Hue University Journal of Science: Natural Science
Vol. 133, No. 1D, 57–61, 2024

pISSN 1859-1388
eISSN 2615-9678

Determination of andrographolide from *Andrographis paniculata* growing in Thua Thien Hue with high performance liquid chromatography

Tran Phuong Ha[1], Phan Thi Diem Tran[1], Le Canh Viet Cuong[1]*

Mientrung Institute for Scientific Research, Vietnam National Museum of Nature, VAST, 321 Huynh Thuc Khang, Hue, Thua Thien Hue, Vietnam

* Correspondence to Le Canh Viet Cuong <vietcuongtnmt@gmail.com>

(*Received: 10 October 2024; Revised: 05 November 2024; Accepted: 02 December 2024*)

Abstract. *Andrographis paniculata* (Acanthaceae) is a medicinal plant commonly used in Vietnam in the 1980s. The chemical composition of the plant includes a group of diterpene lactones with antimicrobial, cytotoxic, antidiabetic, and anti-inflammatory activities. In this group, andrographolide is a typical compound. This paper describes a method for quantifying andrographolide in *A. paniculata* grown in Thua Thien Hue by using high-performance liquid chromatography. Andrographolide was analyzed on an RP-C18 column with a methanol/acetonitrile/water (55/10/35, v/v/v) mobile phase, a detecting wavelength of 225 nm, a flow rate of 0.5 mL/min, and an injected sample volume of 10 μL. This method displays linearity for andrographolide in the range of 200–1,000 μg/mL, and the recovery of andrographolide is 99.63 ± 0.88 %. The contents of andrographolide in root, stem, and leaf of *A. paniculata* grown in Vietnam were determined for the first time, and the values are 0.03 ± 0.007%, 0.57 ± 0.021%, and 3.98 ± 0.034%, respectively.

Keywords: Andrographolide, *Andrographis paniculata*, HPLC

1 Introduction

Andrographis paniculata (Xuyên tâm liên) belongs to family Acanthaceae. The aerial parts of this plant are used to treat dysentery, gastritis, enteritis, colds, sore throat, tonsillitis, pneumonia, cervical ulcers, high blood pressure and burns [1, 2]. Research on the chemical composition of *A. paniculata* shows that andrographolide is the main component of the leaves of this medicinal herb with a content ranging from 2.76 to 4.39% [3]. Besides, andrographolide has been reported to possess interesting biological activities such as anti-cancer, anti-virus, and anti-inflammatory effect [4–8]. However, publications on the andrographolide content in *A. paniculata* in Vietnam are still very limited. This is the first complete report on andrographolide content in parts of *A. paniculata* native to Vietnam.

2 Experimental

2.1 Materials

The root, stem, and leaf parts of *A. paniculata* were collected in August, 2024, in Huong Van, Huong Tra, Thua Thien Hue. The scientific name was identified at Mien Trung Institute for Scientific Research and Vietnam National Museum of Nature, and the voucher specimen was deposited at Mien Trung Institute for Scientific Research.

Andrographolide was purchased from Sigma –Aldrich. The andrographolide standard was dissolved in a mobile phase at concentrations of 10, 200, 400, 500, 600, 800, and 1,000 μg/mL. All andrographolide solutions were filtered through a 0.45 μm nylon syringe filter. The HPLC-grade solvents were also purchased from Sigma – Aldrich.

2.2 Preparation of methanol extracts

An exact amount (1.0 g) of finely ground *A. paniculata* sample powder was subjected to ultrasonic extraction with 20 mL of methanol 99%, 3 times, each for 30 minutes. The resulting mixture was filtered through Whatman filter paper. Then, the solutions were evaporated with a Buchi R-300 rotary vacuum evaporator, followed by dissolving in a 100 mL volumetric flask with methanol. Finally, the solution was filtered through a 0.45 μm nylon syringe filter prior to injection into the HPLC system.

2.3 HPLC conditions

Chromatographic analysis (HPLC Shimadzu - Japan) was carried out by using a RP 5 μm C18, 4.6 × 250 nm, detector SPD-M20A, LC-20AD, SIL-20A, and the computer integrated with labsolution software. The Excel software was employed for data assessment. The HPLC specification and chromatographic conditions are given in Table 1.

Table 1. HPLC specifications for phytochemical analysis

Chromatographic conditions	
Concentrations (μg/mL)	200 to 1000
Mobile phase (v/v/v)	methanol/acetonitrile/ water (55/10/35)
Flow rate (mL/min)	0.5
Injection volume (μL)	10
Standard R_t (min)	9.6 ± 0.03
Detection wavelength (nm)	225
Analysis time (min)	20

3 Results and discussion

The HPLC system with PDA (photodiode array detector) can detect substances with an absorbance in the range of 190–800 nm. The UV spectrum in Fig. 1 shows that the active substance andrographolide is maximally absorbed at the 225 nm wavelength.

The HPLC profiles for andrographolide indicate a single peak at a retention time of 9.6 ± 0.03 min (Table 2 and Fig. 2). The system suitability tests were carried out on a prepared andrographolide standard solution (n = 5) with a 10 μL injection volume. All results were obtained in the acceptable range (RSD = 0.34%).

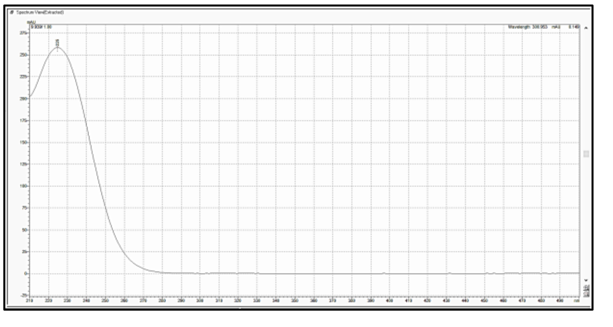


Fig. 1. UV Spectrum of andrographolide

Table 2. Retention time of andrographolide

Number	Retention time (min)	$R_{t.av}$ (min)	Repeatability of retention time (RSD %)
1	9.59	9.60	0.34
2	9.58		
3	9.62		
4	9.56		
5	9.65		
6	9.62		

pISSN 1859-1388
eISSN 2615-9678

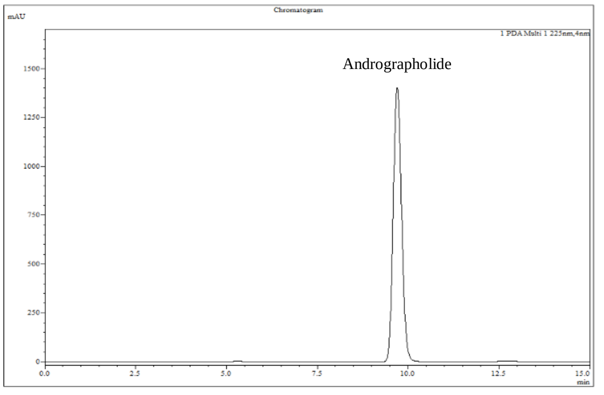


Fig. 2. HPLC chromatogram of andrographolide

The linearity regression equation for andrographolide (y = 33908 × x – 29256) shows a good linear relationship between concentrations and peak areas over the concentration range of andrographolide from 200 to 1000 µg/mL, and the correlation coefficient (R) is 0.9997 (Table 3 and Fig. 3) (the evaluation of each point was repeated three times)

Table 3. Regression equation, regression coefficient, LOD (limit of detection) and LOQ (limit of quantification) of andrographolide

standard solution of andrographolide prepared for calculation of LOD and LOQ					
Concentration (µg/mL)	200	400	600	800	1000
Peak area (mAU)	6913076	13399201	20320911	26847886	34096777
Regression equation	y = 33908 × x – 29256				
Regression coefficient	R^2 = 0.9997				
LOD (µg/mL)	3.97				
LOQ (µg/mL)	12.03				

The LOD (which is the lowest amount of an analyte in a sample that can be detected but not necessarily quantified) is 3.97 µg/mL. The LOQ value (which is the lowest amount of analyte in a sample) is 12.03 µg/mL.

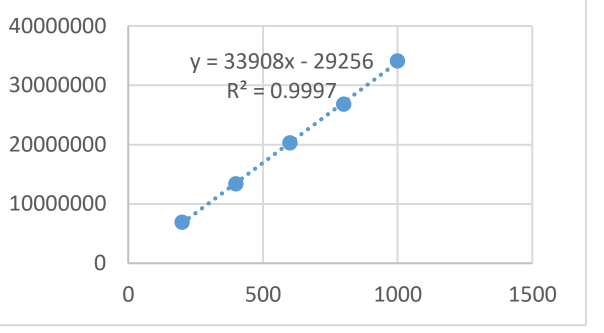


Fig. 3. Calibration curve for andrographolide at 225 nm

The accuracy was determined by using a recovery test at three concentration levels (Table 4). The recovery was determined by subtracting the values obtained for the control matrix preparation from the samples prepared with the added standards, divided by the amount added, and then multiplied by 100. The recovery was found to be 99.63 ± 0.88%, indicating a good accuracy of the method.

Table 4. Results of survey of recovery of andrographolide

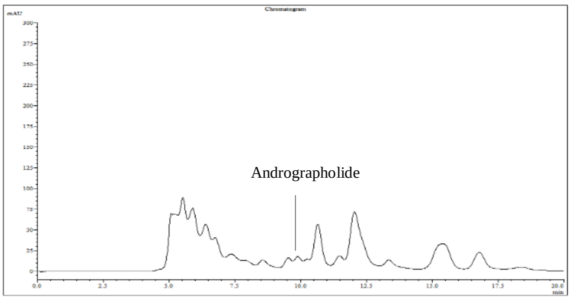
Amount added (µg)	Amount recoveries (µg)	Recovery (%)	$R_{av} \pm SD$
10	9.90 ± 0.17	98.96	99.63 ± 0.88
200	198.63 ± 1.82	99.32	
500	503.11 ± 1.41	100.62	

The distribution of andrographolide in the root, stem, and leaf parts of *A. paniculata* is shown in Table 5 and Fig. 4. The highest amount of andrographolide in methanol extracts from the leaves of *A. paniculta* was found.

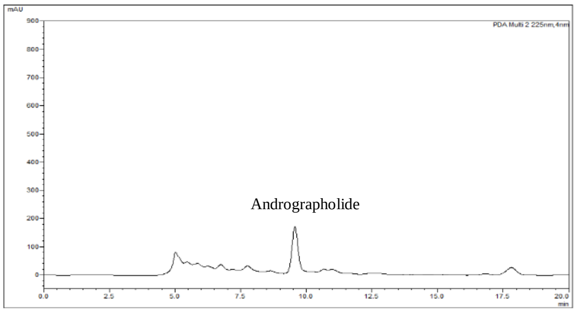
Table 5. Andrographolide contents from plant parts of *A. paniculata* in Thua Thien Hue

Plant parts	Andrographolide, %
Root	0.03 ± 0.007
Stem	0.57 ± 0.021
Leaf	3.98 ± 0034

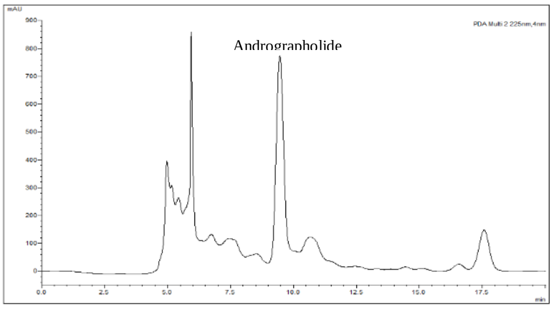
DOI: 10.26459/hueunijns.v133i1D.7640



(a)



(b)



(c)

Fig. 4. HPLC chromatogram of andrographolide from: (a) root; (b) stem; (c) leaf of *Andrographis paniculata* growing at Huong Van, Huong Tra, Thua Thien Hue

A report by Patarapanich et al. displays that the content of andrographolide in *A. paniculata* leaves in Thailand ranges from 2.76 to 4.39%, with the highest (4.39 ± 1.02%) from the south and the lowest from the north part of the country [3]. Another report shows that the andrographolide content in *A. paniculata* leaves and stems is 17.45 ± 0.16 and 8.37 ± 0.12 (mg/g dry weight), respectively [9]. Our results and those reported previously show that the andrographolide content is the highest among the diterpene lactones of *A. paniculata*, and higher in leaves than in stems and roots.

4 Conclusion

The quantification of andrographolide in methanol extracts from different parts of *A. paniculata* was conducted by using the HPLC method. The andrographolide content from roots, stems, and leaves is 0.03 ± 0.007%, 0.57 ± 0.021%, and 3.98 ± 0.034%, respectively. These results can provide a theoretical basis for further research on andrographolide from *A. paniculata*. This is the first report on the content of andrographolide in the roots, stems, and leaves of *A. paniculata* growing in Vietnam. The HPLC method in this study shows specificity and selectivity with linearity in the working range and a good precision and accuracy, making it very suitable for the determination of andrographolide from the extracts of *A. paniculata*.

Acknowledgement

This research was the result of Provincial Science and Technology Project [No. TTH.2021-KC.19] funded by Vietnam Governmental Budget under regulation of Thua Thien Hue province.

References

1. Materials NIoM. Checklist of medicinal plants in Vietnam. Science and Technics Publishing House. 2016;1:1051.
2. Chi VV. Dictionary of Medicinal Plants in Vietnam. Medical Publishing House. 2012;2:1224-5.
3. Patarapanich C, Laungcholatan S, Mahaverawat N, Chaichantipayuth C, Pummangura S. HPLC determination of active diterpene lactones from *Andrographis paniculata* Nees planted in various

pISSN 1859-1388
eISSN 2615-9678

seasons and regions in Thailand. Thai J Pharm Sci. 2007;31:91-9.

4. Hu J, Li Y, Xie X, Song Y, Yan W, Luo Y, et al. The therapeutic potential of andrographolide in cancer treatment. Biomedicine & Pharmacotherapy. 2024;180:117438.
5. Jiang M, Sheng F, Zhang Z, Ma X, Gao T, Fu C, et al. *Andrographis paniculata* (Burm.f.) Nees and its major constituent andrographolide as potential antiviral agents. Journal of Ethnopharmacology. 2021;272:113954.
6. Jothi S, Mohtar N, Masarudin MJ, Parumasivam T. Novel inhaled andrographolide for treatment of lung cancer: In vitro assessment. Heliyon. 2024;10(9):e30761.
7. Liu Y-f, Feng Z-q, Chu T-h, Yi B, Liu J, Yu H, et al. Andrographolide sensitizes KRAS-mutant colorectal cancer cells to cetuximab by inhibiting the EGFR/AKT and PDGFRβ/AKT signaling pathways. Phytomedicine. 2024;126:155462.
8. Fu K, Chen H, Wang Z, Cao R. Andrographolide attenuates inflammatory response induced by LPS via activating Nrf2 signaling pathway in bovine endometrial epithelial cells. Research in Veterinary Science. 2021;134:36-41.
9. Pholphana N, Rangkadilok N, Thongnest S, Ruchirawat S, Ruchirawat M, Satayavivad J. Determination and Variation of Three Active Diterpenoids in *Andrographis paniculata* (Burm.f.) Nees. Phytochem Anal. 2004;15(6):365-71.