ISSN : 0974-7419 Volume 11 Issue 4

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 11(4) 2012 [162-168]

Fingerprint chromatogram analysis of methanol extracts of *Gardenia jasminoides* and *Arctium lappa* by high performance liquid chromatography

Ping-Chung Kuo*, Yu-Lin Chang

Department of Biotechnology, National Formosa University, 64 Wenhua Road, Huwei, Yunlin, Taiwan, (REPUBLIC OF CHINA)

E-mail : pcckuoo@sunws.nfu.edu.tw

Received: 12th January, 2012 ; Accepted: 6th February, 2012

ABSTRACT

In the present research, the indicator compounds in *Gardenia jasminoides* Ellis and *Arctium lappa* L. were purified and used as standards to analyze the crudes and concentrated herbal extracts of local GMP manufacturers with the aid of high-performance liquid chromatography. The methods validation examinations were performed to confirm that these methods were precise and reliable. Thus they were subjected into the fingerprint chromatogram analyses of *G. jasminoides* and *A. lappa* and the results suggested that the chromatographic fingerprints could improve the products quality of the traditional Chinese medicine preparations.

KEYWORDS

Fingerprint chromatogram;
Indicator compound;
Traditional Chinese medicine;
HPLC;
Methods validation.

INTRODUCTION

The traditional Chinese medicines (TCMs) are well known to contain multiple chemical components and each compound may be related to the medicine's bioactivity. Several factors, including the various origins, different processing procedures, preserved conditions and deposited years may lead to the difference in the contents of chemical constituents and compound denominations of herbal medicine, which have direct impact on the therapeutic effects. Thus discrimination of an herbal material's origin as well as determination of its bioactive ingredients is crucial in order to ensure its authenticity, quality, safety and efficacy[1-6]. Despite of its existence and continuous uses over centuries, traditional herbal medicines have not been officially recognized in most countries due not only to the lack of research data to support its safety and efficacy but also to a lack of adequate or accepted research methodology for its evaluation[7]. Nowadays there are two major modes for quality control of traditional Chinese medicines. First one is selecting some known active constituents or indicator compounds from the herbal drug as the qualitative and quantitative targets to assess its authenticity and inherent quality. However, for herbal medicines derived from natural products with the inherent uncertainty feature of their secondary metabolic substances, the drawbacks of such a quality control mode have arisen more and more frequently. Therefore a practical approach, chromatography fingerprinting generated from a comprehensive mode was subjected to control the quality of herbal products and the com-

plex formulation of herbal medicines. The fingerprinting procedures have emerged to be efficient for serial analyses in quality control and stability tests of herbal medicine. It also emphasizes the integral formulation of pharmacologically active components and characteristic phytochemical compounds of samples with similar or different attributions[8-12]. The quality consistency and stability of herbal extracts or products can be assessed by their integral fingerprint patterns in quantified operation procedures. Therefore, it was formally introduced by WHO in Munich in 1991[13], and subsequently accepted by many countries. In the present study, the indicator compounds in *Gardenia jasminoides* Ellis and *Arctium lappa* L. had been extracted, purified, and identified for their chemical structures. In addition, the indicator compounds were used as standards to quantitatively analyze these traditional Chinese medicines with the aid of high performance liquid chromatography (HPLC) and the validation examinations were performed to confirm that these methods were precise and reliable for quality evaluation. Thus they could be utilized to control the quality of Chinese herbal preparations and the fingerprint chromatogram analyses of the methanol extracts of different herbal preparations of *G. jasminoides* and *A. lappa* were accomplished under the assistance of these methods.

EXPERIMENTAL

General

All the solvents including the HPLC-grade methanol were purchased from Merck KGaA (Darmstadt, Germany). The indicator compounds geniposide, arctigenin, and arctiin were purified by our lab and the chemical structures were identified by comparison of their spectroscopic and physical data with those reported in the literature. The purity of all indicator compounds, as determined by HPLC, was better than 97.0 %[14]. Plant materials were extracted using a Major Science LM-570R shaking incubator. High performance liquid chromatography (HPLC) was performed on a Shimadzu LC-20AT series pumping system equipped with a Shimadzu SPD-20A UV-Vis detector, a Gemini 5u C18 column (4.6 mm × 250 mm, 5 μm), and a SIL-10AF autosampling system.

Plant material

Two batches of plant materials *Gardenia jasminoides* (fruits) and *Arctium lappa* (seeds) were purchased in the herbal markets in Tainan and Yunlin, Taiwan, and named as GJT, GJY, ALT, and ALY, respectively. Three different concentrated herbal extracts of *G. jasminoides* and *A. lappa* were purchased from the local herbal GMP manufacturers (Companies A-C) in Taiwan, and named as GJA, GJB, GJC, ALA, ALB, and ALC, respectively. The voucher specimens and herbal extracts were deposited in the herbarium of Department of Biotechnology, National Formosa University, Yunlin, Taiwan.

Sample preparations for HPLC analysis

All the analytical samples (1.0 g) including GJT, GJY, GJA, GJB, GJC, ALT, ALY, ALA, ALB, and ALC, were extracted with 10 mL methanol in a 50 mL flask respectively. The samples were extracted in a shaking incubator at 25 ºC and 150 rpm for 30 min. The final volume was raised to 50 mL after the solution was filtered and kept at 4 ºC for the analyses. The sample solutions were then filtered through a membrane (0.45 μm) before injection into a HPLC to quantifying the bioactive constituents according to the following procedures.

Chromatography

The elution gradient for the HPLC analysis of *G. jasminoides and A. lappa* consisted of two solvent compositions: methanol (solvent A) and water (solvent B). Gradient elution for solvent A was as follows: maintenance at a concentration of 40 % for the first 5 min, then increase to 65 % in the next 20 min, then decrease to 40 % in 10 min, and hold for 10 min for the next injection (5 μL each). The column and auto-sampler were set at ambient temperature; flow rate was 0.5 mL/min. The eluent was monitored by a UV detector at 240 nm for geniposide, and at 280 nm for arctigenin and arctiin.

Preparation of standard solutions and calibration curves

G. jasminoides

The reference compound geniposide was purified by our lab and its purity was determined by HPLC as

Full Paper

99.8 %. The methanolic standard solution was prepared with 1001.46 µg/mL of geniposide and stock solution was prepared by mixing and diluting the original standard solution with methanol to achieve final concentrations of 50.07 µg/mL and then kept in brown glass bottles and stored at 4 ºC for further analysis. Calibration curve was constructed by plotting the peak-area versus analyte standard solutions at various concentrations. In the present study, the concentration ranges were 50.07–1001.46 µg/mL for geniposide.

A.lappa

The reference compounds, arctigenin and arctiin were purified by our lab and their purities were determined by HPLC as 97.4 and 98.9 %, respectively. The methanolic standard solutions were prepared with 486.01 and 492.60 µg/mL of arctigenin and arctiin and the stock solutions were prepared by mixing and diluting the original standard solution with methanol to achieve final concentrations of 9.72 and 9.85 µg/mL and then kept in brown glass bottles and stored at 4 ºC for further analysis. Calibration curves were constructed by plotting the peak-area versus analyte standard solutions at various concentrations. In the present study, the concentration ranges were 9.72–486.01 µg/mL for arctigenin and 9.85–492.60 µg/mL for arctiin.

Methods Validation: Reproducibility, precision, and limit of detection and quantification

The reproducibility and precision were measured by repeatedly injecting a ready-made sample pool and expressed as the relative standard deviation of the results. Analyses with three different concentrations were performed. To determine the intra-day variance, the assays were carried out on the same samples at different times during 1 day. Inter-day variance was determined by assaying the spiked samples over three consecutive days at the same time each day. The limit of detection (LOD) was determined as the lowest concentration that could be detected with acceptable accuracy and precision, which was achieved from the plot three times above the noise level.

Methods Validation: Recovery

The recovery from the samples was evaluated using three different concentrations covering the linear range of the standard curve. After the samples were processed according to the methods mentioned earlier, the resulting peak heights were compared to the standard compounds carried in mobile phase to provide the recovery values.

Statistical analysis

The data were statistically analyzed by Excel software and the analytical results are expressed as mean ± standard deviation (SD). Relative standard deviations (RSD) were calculated from these values.

RESULTS AND DISCUSSION

Optimization of the HPLC condition

In the development of the HPLC method for the fingerprint analysis and determination of indicator compounds of *G. jasminoides* and *A. lappa*, several solvent systems (methanol-water, methanol-water-formic acid, methanol-water-acetic acid, acetonitrile-water, acetonitrile-water-formic acid, acetonitrile-water-acetic acid) and separation columns (Zorbax ODS-4, Inertsil ODS-3, HyperClone 5u ODS, and Gemini 5u C18) were evaluated and compared. The methanol-water system gave more symmetrical peaks and best separation compared with other solvent system. Among the columns examined, the Gemini 5u C18 column was displayed to be the most suitable and gave good peak

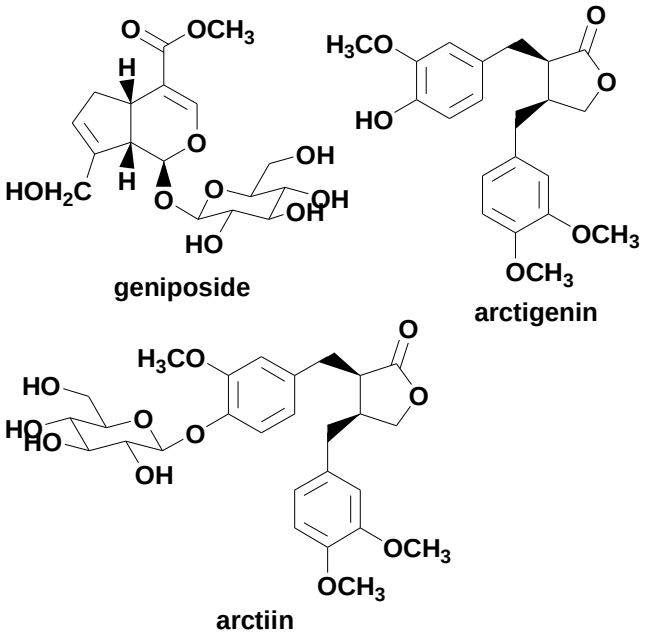

Figure 1 : Chemical structures of the indicator compounds geniposide, arctigenin, and arctiin.

separation and sharp peaks. Detection wavelength was also optimized in this work. Due to the simple chromophore in its chemical structure (Figure 1), geniposide show only absorption at 240 nm, whereas arctigenin and arctiin generally show more significant UV absorptions at 280 nm based on the lignan basic skeleton (Figure 1). The maximum number and the heights of the peaks of the constituents were obtained and the baseline of chromatogram was stable. Based on the results, an optimized HPLC analytical condition was designed as described above.

Method validation

The reproducibility and precision of a quantitative method are the degrees of agreement among the individual test results when the procedure is applied repeatedly to multiple samplings. The calibration curve parameters and limits of detection (LOD) for the three indicator compounds identified in *G. jasminoides* and *A. lappa* were displayed in TABLE 1. The reproducibility of the analytical method was performed and the results showed that it was satisfactory with the RSDs below 0.98 % for any of the three indicator compounds geniposide, arctigenin, and arctiin. All of the correlation coefficients (r^2) of the calibration curves for the three compounds were > 0.9999. The LOD values of geniposide, arctigenin, and arctiin were 50.15, 48.60, and 49.30 ng/mL, respectively. The precision of the HPLC method developed for *G. jasminoides* and *A. lappa* was evaluated through the intra-day and inter-day experiments. Among the linear ranges, the RSDs for the three indicator compounds of the intra-day and inter-day precisions were found to be less than 1.59 and 2.15 %, respectively (TABLE 2). A recovery test was used to evaluate the accuracy of this method. The recovery of the indicator compounds was determined by the addition of a sample with known concentration to the standard solution, and the mean recovery rate was found to be in the ranges from 83.70 to 114.04 % with satisfactory RSDs in the ranges between 0.23 and 1.51 % (TABLE 3).

TABLE 1 : Calibration curve parameters and limits of detection (LOD) for the three indicator compounds identified in *G. jasminoides and A. lappa*.

Compound	Calibra Tion curve	Correla tion coeffi cients (r^2)	Linear range (µg/mL)	LOD (ng/mL) (n = 3)	Reprodu cibility RSD (%)
Geniposide	y 45547 x–340188	0.99999	10.01–1001.46	50.15	0.94
Arctigenin	y= 22137x +10548	0.99999	9.72–486.01	48.60	0.47
Arctiin	y 16385x–45785	0.99997	9.85–492.60	49.30	0.98

TABLE 2 : Precision of the HPLC method developed for *G. jasminoides and A. lappa*.

Compound	Concentration µg/mL	Intra-day Mean ± SD (RSD %)	Inter-day Mean ± SD (RSD %)
Geniposide	50.07	51.91 ± 0.23 (0.43)	51.82 ± 0.50 (0.97)
	100.15	98.21 ± 0.54 (0.55)	97.55 ± 0.89 (0.91)
	1001.46	1001.56 ± 0.56 (0.06)	1006.88 ± 9.93 (0.99)
Arctigenin	9.72	9.89 ± 0.16 (1.59)	10.13 ± 0.22 (2.15)
	48.60	48.41 ± 0.57 (1.19)	48.83 ± 0.53 (1.09)
	486.01	486.02 ± 1.47 (0.30)	489.06 ± 4.28 (0.88)
Arctiin	9.85	10.76 ± 0.06 (0.59)	10.81 ± 0.08 (0.78)
	49.26	48.27 ± 0.32 (0.66)	48.15 ± 0.29 (0.60)
	492.60	492.68 ± 1.95 (0.40)	499.48 ± 6.42 (1.29)

TABLE 3 : Recovery of the three indicator compounds using in the HPLC method developed for *G. jasminoides and A. lappa*.

Compound	Spiked concentration µg/mL	Recovery (%)	RSD (%)
Geniposide	18.78	83.70 ± 0.20	0.23
	51.82	95.39 ± 0.59	0.62
	97.55	95.80 ± 0.75	0.78
Arctigenin	3.64	114.04 ± 0.99	0.87
	10.13	104.92 ± 0.57	0.54
	48.83	103.91 ± 0.79	0.76
Arctiin	6.99	92.75 ± 1.40	1.51
	10.81	94.91 ± 0.71	0.75
	48.15	99.22 ± 0.26	0.26

Analysis of chromatographic fingerprints of the analytical samples

The HPLC chromatographic fingerprint analysis was applied to assess the quality of the plant materials and herbal extracts. Chromatograms of the five

Full Paper

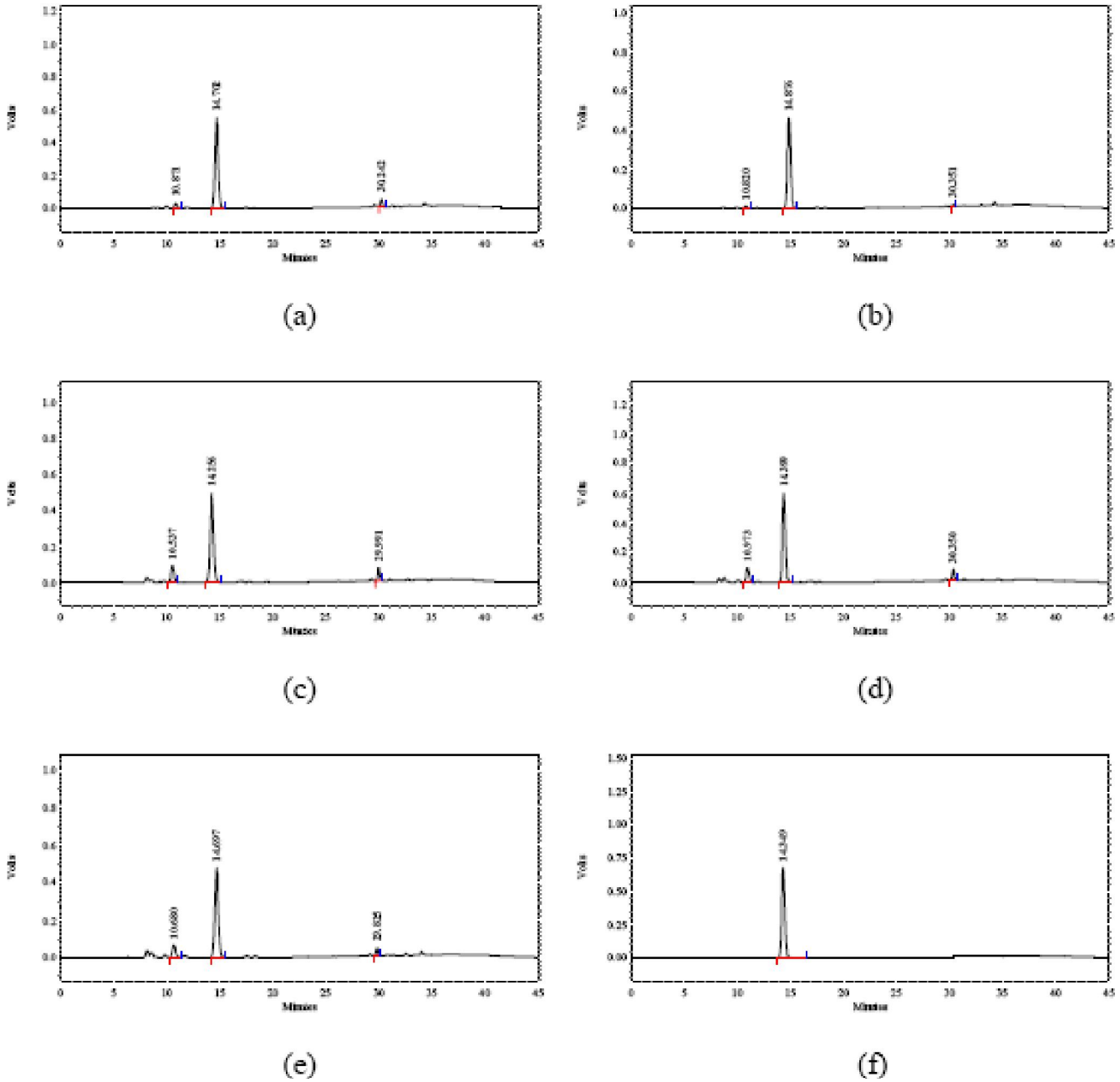

Figure 2 : HPLC fingerprint profiles of five methanol extracts of *G. jasminoides*. (a) Raw materials purchased in Tainan (GJT); (b) Raw materials purchased in Yunlin (GJY); (c) Herbal extract produced by Company A (GJA); (d) Herbal extract produced by Company B (GJB); (e) Herbal extract produced by Company C (GJC); (f) Reference compound geniposide.

samples of *G. jasminoides* and *A. lappa* and the indicator compounds were displayed in Figure 2 and 3, respectively. In Figure 2 (a)-(e), three common peaks in the five chromatograms were selected and among them one characteristic peak, geniposide was identified by matching its retention time (RT) and UV spectrum to the reference standard (Figure 2 (f)). All the peaks exhibited in Figure 2 (a)-(e) were very similar and it could be deduced from these analyses that the plant origins and preparative procedures did not have significant impacts on the chemical composition of *G. jasminoides*. With similar steps as described above, five peaks were selected and arctigenin and arctiin were identified for *A. lappa* in Figure 3 (a)-(e). Arctigenin is the aglycone of arctiin, so that arctiin may be hydrolyzed to afford arctigenin in the preparative procedures. Thus the differences of the contents for the indicator compounds were found among the ana-

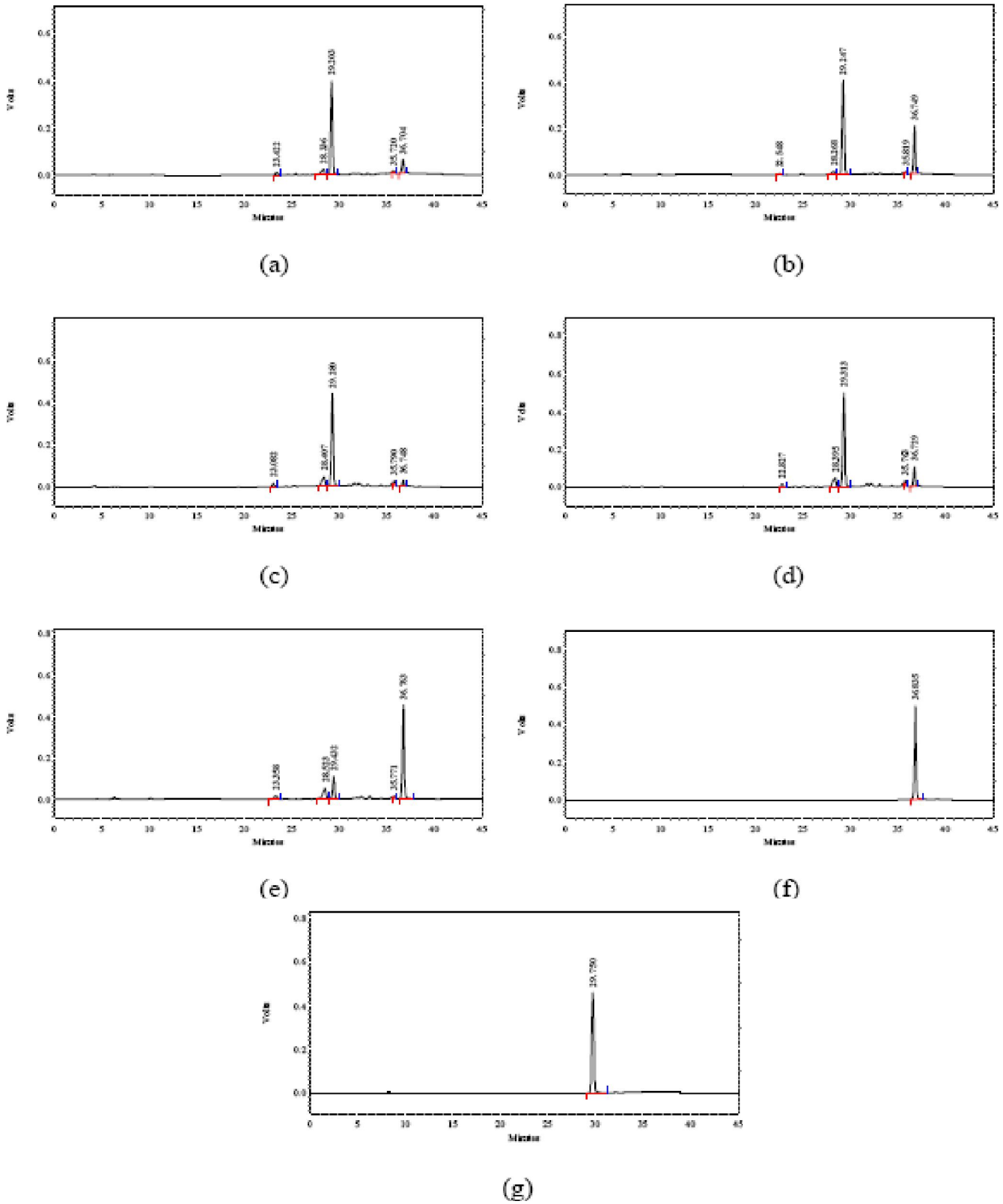

Figure 3 : HPLC fingerprint profiles of five methanol extracts of *A. lappa*. (a) Raw materials purchased in Tainan (ALT); (b) Raw materials purchased in Yunlin (ALY); (c) Herbal extract produced by Company A (ALA); (d) Herbal extract produced by Company B (ALB); (e) Herbal extract produced by Company C (ALC); (f) Reference compound arctigenin; (g) Reference compound arctiin.

lytical samples. The contents of arctigenin in samples ALY and ALC were significantly more and that of arctiin was comparatively low in ALC. It suggested that the Company C may adapt the processes that would hydrolyze the arctiin in higher percentages. The presented chemical fingerprints may enable drug manufacturers to adjust the proportion of herb to prepare a standardized product with consistent biological activity. Thus, the fingerprint developed for different samples represents a detailed chemical profile which may be useful in the identification as well as quality evaluation of the drugs.

Although the contents of the marker compounds in the samples varied with their sources, the chemical profiles among the analytical samples were very similar. The chromatographic fingerprints showed similar compositions and full sets of detectable components, in which not only most of the common characteristic peaks were present but also the peak-to-peak distribution patterns were stable and consistent. Thus, it could be concluded that the samples from different origins have sufficient similarity in chemical composition to ensure the quality stability of the traditional Chinese medicines. Furthermore, the established fingerprint methods of *G. jasminoides* and *A. lappa* were convenient and feasible as tools for species authentication and quality assessment of the raw materials and herbal extracts.

CONCLUSION

Traditional plant medicines were widely used from the ancient periods, such as people in China, India, and European countries were accustomed to take some medicinal plants to cure some diseases. Empirically, the inexact denomination of a plant is the main fault that was reported in the traditional pharmaceuticals markets. It can occur when plants are harvested or handled. A great deal of errors was found in the starting material collected from the market. Traditional Chinese medicinal products based on plants were collected from commercial manufacturers and the problems found in these products were related to the naming of the constituent plants and the conformity of the product with the declared composition. The developed chromatographic fingerprints could serve as useful techniques in differentiation of the plants from adulterants and also in the improvement of the products quality of the traditional Chinese medicine preparations.

ACKNOWLEDGEMENTS

The authors are grateful to the financial support of this research from National Science Council, Taiwan, ROC.

REFERENCES

[1] P.Zou, Y.Hong, H.L.Koh; J.Pharm.Biomed.Anal., **38**, 514-520 **(2005)**.
[2] L.F.Hu, S.P.Li, H.Cao, J.J.Liu, J.L.Gao, F.Q.Yang, Y.T.Wang; J.Pharm.Biomed.Anal., **42**, 200-206 **(2006)**.
[3] B.Y.Li, Y.Hu, Y.Z.Liang, P.S.Xie, Y.Ozaki; Analyst., **131**, 538-546 **(2006)**.
[4] K.Y.L.Yap, S.Y.Chan, C.S.Lim; Food Res.Int., **40**, 643-652 **(2007)**.
[5] Y.Zhao, Z.W.Li, X.Zhou, Z.W.Cai, X.J.Gong, C.Y.Zhou; J.Pharm.Biomed.Anal., **48**, 1230-1236 **(2008)**.
[6] W.J.Kong, Y.L.Zhao, X.H.Xiao, C.Jin, Z.L.Li; Phytomedicine, **16**, 950-959 **(2009)**.
[7] World Health Organization; General Guidelines for Methodologies on Research and Evaluation of Traditional Medicines, Geneva, **(2000)**.
[8] T.P.Layloff; Pharm.Tech., **15**, 146-148 **(1991)**.
[9] R.D.Kirchhoefer; The Journal of AOAC International, **75**, 577-580 **(1992)**.
[10] W.J.Welsh, W.Lin, S.H.Tersigni, E.Collantes, R.Duta, M.S.Carey, W.L.Zielinski, J.Brower, J.A.Spencer, T.P.Layloff; Anal.Chem., **68**, 3473-3482 **(1996)**.
[11] P.Valentão, P.B.Andrade, F.Areias, F.Ferreres, R.M.Seabra; J.Agric.Food Chem., **47**, 4579-4582 **(1999)**.
[12] P.S.Xie; Trad.Chin.Drug Res.Clin.Pharm., **12**, 141-151 **(2001)**.
[13] World Health Organization; Guidelines for the Assessment of Herbal Medicines, Geneva, **(1991)**.
[14] Y.L.Chang; "Purification of Indicator Compounds from Traditional Chinese Medicines and Their Application on The Quality Control of Medicinal Products," Master's Thesis, National Formosa University, Yunlin, Taiwan, **(2010)**.