3.3. Quantification of BBR in viscous extract samples

From the developed method, quantification of BBR in viscous extract samples was carried out. Preparation of the test sample solution and conduct chromatography under the conditions described on the 3 samples. The result of BBR content in Tan thong phong viscous extracts was 0.88 ± 0.06%.

4. Conclusion

The method for quantification of berberine hydrochloride in Tan thong phong viscous extracts by HPLC had been developed and validated analytical: Shim-pack GIST C_{18} column (250 mm x 4.6 mm, 5 µm), flow rate: 1 mL/min, column temperature: 20°C, and mobile phase acetonitrile : orthophosphoric acid 0.05% (gradient elution). Analytical method with high sensitivity, specificity, linear ranging from 11.29 to 361.23 µg/mL (r = 0.9999), good repeatability and intermediate precision (RSD < 3.7%), high accuracy (97.77% to 100.36%), LOD and LOQ were 0.052 µg/mL and 0.172 µg/mL, respectively. The berberine hydrochloride content in Tan thong phong viscous extracts was 0.88 ± 0.06%. It was necessary to continue investigating the berberine hydrochloride content in Tan thong phong viscous extracts to develop quantitative criteria and quality standards for this viscous extract.

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METHOD DEVELOPMENT FOR QUANTIFICATION OF PAEDEROSIDIC ACID FROM *PAEDERIA SCANDENS* BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Summary

Method Development for Quantification of Paederosidic Acid from *Paederia scandens* by High Performance Liquid Chromatography

A simple and sensitive high performance liquid chromatography (HPLC-DAD) method for quantification of paederosidic acid from *Paederia scandens* aerial part was developed and validated. The chromatographic separations were obtained by a Shimadzu C18 column (250×4.6mm, 5µm) using isocratic mode with 0.1% phosphoric and acetonitrile (85:15, v/v). Detection was set at a wavelength of 234 nm. The validation results displayed good accuracy, repeatability, linearity, and selectivity. The contents of paederosidic acid in *Paederia sc* acid *andens* samples were 0.0075 -0.2339%. The obtained results provide useful information for the quality testing of the *Paederia scandens* in Vietnam.

Keywords: *Paederia scandens* (Lour.) Merr., *Paederosidic acid, HPLC.*

1. Introduction

Paederia scandens (Lour.) is a perennial herb belonging to the *Paederia* L. genus of Rubiaceae. The vine Paederia scandens is widely distributed in Asia, particularly in Vietnam [1]. Many parts of the plant, involving the roots, leaves, barks, and fruits

have been used to treat toothaches, chest pain, piles, spleen inflammation, rheumatoid arthritis, and bacillary dysentery, as well as as a depressant and emetic [2]. In addition, in recent years, a large number of bioactive compounds have been found in Paederia scandens, including iridoids and glycoside derivatives [1],[3]. Paederosidic acid is an active component isolated from *Paederia scandens* that have displayed a wide spectrum of biological properties, such as anti-cancer, anti-inflammatory, anti-diarrheal and anti-tumor [4],[5].

Analytical technique is an approach that is used to determine a chemical or physical property of a chemical substance, chemical element, or mixture. There is a broad selection of techniques used for analysis, from simple weighing to advanced techniques using highly specialized instrumentation. Among them, liquid chromatography is an ideal analytical technique for chemical compounds in natural material, espicially in *Paederia scandens* [6]. In this paper, a fast, accessible high performance liquid chromatography (HPLC) were proposed for the quantitative analysis of paederosidic acid in *Paederia scandens* and evaluate the content of this compound in some samples obtained in Vietnam, contributing to standardizing the quality of medicinal herbs in Vietnam.

2. Experimental

2.1. Plant material

Paederia scandens (Lour.) Merr. were collected in Vietnam in March 2021 and **botanically identified by MSc. Nguyen Van Hieu, Department of Botany, National Institute of Medicinal Materials** (NIMM), Hanoi, Vietnam**, where the voucher specimen (ML-260321) was deposited.** After collection, the plant were air-dried under the shade and dried at 45°C, then ground into fine powder.

2.2. Chemicals

Paederosidic acid standard (CAS: 18842-98-3, Lot No: CFN92524) with 98% purity was purchased from Chemfaces (China).

Acetonitrile and ortho-phosphoric acid of HPLC grade were provided by Merck (Germany); all other solvents for sample preparation were of analytical grade.

2.3. Instrument and chromatographic conditions

Various chromatographic conditions, including mobile phase composition, solvent ratio, flow rate was examined along with references to relevant articles and journals [7],[8],[9],[10]. In the paper, HPLC analyses were performed on an HPLC system (Shimadzu, Japan) equipped with vaccum degasser, quaternary gradient pump, auto-sampler, Diode Array detector. A Shimadzu C_{18} column (250×4.6mm, 5µm) were used for separation of compounds. The mobile phase was run in an isocratic mode with 0.1% acid phosphoric and acetonitrile (85:15, v/v) at a flow rate of 0.8 mL min^{-1}. The injection volume was 20 µL. Detection was set at a wavelength of 234 nm. The run time was 30 min.

2.4. Optimization of sample preparation

Different extraction factors, including extraction solvent, concentration of solvent, extraction method and extraction time were tested. Each sample was analyzed in triplicate to determine the mean content (%). Finally, each dried sample (1 gam powder) was sonicated with 100 mL methanol 50% for 30 min. After that, the extraction solution was cooled and filtered through a filter. The extraction solution was centrifuged at a speed of 4000 rpm for 10 min, and the supernatant was accumulated; the solvent was vaporized under vacuum and stored at 4°C in the refrigerator for following evaluation.

2.5. Method validation

The analytical method was validated for system suitability, specificity, calibration curve, accuracy, precision, dectection limit and quantitation limit following the current ICH guidelines [11].

2.6. Calculation

Calculate the percentage of paederosidic acid in the tested sample taken:

$$X\,\% = \frac{C.V.P}{m.(100 - W) * 10}$$

C: the concentration of analyzed compound in the sample solution from the calibration curve equation (µg/ml); V: volume of the sample solution (mL); m: weight of tested sample taken to prepare the sample solution (mg); W: the moisture of powder (%); P: purity of standard (%).

3. Results and Discussion

3.1. Optimization of the extraction methods

Ultrasonic and reflux extraction were evaluated to achieve a good extraction efficiency. The ultrasonic approach was proven to be more successful and was so employed in subsequent studies.

As extraction solvents, methanol, ethanol, and aqueous ethanol and methanol solutions (50% ethanol, 50% methanol) were studied. Among these solvents, aqueous methanol solution performed the best for sample solution preparation and was chosen as the extraction solvent.

Different extraction times (15 minutes, 30 minutes, 45 minutes, and 60 minutes) were also tried, with 30 minutes being chosen.

The following extraction conditions were established: samples (1 g of powder) were extracted by sonication extraction using methanol 50% (100 mL), and the extraction period was 30 minutes (Table 1).

Table 1. The results of optimization of the extraction method

Compounds	Paederosidic acid (%)
Extraction method (Extraction solvent: methanol, V=100 mL, extraction time 30 min)	
Reflux	0.1934 ± 0.012
Sonication	0.2321 ± 0.015
Extraction solvents (Extraction method: sonication, V= 100 mL, extraction time: 30 min)	
MeOH	0.2011 ± 0.022
MeOH 50%	0.2345 ± 0.024
EtOH	0.1323 ± 0.028
EtOH 50%	0.1966 ± 0.017
Extraction time (Extraction solvent: MeOH 50%, V= 100 mL, extraction method: sonication)	
15 min	0.1939 ± 0.017
30 min	0.2352 ± 0.005
45 min	0.2374 ± 0.013
60 min	0.2365 ± 0.022

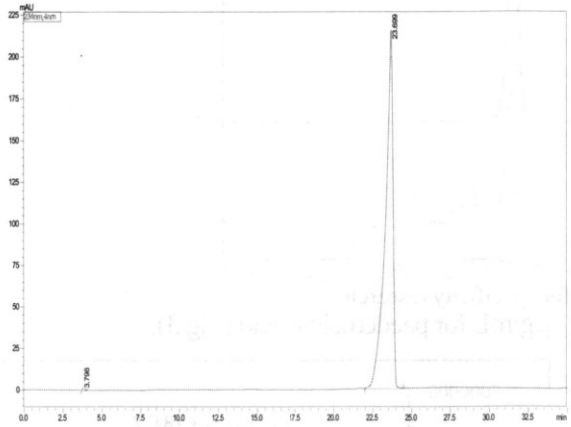
3.2. Method validation of quantitative analysis

3.2.1. Optimization of chromatographic conditions:

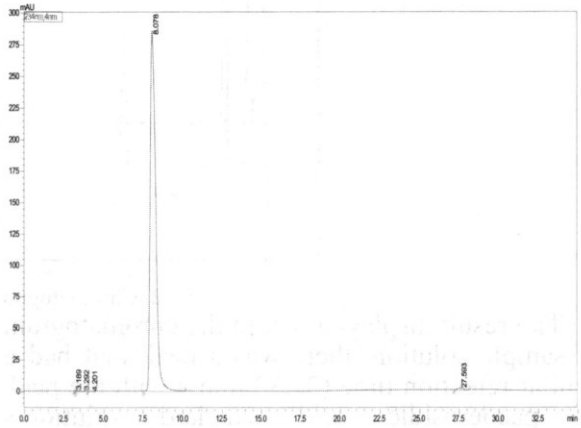
Different mobile phase composition has been tested. With the conditions of mobile phase MeOH: H_3PO_4 0.1% (3:7, v/v), the chromatogram in Fig 1A showed the standard peak was asymmetrical. Changing the mobile phase to ACN : H_3PO_4 0.1% (2:8,v/v), standard peak in fig 1B was better but when applied in the test sample paederosidic acid peak in fig 1C has not been completely separated from the other peaks. With the modified of the solvent ratio ACN: H_3PO_4 0.1% (15:75, v/v), the results found that the standard peak was symmetrical (fig 1D) and was also seperated from the adjacent peaks in the test sample (fig 1E).

After the experiment, the following HPLC conditions were used: HPLC analyses were performed on an HPLC system (Shimadzu, Japan) equipped with vaccum degasser, quaternary gradient pump, auto-sampler, Diode Array detector. A Shimadzu C_{18} column (250×4.6mm, 5µm) were used for separation of compounds. The mobile phase was run in an isocratic mode with 0.1% acid phosphoric and acetonitrile (85:15, v/v) at a flow rate of 0.8 mL min^{-1}. The injection volume was 20 µL. Detection was set at a wavelength of 234 nm. The run time was 30 min.

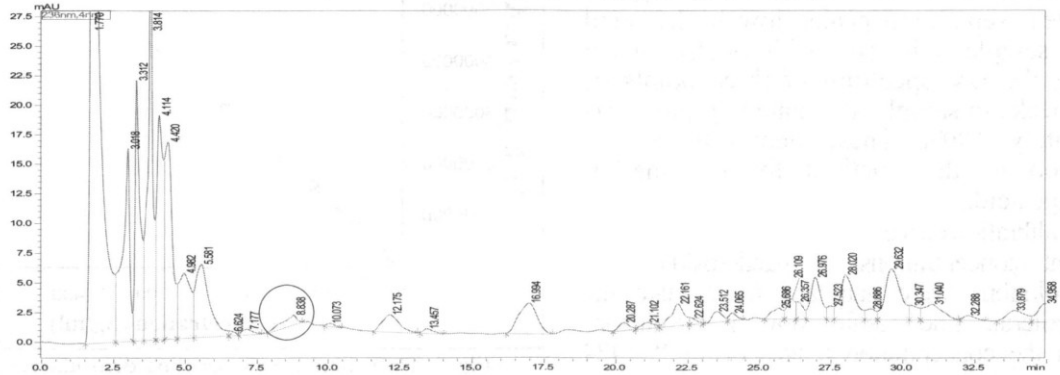
a) MeOH: H_3PO_4 0.1% (30:70)



b) ACN: H_3PO_4 0.1% (20:80)



c) ACN: H_3PO_4 0.1% (20:80) (in the test sample)



d) N: H_3PO_4 0.1% (15:75)

e) ACN: H_3PO_4 0.1% (15:75) (in the test sample)

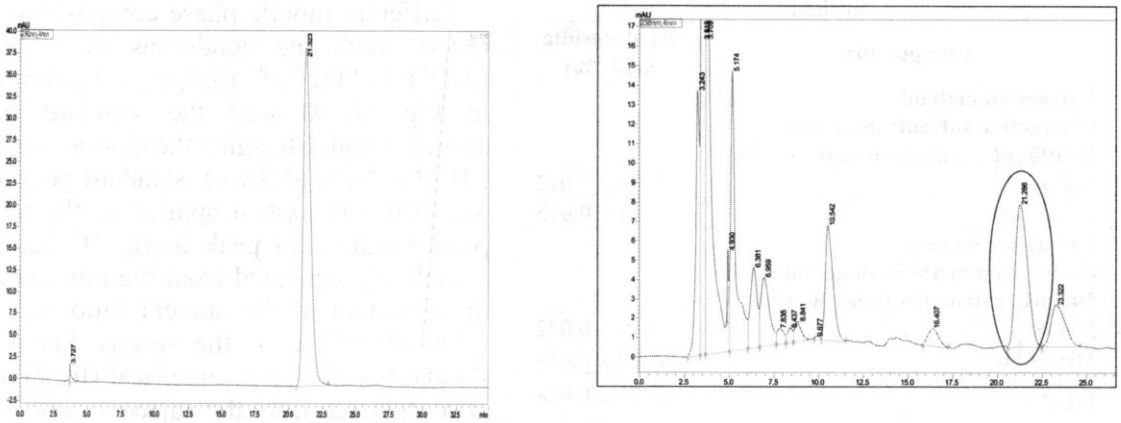


Fig. 1. The experiment for the chromatographic conditions

3.2.2. System suitability:

Analyzing the paederosidic acid standard solution 6 times, consecutively. The results showed that RSD of retention time, peak area are 0.21% and 0.22%, respectively These indicate that the developed method ensures system suitability criterion. The system validation was performerd on the standard solution with the concentration of 53 µg/mL.

3.2.3. Specificity:

The experiment was performed with the blank, standard and sample solutions according to the analytical procedure (section 2.2). The obtained chromatograms showed in Fig 2.

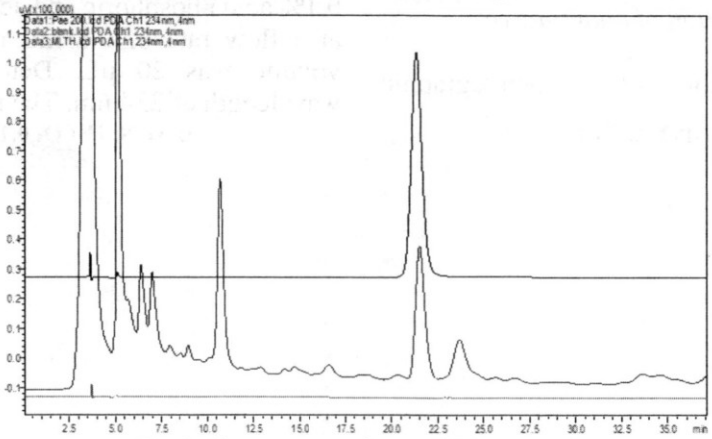


Fig. 2. Chromatogram for specificity research

The result displayed that in the chromatogram of sample solution, there was a peak that had a similar retention time (21.39 mins) with the peak of paederosidic acid standard solution's chromatogram and did not appear in the blank solution's chromatogram. The obtained chromatograms showed clear separation peaks, low background noise for sample solution. Additionally, when comparing the UV spectrum of three points of analyzed peaks in sample, the matching ratio was approximately 100% These demonstrated the specification of this method for the marker paederosidic acid.

3.2.4. Calibration curve:

Different concentrations of paederosidic acid standard solutions were analyzed to validate the linearity criteria. The result showed satisfactory linearity in the concentration ranges of 13.25 -424 µg/mL for paederosidic acid (Fig 3).

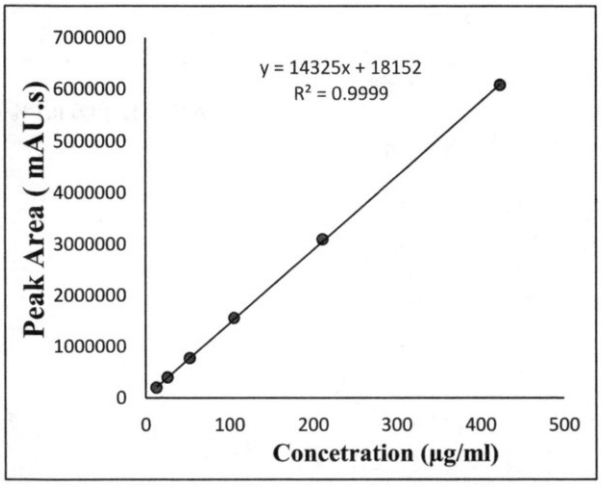


Fig. 3. The calibration curves of paederosidic acid

Linear correlation coefficient (R^2) values were above 0.999, as well as each calculated concentration of each calibration point being within ±15% of the true value. Therefore, it can be concluded that this developed method conforms the linearity.

3.2.5. Precision:

The intra- and inter-day precision have been summarized in Table 2 . The result of quantifying six samples showed that RSD of sample content are acceptable for all analytes. Therefore, the developed method fits the precision criteria.

Table 2. The result of precision test.

Sample	Intra-day			Inter-day		
	Weight (g)	Peak area (mAU)	Content (%)	w (g)	Peak area (mAU)	Content (%)
1	1.0303	1341340	0.2345	1.0273	1292396	0.2278
2	1.0157	1325767	0.2351	1.0303	1341340	0.2345
3	1.0259	1300908	0.2283	1.1002	1448194	0.2385
4	1.0912	1440293	0.2378	1.0107	1275301	0.2285
5	1.0144	1282295	0.2276	1.005	1308424	0.2358
6	1.0645	1330237	0.2250	1.0121	1304950	0.2335
Mean			0.2314			0.2331
RSD (%)			**2.19**			**1.80**

3.2.6. The limit of detection (LOD) and limit of quantification (LOQ):

The sensitivity of the method was determined about the limit of detection (LOD) and the limit of qualification (LOQ) by comparing the height of a sample peak and the height of a noise peak. LOD and LOQ were calculated by diluting sample solutions until signal-to-noise ratios of 3 and 10, respectively. For paederosidic acid, the LOD, LOQ were found to be 0.18 and 0.54 µg/mL.

3.2.7. Accuracy:

The accuracy of the method was tested by measuring the recovery of three different concentration levels of standard addition (50, 100, and 150%) which added into a known test sample then extraction.. Each concetration level was analyzed in triplicate. The results were presented in Table 3. The result was in good agreement with acceptable values for validation of an analytical procedure (the average recovery was in the allowed range 95% - 105%).

Table 3. The results of the accuracy test

Experiment no	Amount added (µg/ml)	Recovery concentration (µg/ml)	Recovery rate (%)	Mean ± SD (%)
1	44.029	42.847	97,315	97,347±0,166
2	44.029	42.940	97,527	
3	44.029	42.796	97,200	
4	88.056	88.545	100,555	100,682±0,110
5	88.056	88.717	100,750	
6	88.056	88.708	100,740	
7	146.083	150.716	103,171	103,669±0,440
8	146.083	151.938	104,008	
9	146.083	151.673	103,827	

3.2.8. Evaluating the contents of paederosidic acid in the collected sample:

The proposed validated HPLC method was applied for detection and qualification of paederosidic acid from *Paederia scandens* which was harvested in the different region in Vietnam. Each sample was analyzed in triplicate to determine the mean content (%). The results were summarized in Table 4.

Table 4. Content of paederosidic acid in 8 samples collected in Vietnam

No	Sample Name	Sample Information		Moisture contents (%)	Paederosidic acid content (M±SD) (%), (n=3)
		Scientific name	*Place*		
1	MLTB1	*P. scandens*	Dinh Phung, Kien Xuong, Thai Binh	9.04	0.0299 ± 0.005
2	MLTB2	*P. tomentosa*	Kien Xuong, Thai Binh	9.17	0.0246 ± 0.002

3	MLTB3	*P. scandens*	Hoa Binh, Kien Xuong, Thai Binh	9.53	0.0279 ± 0.003
4	MLTB4	*P. scandens*	Quang Lich, Kien Xuong, Thai Binh	9.02	0.0075 ± 0.007
5	MLTB5	*P. scandens*	Binh Minh, Kien Xuong, Thai Binh	9.89	0.0183 ± 0.006
6	MLTH	*P. scandens*	Thanh Hoa	8.08	0.2339 ± 0.002
7	MLML	*P. tomentosa*	Muong Lat, Thanh Hoa	9.52	0.0166 ± 0.005
8	MLĐN	*P. scandens*	Dong Nai	8.26	0.0629 ± 0.004

Table 4 showed that the content of paederosidic acid varied from 0.0075 -0.2339% with the MLTH sample had the highest content of paederosidic acid (reaching 0.2339%), besides that of the *P. tomentosa* sample had a lower concentration of 0.0166% than that of the *P. scandens* sample collected in Thanh Hoa. This differential may be because of the harvesting season, as well as the growing area's climatic and soil conditions.

Paederia scandens (Lour) Merrill is a climbing plant, widely grown around Vietnam and also in India, China, Japan, Philippines and USA. Extracts of the roots, leaves, bark, or fruits are used to treat abscessed teeth, chest pains, piles, kidney and liver inflammation, and as a depressant and purgative [1]. Over time, researches are revealed that glucosides, like paederosidic acid, isolated from *Paederia scandens*, have some pharmacological effects and are applied to support the treatment of diseases such as cancer, inflammation, convulsant... [5],[12].

Along with the research interest in the pharmacological effects of *Paederia scandens*, ensuring and standardizing the source of medicinal herbs also plays a very important role. Studying on standardization of *Paederia scandens* can not only support in establishing the appropriate concentration of *bioactive* compounds contained in herbal medications, but also certifying their quality. Until recently, there are very few studies evaluating paederosidic acid content at different regions. Most of the studies focus on isolating the chemical composition and pharmacological effects of *Paederia scandens* species. A suitable method for quantitative analysis of *Paederia scandens* plant is mandatory. Liquid chromatography is an accessible and popular technical method for qualitative and quantitative analysis of active components in the herbal drugs [10],[13]. The outcomes in this study will be beneficial in standardization and evaluation quality of *Paederia scandens* in Vietnam.

4. Conclusion

A simple, accurate HPLC-DAD method has been developed to quantify paederosidic acid in *Paederia scandens* collected in Vietnam. The method was successfully validated with good linearity, precision, repeatability and accuracy. Utilizing this HPLC method, the paederosidic acid contents in *Paederia scandens* sample were measured. The results showed a trend of range in paederosidic acid contents, which could be a supportive marker for an proper harvest site and time. Finally, the HPLC method may efficiently provide to the quality control of this valuable herb in the time to come.

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