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Analysis of *N-cis*- and *N-trans*-Feruloyl 3-Methyldopamine in *Achyranthes bidentata* by HPLC

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ABSTRACT

Huai-niu-xi is the dried root of *Achyranthes bidentata* (Amaranthaceae) and a commonly used herbal medicine. It possesses analgesic, anti-inflammatory, diuretic, anti-bacterial, and anti-convulsive activity. In clinical use, it was used to strengthen the sinew and bones, supplement the

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liver and kidney, and cure knee pain. A simple, rapid, and accurate high-performance liquid chromatographic (HPLC) method was developed for the assay of *N-cis*-feruloyl 3-methyldopamine (*cis*-FMD) and *N-trans*-feruloyl 3-methyldopamine (*trans*-FMD) in Huai-niu-xi. The HPLC system uses an Inertsil ODS-P (5 μm, 250 mm × 4.6 mm I.D.) column with gradient elution, with acetonitrile and water as the mobile phase. A detector was set at 300 nm. Calibration graphs were constructed in the range 8.0–160.0 $\mu g\,mL^{-1}$ for *cis*-FMD and 16.4–246.0 $\mu g\,mL^{-1}$ for *trans*-FMD. Regression equations revealed good linear relationships (correlation coefficients: 0.9997–0.9998) between the peak-area to each constituent. The contents of two constituents of *A. bidentata* in six samples have been determined. This study using HPLC, also compared with those similar herbal medicines, *Cyathula officinals* (Amaranthaceae) and *Strobilanthes forrestii* (Acanthaceae), commonly used as an adulterant.

Key Words: *Achyranthes bidentata*; *N-cis*-feruloyl 3-methyldopamine; *N-trans*-feruloyl 3-methyldopamine; HPLC.

INTRODUCTION

Niu-xi, also known as Huai-niu-xi, is the dried root of *Achyranthes bidentata* (Amaranthaceae) and a commonly used herbal medicine. It possesses analgesic, anti-inflammatory, diuretic, anti-bacterial, and anti-convulsive activity. In clinical use, it was used to strengthen the sinew and bones, supplement the liver and kidney, and cure knee pain.[1]

Several herbal medicines such as Niu-xi are substituted or adulterated on the Taiwan markets. There are two species, *Cyathula officinals* (Amaranthaceae) and *Strobilanthes forrestii* (Acanthaceae) which are also used as Niu-xi and known as Chuan-niu-xi and Tu-niu-xi, respectively. Therefore, the origin of Niu-xi is complicated and not well known. The identification of medicinal herbs is usually based on the apparent characteristics and the examination of herbal tissue. However, these procedures are difficult and tedious. High-performance liquid chromatography (HPLC)[2–4] and capillary electrophoresis[5,6] are more promising techniques, because it allows high resolution and a rapid and reproducible determination, even of trace amounts of compounds. So, a simple and rapid method is needed for a routine analysis of herbal medicines.

A few methods, HPLC and thin-layer chromatography, scanning for the analysis of oleanolic acid in *A. bidentata* have been reported.[7,8] However, oleanolic acid was also contained in many plants, and this constituent could not be used for identification and quality control of *A. bidentata.* Two phenolic

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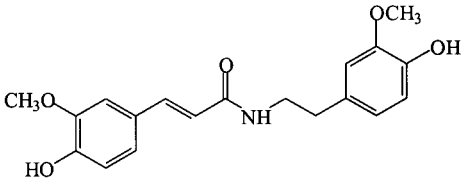
amides, *N-trans*-feruloyl 3-methyldopamine (*trans*-FMD)[9] and the isomer of *trans*-FMD, *N-cis*-feruloyl 3-methyldopamine (*cis*-FMD),[10] have been isolated from *A. bidentata* and their chemical structure was identified by spectroscopic methods (Fig. 1).

In this study, an HPLC method was developed for the determination of the *cis*-FMD and *trans*-FMD of *A. bidentata.* The contents of two constituents in six samples from markets have been determined. Additionally, comparisons were made among *A. bidentata, C. officinals* and *S. forrestii* by their HPLC chromatograms.

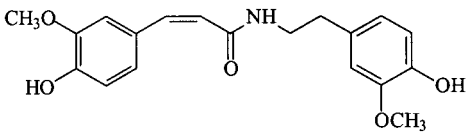
EXPERIMENTAL

Plant Materials

Plant materials of *A. bidentata* were collected in Taichung, Taiwan in September 1997, and identified by Professor Chung-Chuan Chen, Institute of Chinese Pharmaceutical Science, China Medical College, Taichung, Taiwan. Groups of six, three, and three samples of *A. bidentata*, *C. officinals* and *S. forrestii* were also obtained from markets in Taichung in July 2001. All samples were identified by comparative microscopy studies.[11]



N-trans-feruloyl 3-methyldopamine



N-cis-feruloyl 3-methyldopamine

Figure 1. The chemical structures of *N-cis*-FMD and *N-trans*-FMD.

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Reagents

Cis- and *trans*-FMD were isolated from the dried roots of *A. bidentata.* Acetonitrile (HPLC grade) was purchased from Labscan (Dublin, Ireland). Ultra-pure distilled water with a resistance greater than 18 MΩ was used. Methanol, chloroform, and *n*-hexane were of industrial grade.

High-Performance Liquid Chromatographic System

The HPLC was performed on a Hitachi Model L-6200 Intelligent pump system equipped with a Hitachi Model L-3000 Photo Diode Array and a Shimadzu SIL-9A auto-injector. The detector was set at 300 nm. Satisfactory separation of the marker substance was obtained with a reversed phase column (Inertsil ODS-P, 5 μm, 250 mm × 4.6 mm I.D., GL Sciences Inc., Tokyo, Japan), eluted at a rate of 1.0 mL/min with a linear solvent gradient of A–B (A = acetonitrile; B = water) varying as follows: 0 min, 25 : 75; 14 min, 30 : 70 and 30 min, 35 : 65.

Preparation of Standard Solution

To prepare standard solutions (containing *cis*- and *trans*-FMD), an accurately weighed amount of *cis*- and *trans*-FMD standard was dissolved in methanol for HPLC. Five concentrations were chosen, with the range 16.4–246.0 and 8.0–160.0 $\mu g\,mL^{-1}$, respectively. Calibration graphs were subsequently plotted for linear regression analysis of the peak-area vs. concentrations.

Preparation of Sample Solution

The air-dried roots of *A. bidentata*, *C. officinals*, and *S. forrestii* (600 g) were exhaustively extracted with methanol (2 L) three times at room temperature. for 7 days. Then, the combined methanol extract was filtered and then concentrated under reduced pressure. The crude methanol extract was suspended in water and partitioned with chloroform (400 mL) three times to afford the chloroform fraction. The combined chloroform extract was also concentrated under reduced pressure. A 180 mg sample of the chloroform fraction was dissolved in 5 mL of methanol. This solution was filtered through a 0.45 μm syringe filter (Gelman Sciences, Ann Arbor, MI) before use.

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Preparation for Recovery Studies

Three different concentrations of markers: 5, 10, and 20 $\mu g\,mL^{-1}$ for *cis*-FMD, and 5.2, 10.3, and 20.6 $\mu g\,mL^{-1}$ for *trans*-FMD were added to the extractions of plant materials, respectively. All samples were filtered through a 0.45 μm syringe filter (Gelman) and injected for HPLC analysis, to calculate the concentration of *cis*- and *trans*-FMD from their calibration graphs.

RESULTS AND DISCUSSION

The detection wavelength of 300 nm was chosen because *cis*- and *trans*-FMD have better absorption at this wavelength. The photodiode array detection facilitated the identification and confirmation of these two constituents. Figure 2 presents a chromatogram showing the separation of

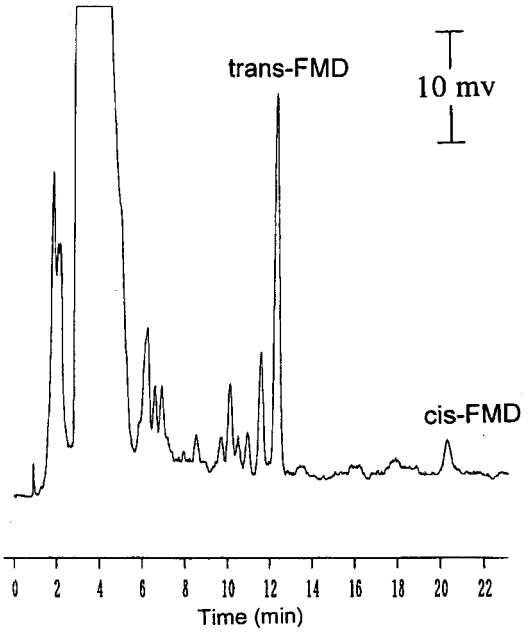

Figure 2. Chromatogram of methanol extract of *A. bidentata*. *cis*-FMD: *N-cis*-feruloyl 3-methyldopamine; *trans*-FMD: *N-trans*-feruloyl 3-methyldopamine. HPLC conditions, column: Inertsil ODS-P, 5 μm, 25 cm × 4.6 mm I.D.; mobile phase: A–B (A = acetonitrile; B = water), 0 min, 25 : 75, 14 min, 30 : 70 and 30 min, 35 : 65; flow rate: 1.0 mL/min; detection wavelength: 300 nm.

the constituents with the retention times of 12.3 min for *trans*-FMD and 20.1 min for *cis*-FMD. When the sample solution was injected directly and analyzed, the whole analysis was finished within 21 min. In our solvent system, we found the cis-form is less polar than the trans-form. By examining the structures with Chem 3D, we observed that the distance between the two hydroxyl groups are 10.866 Å for cis-form and 12.535 Å for trans-form. This means that the cis-form is folding more together than the trans-form, which supports our results.

Calibration graphs were constructed in the range 16.4–246.0 $\mu g\,mL^{-1}$ for *trans*-FMD and 8.0–160.0 $\mu g\,mL^{-1}$ for *cis*-FMD. The regression equations of these curves and their correlation coefficients (r), coefficients of determination (R^2) were calculated as follows: *trans*-FMD, $y = 1.00$ E04x − 5.03E04, 0.9998, 99.96% and *cis*-FMD, $y = 8.06$E03x − 8.71E03, 0.9997, 99.94%. It showed good linear relationships between the peak areas and the concentration. A signal three times higher than the peak noise height was regarded as the detection limit. The detection limits of these three constituents were 1.5 and 1.2 $\mu g/mL$ for *cis*- and *trans*-FMD, respectively.

To assess the precision of these methods, we injected standard solutions of *cis*- and *trans*-FMD, respectively, five times on the same day and for a period of 5 days. The precision RSDs of the proposed method of the two constituents, on the basis of peak-area ratios were 1.46–2.78% for intra-day and 2.06–3.89% for inter-day, respectively. All of these data indicates that the precisions are acceptable (Table 1). The results for the recoveries of *cis*- and *trans*-FMD ranged from 80.6% to 90.7% (Table 2). The RSDs of recoveries of two constituents ranged between 2.1–4.3%. These assessments indicates good accuracy with this method.

Table 1. Intra-day and inter-day assay variations of two constituents.

Constituent	Concentration ($\mu g\,mL^{-1}$)	Intra-day[a] RSD (%)	Inter-day[a] RSD (%)
Cis-FMD	5.0	2.48	3.89
	20.0	1.99	3.14
	80.0	2.70	3.05
Trans-FMD	5.2	2.15	3.78
	20.6	2.78	2.84
	41.2	1.46	2.06

[a]$n = 5$.

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Table 2. Recoveries of two constituents in *A. bidentata*.

Constituent	Amount added ($\mu g\,mL^{-1}$)	Recovery (%)	Mean ± SD (%)	RSD (%)
Cis-FMD	5.0	84.5	83.0 ± 1.7	2.1
	10.0	80.6		
	20.0	83.9		
Trans-FMD	5.2	89.1	87.3 ± 3.8	4.3
	10.3	82.0		
	20.6	90.7		

Note: $n = 3$.

When the sample solution was analyzed by HPLC, the peaks were identified by comparison of the retention time with those obtained from authentic samples of *A. bidentata.* The comparison graph of the contents of *cis*- and *trans*-FMD in 6 crude drugs of *A. bidentata* are given in Fig. 3. The contents of two constituents ranging between 0.00008–0.00057% and 0.0006–0.0079% for *cis*- and *trans*-FMD, respectively, were extracted from crude drugs. The result showed higher differences of contents among crude drugs. Examination of the different contents of each drug reveals the origin of *A. bidentata*, and the establishment of the good manufacturing and/or agriculture procedures can be based on the contents of these constituents.

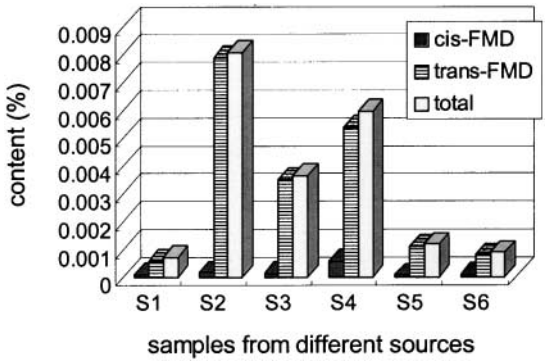

Figure 3. The contents of six constituents in six *A. bidentata* (S1–S6) obtained from different sources. (View this art in color at www.dekker.com.)

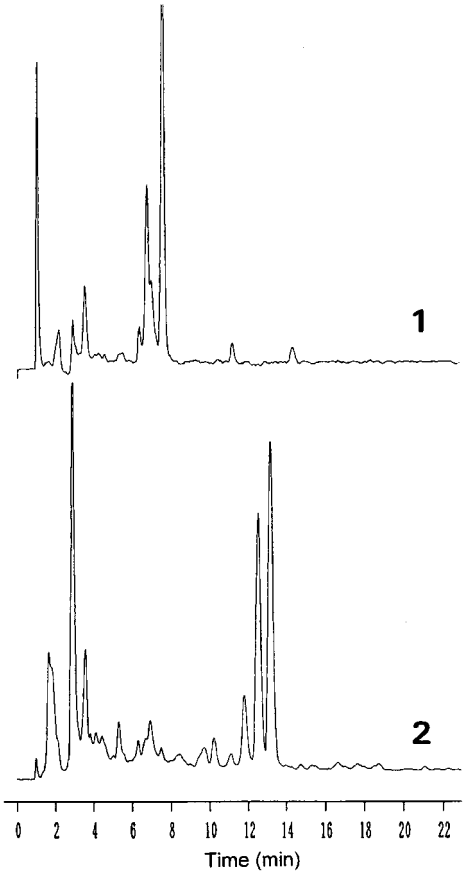

Figure 4. Chromatograms of *S. forrestii* (1) and *C. officinals* (2). For HPLC conditions, see Fig. 1.

Figure 4 showed the chromatograms of *C. officinals* and *S. forrestii.* We could differentiate among the chromatograms of *A. bidentata*, *C. officinals* and *S. forrestii formosanum.* Since there are differences among these three herbs, it is suggested that identification steps should be taken before medical use.

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