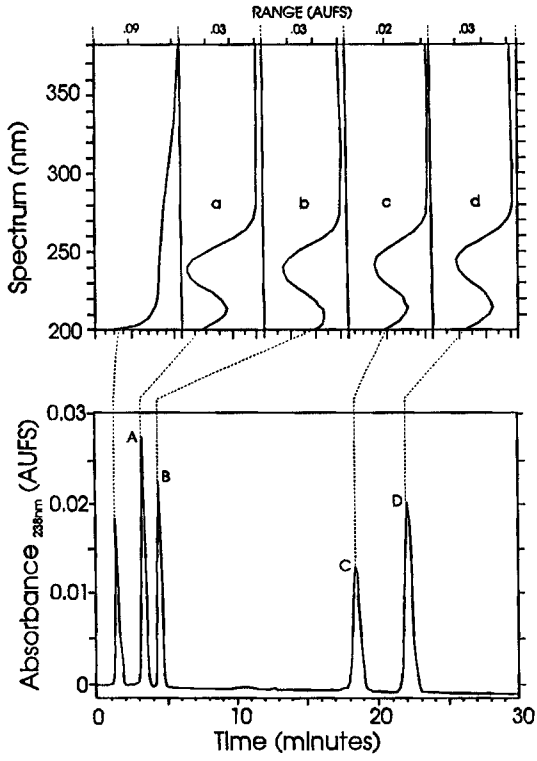

Fig. 1. Chromatogram and UV spectra of authentic compound. A: geniposidic acid; B: gardenoside; C: geniposide; D: genipin.

Statistics

ANOVA with post hoc analysis was used to compare variations between ZZ and SZ and those resulted by different extraction methods.

RESULTS AND DISCUSSION

Fig. 1 shows the chromatogram and UV spectra of authentic geniposide, genipin, gardenoside and geniposidic acid. The peaks corresponding to these four compounds

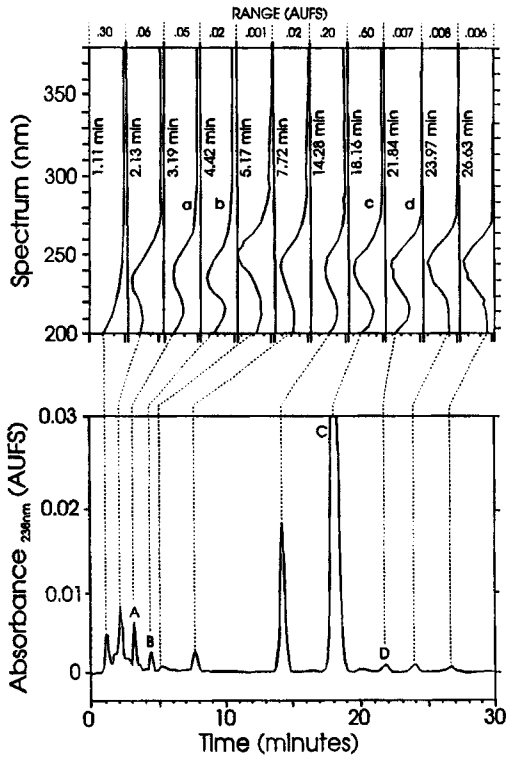

Fig. 2. Chromatogram and UV spectra of water extract of *Gardenia jasminoides* Ellis. A: geniposidic acid; B:gardenoside; C: geniposide; D: genipin.

were confirmed by the retention times and the UV spectra obtained with photodiode-array detection. The retention times of geniposide, genipin, gardenoside and geniposidic acid were found to be 3.5, 4.7, 18.3 and 22.1 min, respectively. The spectra's characteristics suggested an absorption maxima at 238 nm for geniposide, gardenoside and geniposidic acid, and 243 nm for genipin in this mobile phase.

The equations of the calibration curve for geniposide, genipin, gardenoside and geniposidic acid were $y = 0.0232x + 0.0147$ ($r^2 = 0.998$), $y = 0.0158x - 0.0012$ ($r^2 = 0.999$), $y = 0.0143x + 0.0033$ ($r^2 = 0.999$) and $y = 0.0179x + 0.0004$ ($r^2 = 0.999$)

TABLE I

Contents of geniposide, genipin, gardenoside and geniposidic acid in different extracts of *Gardenia jasminoides* Ellis (ZZ) and *Gardenia jasminoides* Ellis var. *grandiflora* Nakai (SZ).

extract solution		geniposide	genipin	gardenoside	geniposidic acid
Water	ZZ	56.03±0.62	1.72±0.01	2.16±0.04	1.79±0.01
	SZ	79.76±1.60[a]	1.88±0.04[a]	3.37±0.21[a]	6.38±0.13[a]
Methanol	ZZ	48.52±0.77	n.d.	n.d.	0.63±0.16
	SZ	76.49±1.90[a]	n.d.	n.d.	3.98±0.40[a]
50% Ethanol	ZZ	54.74±0.98	1.39±0.16	1.22±0.04	0.79±0.01
	SZ	80.01±2.52[a]	2.63±0.07[a]	1.41±0.05[a]	4.11±0.14[a]
0.1 M HCl	ZZ	56.76±1.06	3.64±0.04	0.62±0.03	1.76±0.06
	SZ	82.80±1.88[a]	3.62±0.21[a]	0.91±0.05[a]	8.34±0.27[a]
0.1 M NaOH	ZZ	n.d.	n.d.	n.d.	6.74±0.26
	SZ	n.d.	n.d.	n.d.	9.17±0.45[a]

[a]Significantly different ($p < 0.05$) from ZZ.
Data are expressed as mean±SD (mg/g, n=4).
n.d.: not detectable

respectively, where x is the amount of compound analyzed and y is response in peak area. The detection limits for the four compounds, at a signal-to-noise ratio of 4, were 1 ng for geniposide, gardenoside and geniposidic acid, and 3 ng for genipin.

Fig. 2 shows the chromatogram and UV spectra of the water extract of ZZ. The peak corresponding to geniposide, genipin, gardenoside and geniposidic acid were confirmed by both the retention time and the UV spectra. Table I summarizes the contents of geniposide, genipin, gardenoside and geniposidic acid in ZZ and SZ obtained from the different solvents. Significant difference was observed between the two

variations of *Gardenia jasminoides* with SZ being consistently higher in content of these components. The method of extraction also gave very significant differences in quantity of component extracted. Highest yield of geniposide and genipin was obtained with 0.1 M HCl extraction, whereas highest yields of geniposideic acid, gardenoside were obtained from 0.1 M NaOH and water extraction, respectively.

In conclusion, the proposed technique should be useful to quantitate the content of quality of either ZZ or SZ.

REFERENCES

1. Juangsu New Medical College, Zhong Yao Da Ci Dian (Dictionary of Chinese Materia Medical), Shanghai Scientific and Technological Publishers, Shanghai, 1985.

2. Y. Kimura, H. Okuda and S. Arichi, Chem. Pharm. Bull., 30: 4444-4447(1982)

3. C.J. Wang, S.W. Wang and J.K. Lin, Cancer Lett., 60: 95-102(1991)

4. T.H. Tseng, C.Y. Chu and C.J. Wang, Cancer Lett., 62: 233-242(1992)

5. T. Miwa, Jap. J. Pharmacol., 2: 102-108(1953)

6. T. Miwa, Jap. J. Pharmacol., 2: 139-143(1953)

7. T. Miwa, Jap. J. Pharmacol., 3: 1-5(1953)

8. T. Miwa, Jap. J. Pharmacol., 4: 69-81(1954)

9. F.T.K. Lau and R.C.K. Pak, Asia Pacific J. Pharmacol. 1: 91-98(1986)

Received: September 25, 1993
Accepted: November 2, 1993