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ISOLATION AND SIMULTANEOUS QUANTIFICATION OF PHENOLICS FROM *FLOS LONICERAE* BY HPLC-DAD

Tran Thi Huyen[1,2], Phung Thai Han[3], Huynh Loi[4,*], Le Van Ut[4], Tran Huyen Tran[4], Huynh Ngoc Thuy[3]

[1]*School of Medicine, Vietnam National University of Ho Chi Minh City, Vietnam;*

[2]*Research Center for Genetics and Reproductive Health - CGRH, School of Medicine, Vietnam National University of Ho Chi Minh City, Vietnam;*

[3]*University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam;*

[4]*Faculty of Pharmacy, Binh Duong University, Vietnam*

*Corresponding author: huynhloivn@gmail.com

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Summary

Isolation and Simultaneous Quantification of Phenolics from *Flos Lonicerae* by HPLC-DAD

The aim of this study was to isolate, elucidate the structures, and develop an HPLC method for the simultaneous quantification of phenolic compounds in *Flos Lonicerae*. The isolation of the main compounds was carried out by using column chromatography. The structures were elucidated by spectroscopic methods. The isolated compounds, including chlorogenic acid (CA) and 3,5-dicaffeoyl quinic acid (DCQA), were used as reference compounds to develop a method for quantification. The developed method was validated for linearity, limit of detection (LOD), limit of quantitation (LOQ), system suitability, specificity, precision and accuracy. The method was linear with r^2 = 0.9999. The Limit of Detection and the Limit of Quantification were 0.005; 0.005 µg/mL and 0.016; 0.016 µg/mL for CA and DCQA, respectively. The method was successfully applied for the analysis of two major phenolic acids in four samples of *Flos Lonicerae* collected from various region in Vietnam. The contents were 4.30 - 5.60% and 1.97 - 2.99% for CA and DCQA, respectively. Our study may be a good suggestion for the quality control and standardization of *Flos Lonicerae* in the future.

Keywords: *Kim ngân, Flos Lonicerae; 3,5-dicaffeoyl quinic; Chlorogenic acid.*

1. Introduction

The Caprifoliaceae (Honeysuckle family, Latin for "goat leaf") consists of 5 genera including 220 species which are widely distributed in temperate and subtropical zones [1],[2]. More than 140 chemical compounds from *Lonicera japonica* have been isolated, with the main compositions being essential oils, organic acids, saponins, iridoids, and flavonoids. The essential oils exist in the aerial parts, flowers (fresh and dry), leaves, and vines. The contents and components of essential oils are different due to the habitat, harvest time, medicinal parts, extraction methods, and processing. Organic acids are isolated in *Lonicera japonica* including chlorogenic acid (CA), isochlorogenic acid, caffeic acid, hexadecanoic acid, hexadecanoic acid, myristic acid, 3,5-O-dicaffeoyl quinic acid, 4,5-O-dicaffeoylquinic acid, 3,4-O-dicaffeoyl quinic acid (DCQA), 1,3-O-dicaffeoylquinic acid, 3-ferulicoyl quinic acid, and 4-ferulicoyl quinic acid. About 30 flavones have been isolated from *Lonicera japonica* including quercetin-3-O-β-D-glucoside, luteolin-7-O-β-D-glucoside, luteolin-7-O-β-D-galactoside, hyperoside corymbosin, 5-hydroxy-3′,4′,7′-trimethoxylflavone, biflavones, 3′-O-methyl loniflavone and loniflavone. More than 30 iridoids have been found in *Lonicera japonica*, such as loganin, sweroside, secoxyloganin, secologanin, kingiside, ketologanin, 7α-morroniside, 7β-morroniside, and secologanoside. The types of saponins from *Lonicera japonica* are oleanane and hederagenin. Extracts from *Lonicera japonica* have shown various activities, including anti-inflammatory, antiviral, antibacterial, antioxidant, hepatoprotective, and anti-tumor activities...[3] The leaf-bearing stem is an antibacterial and antiallergic agent, and is recommended for the treatment of boils, impetigo, urticaria, allergic rhinitis, fever, malaria, erythema, measles, diarrhea, dysentery, syphilis, rheumatism, and lichen tropics [4]. The daily dose is 4 to 8 g of flowers or 10 to 20 g of stems and leaves in the form of a decoction, infusion, extract, or alcoholic maceration [4]. Up to date, caffeoylquinic acid (CQA) and CQA-based compounds have been exhibited to show important health benefits such as antioxidant, antihistaminic, antibacterial, antiviral, and anticancer activities. As a part of our continuous effort to develop natural products with bioactivities, DCQA, a bioactive derivative of CQA, has been shown to possess antiobesity and photoprotective antioxidant abilities [5]. In Chinese Pharmacopoeia 2015, the Flos Lonicerae contains not less than 1.5 per cent of CA and not less than 0.05 per cent of luteolin-7-O-glycose, calculated with reference to the dried drug [6]. In Vietnamese Pharmacopoeia V, it contains not less

than 1.5 per cent of CA, calculated with reference to the dried drug [7]. Chlorogenic acid (5-caffeoyl quinic acid) can be formed by hydrolysis of DCQA which is decomposed by enzymes or chemicals. Therefore, the quantitative analysis of CA only can be not precise. The aim of this study is to isolate the main compounds and develop the HPLC method for simultaneous quantification of these compounds in *Flos Lonicerae*.

2. Materials and methods

2.1. Plant materials

Four samples of *Flos Lonicerae* using for the present study were collected in March 2013. Sample ID A, B were purchased from Cholon traditional herbal markets in Ho Chi Minh City. Sample C was obtained in Hanoi and sample D was purchased in Moc Chau, Son La, in the north of Vietnam. Sample A was used for the isolation of reference compounds and for the validation of the analytical method. The samples were identified by comparing with criteria for *Flos Lonicerae* monograph in Vietnamese Pharmacopoeia V.

2.2. Chemicals and reagents

The reference substances of chlorogenic acid (1) and 3,5-dicaffeoyl quinic acid (2) were isolated from *Flos Lonicerae* in the present study. The structures of the isolated compounds were elucidated by comparing their spectral data (MS and NMR) with published data. The purities of 1 and 2 were 100% and 93%, respectively, as determined by HPLC analysis. HPLC grade solvents including acetonitrile, methanol, formic acid, and double distilled water were purchased from Merck (Merck KGaA, Darmstadt, Germany). *n*-hexane, chloroform, ethyl acetate (EtOAc), and ethanol (Chemsol, Vietnam) were of analytical reagent grade.

2.3. Instruments

The HPLC analysis was performed using a Waters 2695 HPLC system (USA) equipped with an autosampler and coupled to variable UV wavelength detector. The column was a Sunfire C_{18}, 5 µm, 4.6 mm in internal diameter and 250 mm in length (Waters, USA). The isolation was carried out using MPLC Chromatography Systems BioLogic QuadTec™ UV/Vis Detector (USA) With Four Wavelengths Simultaneously and medium-pressure chromatographic column (460 × 35 mm; *silica gel*, 40-63 µm, Merck). Mass spectrometry was performed in a Micromass Quattro Micro API (Waters, Milford, MA, USA). NMR spectra were recorded on Bruker Avance 500 MHz.

2.4. Place of study

This study was held at Laboratory of Department of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh city, Vietnam.

2.5. Methods

2.5.1. Extraction and isolation of reference compounds:

The extraction and isolation were carried out by using various methods such as percolation, liquid-liquid extraction, flash column chromatography, *silica gel* column chromatography, and Medium Pressure Liquid Chromatography (MPLC).

2.5.2. Structure elucidation:

The structures were elucidated by using mass spectra and NMR (1D, 2D) spectra.

2.5.3. The HPLC chromatographic conditions:

The HPLC chromatographic conditions including wavelength, elution condition, and flow rate were investigated in this study.

2.5.4. Preparation of reference solutions:

Stock solutions of 1 and 2 were initially prepared as 500 µg/mL and 465 µg/mL in 70% MeOH, respectively. These stock solutions then were serially diluted to give nine working reference solutions with a concentration range 1 - 500 µg/mL and 1 - 465 µg/mL for 1 and 2. All the dilutions were used for plotting calibration curves of each compound.

2.5.5. Preparation of sample solutions:

The preparation of sample solutions including solvents, temperature, and extraction times were studied.

2.5.6. Validation of the analytical method:

Developed method was validated for linearity, limit of detection (LOD), limit of quantitation (LOQ), system suitability, specificity, precision and accuracy according to International Conference on Harmonization (ICH) guidelines [8].

Linearity was determined by using CA (1) and DCQA (2) reference solutions in 70% methanol at concentrations of 500, 400, 320, 256, 204, 102, 20.5, 10, 1 µg/mL and 465, 372, 297.5, 238, 189.5, 95, 19, 9.5, 1 µg/mL, respectively. Each concentration was analyzed in triplicate using an HPLC method as described above. The calibration curves were obtained by plotting the peak area from the chromatogram versus the concentration of the two reference substances.

Diluted reference substances were made with 70% methanol and were then analyzed using an HPLC method as described above. The LOD was determined at a signal-to-noise ratio (S/N) of 3 and the LOQ was determined at an S/N ratio of 10.

The suitability of the system was determined by a single injection of the solution and five replicate injections of the reference solution and

analyzed using an HPLC method as described above. The retention time (Rt), peak areas (S), number of theoretical plates (N), capacity factor (k'), tailing factor (T) were used as the system suitability parameters.

Seventy percent methanol was used as a blank solution (i). Reference solution was composed of CA and DCQA at the concentration of 0.1 mg/mL to be prepared with 70% methanol (ii). *Flos Lonicerae* sample solution (iii) was prepared as described above. 100 µL of reference solution then was added to 100 µL of *Flos Lonicerae* sample solution (iv). Each solution was analyzed using an HPLC method as described above.

The precision was determined by six analyses of samples which were prepared and analyzed using proposed methods above. The results were expressed as percent relative standard deviation (% RSD).

Accurate amount of standard solutions with three different concentration levels were added to sample solution and then analyzed in triplicate using an HPLC method as described above. The percentage of recovery was calculated as follows:

Recovery (%) = 100 × (amount found - original amount)/amount spiked

The yield (%) of CA and DCQA in samples was calculated as formula

$$X = \frac{St}{Sc} \times Cc \times \frac{k}{m\,(1-h)} \times p \times 100$$

Where: X: yield (%); Sc: peak area of reference compound; St: peak area of reference compound; Cc: concentration of reference compound (mg/ml); k: dilute volume (1.5 mL), m: sample weight (mg); h : loss on drying; p : purity of reference compound (%)

3. Results

3.1. Isolation and elucidation of phenolic compounds

Dried and powdered *Flos Lonicerae* (sample ID A; 3 kg) were percolated with 30 L of 96% EtOH at room temperature. The solvent was removed under reduced pressure to yield 600 g of crude extract, which was suspended in 1.0 L of water and subsequently partitioned with n-hexane, $CHCl_3$, EtOAc, and then *n*-BuOH. Each of the combined organic layers was evaporated to dryness, affording *n*-hexane (Lj-H, 150 g), $CHCl_3$ (Lj-C, 80 g), EtOAc (Lj-E, 30 g), and *n*-BuOH (Lj-B, 120 g) fractions.

The EtOAc fraction (Lj-E, 30 g) was subjected to a flash column chromatography (8 × 60 cm) and eluted using $CHCl_3$-MeOH mixtures (100:1 → 50:50, v/v). The eluates were monitored by TLC analysis to afford five subfractions (Lj-E1 to Lj-E5). Fraction Lj-E4 (5 g) was re-chromatographed over a *silica gel* column chromatography (1.5 × 30 cm), eluted with a gradient solvent system of $CHCl_3$–EtOAc (90:10 → 10:90, v/v), to give five combined subfractions (Lj-E4.1 to Lj-E4.5). Compound **1** (30 mg) was obtained from subfraction Lj-E4.5 by recrystallization. Subfraction Lj-E4.4 (900 mg) was dissolved in distilled water (5 ml) and loaded onto the MPLC loop. The mobile phase was MeOH (solvent A) and water (solvent B). The elution was started with 20% A (0-5 min), 20-50% A (5-50 min). The flow rate was from 5 mL/min while the single detection wavelength was 325 nm. These separations afforded compounds 2 (176 mg). The compound **1** and 2 were used as reference substances for quantitative analysis.

Repeated *silica gel* column chromatography and MPLC of the EtOAc fraction of the ethanolic extract of *Flos Lonicerae* furnished compounds 1 and 2. Compound **1** was obtained as a light-yellow amorphous solid. ESI-MS in the negative mode revealed the presence of a [M-H]- ion at *m/z* 353.54, corresponding to the molecular formula $C_{16}H_{18}O_9$. The NMR spectrum of compound **1** in DMSO was recorded and the resonance values were compared with previously published data [9], compound **1** was assigned as chlorogenic acid (CA).

Table 1. NMR Chemical Shifts of Compounds 1 and 2 in DMSO

C position	DEPT	δ_C (125 MHz; ppm)		δ_H (500MHz; ppm, *J*)	
		1	2	1	2
7	C		176.3		
9″	C		165.6		
9′	C	166.3	166.1		
4′	C	148.5	148.4		
4″	C		148.2		
3′; 3″	C	145.7	145.6		
7″	CH		145.1		7.46 (1H; *d*; 16Hz)
7′	CH	144.6	144.7	7.44 (1H; *d*; 16Hz)	7.42 (1H; *d*; 16 Hz)
1′	C	125.5	125.6		
1″	C		125.5		
6″	CH		121.1		7.05 (1H; *dt*; 7; 3 Hz)
6′	CH	121.2	121.3	6.97 (1H; *dd*; 8; 2Hz)	6.99 (1H; *dt*; 7; 3 Hz)
5″	CH		115.7		6.78 (1H; *dd*; 8; 5 Hz)

C position	DEPT	δ_C (125 MHz; ppm)		δ_H (500MHz; ppm, *J*)	
		1	2	1	2
5′	CH	115.8	115.8	6.74 (1H; *d*; 8.5 Hz)	6.78 (2H; *dd*; 8; 5 Hz)
8′	CH	114.6	114.9	6.22; (1H ; *d*; 16 Hz)	6.16 (1H; *dd*; 8; 2 Hz)
8″	CH		114.8		6.25 (1H. *d*. 16 Hz)
2″	CH		114.7		6.24 (1H; *d*; 16 Hz)
2′	CH	114.6	114.1	7.05 (1H; *d*. 2Hz)’	6.16 (1H; *d*; 16 Hz)
1	C	75.2	72.5		
3	CH	71.5	70.9	3.89 (1H ; *d*. 3Hz)	5.13 (1H; *d*; 7 Hz)
5	CH	71.7	70.7	5.16; (1H; *m*)	5.19 (1H; *m*)
4	CH	73.3	68.0	3.45; (1H ; *dd*, 10; 3)	3.82 (1H; *t*; 7 Hz)
2	CH2	38.1	34.7	1.97; (1H; *dd*, 11.5; 3 H2 ax) 1.60; (1H; *td*; 14; 3Hz)	1.95; 2.12 (2H; *m*)
6	CH2	39.0	34.7	1.81 (2H. *t*. 12 Hz)	1.95; 2.12 (2H; *m*)

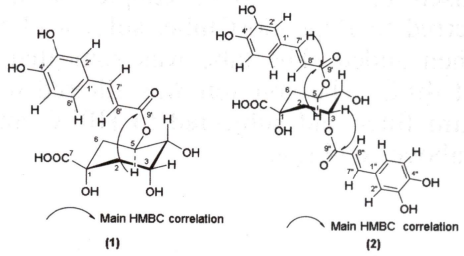

Fig. 1. Chemical structure of Compound 1 and 2

Compound **2** was isolated as grey-white amorphous solid. ESI-MS in the positive mode revealed the presence of a [M+K]+ ion at *m/z* 555.48, and the negative mode of [M-H]- ion at *m/z* = 515.48, corresponding to the molecular formula $C_{25}H_{24}O_{12}$. The NMR spectrum in DMSO of compound 2 was recorded. Compound **2** was assigned as 3,5-dicaffeoyl quinic acid (DCQA) by comparison to reported data previously [10].

3.2. The HPLC chromatographic conditions

3.2.1. Investigation of purity and detection wavelength:

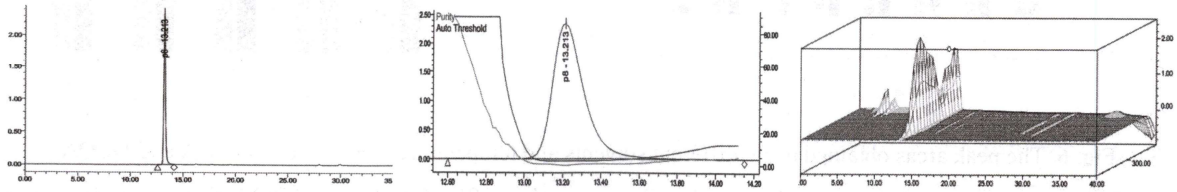

Fig. 2. HPLC chromatogram and purity test of CA (reference substance)
(HPLC condition mentioned in 3.2.2, CA concentration was 100 µg/mL in MeOH)

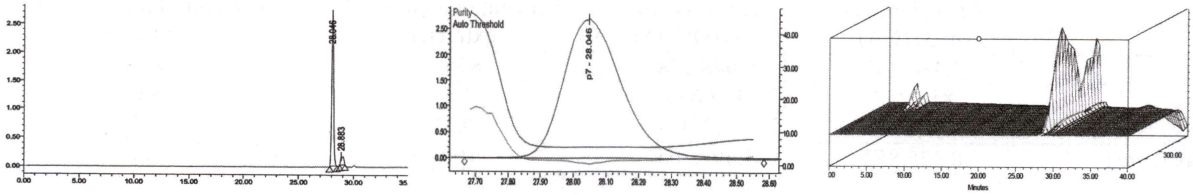

Fig. 3. HPLC chromatogram and purity test of DCQA (reference substance)
(HPLC condition mentioned in 3.2.2, DCQA concentration was 100 µg/mL in MeOH)
The purity of CA and DCQA are 100% and 93%, respectively (Fig. 2 and 3)

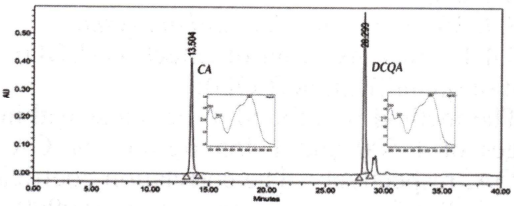

Fig. 4. Chromatogram of HPLC analysis and UV Spectra of both reference substances (CA and DCQA)
At 325 nm, two compounds have the similar maximum absorbance. This wavelength was set for quantitative analysis (Fig. 4).

3.2.2. Investigation of elution conditions:

a. Isocratic elution

The compounds ware not separative in the isocratic elution (Fig. 5).

b. Gradient elution

HPLC analysis was performed on above column eluted with acetonitrile (A) and 0.1% formic acid (B) in gradient elution. The gradient program was as follows: 0-15 min changed from 10%A to 20% A; 15-30 min changed from 20% A to 30% A;. The flow rates were 1.2; 1.0 and 0.8 mL/min. The

column temperature was 30 degrees and the detection wavelength was 325 nm.

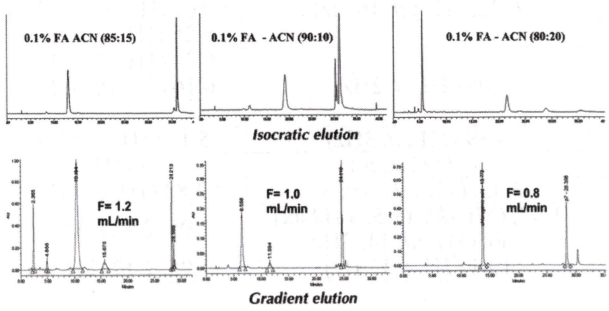

Fig. 5. Chromatogram of HPLC analysis with various elutions and flow rates

Flow rate of 0.8 mL/min was good for separation. The HPLC chromatographic conditions were summarized as below.

The separation of the reference substance mixtures and extracts were assessed by analytical HPLC, using acetonitrile (C) and 0.1% formic acid in water (D) as the mobile phase with the gradient mode of elution. The following multistep linear gradient was applied: 0-15 min, 10% to 20% C; 15-30 min, 20% to 30% C; 30-35 min, 30% to 90% C. The flow rate was 0.8 mL/min and injection volume was 20 µL. Detector wavelength was set at 325 nm and the operating temperature was maintained at 30°C.

3.3. Preparation of sample solution

Methanol and ethanol with various per cents were used for investigation. Sample (40 mg) was transferred to Eppendorf tube, solvent (1.5 mL) was then added. The tube was sonicated for 5 min at 40°C. The solution was filtered using a 0.45 µm filter and subjected to HPLC analysis using above condition.

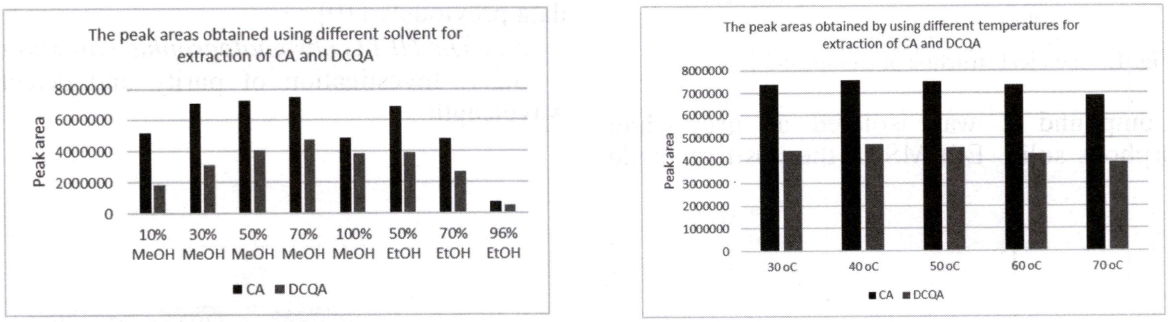

Fig. 6. The peak areas obtained using different solvents and temperatures for extraction of CA and DCQA

The 70% methanol at 40°C is good condition for extraction of both CA and DCQA from sample (Fig. 6).

Table 2. The peak areas obtained in various extraction times

Extraction times	Area (Smean) (n=3) (CA)	Area (Smean) (n=3) (DCQA)	Per cent of area of CA extracted	Per cent of area of DCQA extracted
1	7,486,241	5,048,278	89.9%	91.8%
2	818,257	439,656	9.8%	8.0%
3	21,354	9,271	0.3%	0.2%
Total area	8,325,852	5,497,205	100%	100%

Both CA and DCQA were exhaustively extracted with three times (Table 2).

After investigation of solvent, temperature for extraction, and extraction times, the procedure of extraction was summarized as below.

Flos Lonicerae sample powder (40 mg) was accurately weighed into an Eppendorf (1.5 mL), followed by addition of 1.5 mL of 70% methanol. The mixture was vortexed for 1 minute and extracted twice by ultrasonic-assisted extraction at 40°C for 5 minutes. The mixture then centrifuged for 5 minutes at 5000 rpm; the supernatant was adequately transferred to a 10 mL volumetric flask and completed with 70% methanol to the mark, filtered through a 0.45µm membrane filter before use.

3.4. Method validation and analysis

3.4.1. Linearity, limit of detection (LOD), and limit of quantification (LOQ):

The method was found to be linear within the ranges of 1-500 and 1-465 µg/mL for CA and DCQA, respectively. The correlation coefficients (r2) of all reference substances were 0.9999. The limit of detection (LOD) were 0.005, 0.005 µg/mL and limit of quantification (LOQ) were 0.016, 0.016 µg/mL for CA and DCQA, respectively (Table 3).

Table 3. Linear ranges, regression equations, LOD and LOQ data of CA and DCQA

Compounds	Linear range (μg/mL)	Regression equation*	r2	LOD (μg/mL)	LOQ (μg/mL)
CA	1 - 500	y = 57312x - 247312	0.9999	0.005	0.016
DCQA	1 - 465	y = 76821x - 33109	0.9999	0.005	0.016

*x represents the concentrations of CA and DCQA in μg/mL; y represents the peak area, detected at UV 325 nm.

3.4.2. System suitability:

Table 4. Results of system suitability tests of HPLC method

No of injection	RT		S		k'		N (Theoretical plates)		T (tailing)	
	CA	DCQA	CA	DCQA	CA	DCQA	CA	DCQA	CA	DCQA
1	13.77	28.40	7787433	5048278	8.8	19.3	35000	139380	1.24	1.19
2	13.25	28.10	7859327	5140529	8.5	19.1	34510	136111	1.25	1.19
3	13.34	28.12	7851845	5138359	8.5	19.1	35556	134281	1.25	1.19
4	13.42	28.26	8045917	5269153	8.6	19.2	35154	132736	1.26	1.19
5	13.20	28.07	7890819	5178945	8.4	19.0	33904	136574	1.26	1.19
6	13.44	28.27	8045083	5178098	8.6	19.2	34872	135511	1.26	1.17
Mean	13.40	28.20	7913404	5158894	8.6	19.1	34833	135765	1.26	1.19
RSD (%)	1.53%	0.46%	1.36%	1.40%						

The system is suitable for anlalysis with RSD values less than 2% (Table 4).

3.4.3. Specificity:

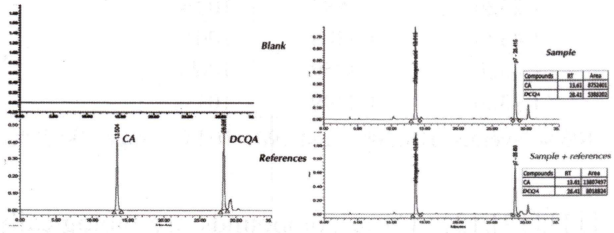

Fig. 7. Chromatogram of specificity test in HPLC method

The control for sample testing, no peak was observed (data not shown), indicating the dissolving solvent (70% methanol, blank) did not interfere with the results for the two compounds separation. The results indicated that quantitative method was validated for specificity (Fig. 7).

3.4.4. Precision:

Table 5. Precision test of HPLC method

	Sample weight (mg)		Peak area		Amount (mg)		Percent (*)	
Sample No	CA	DCQA	CA	DCQA	CA	DCQA	CA	DCQA
1	40	40	8777715	5356185	7.86	3.51	4.2%	1.94%
2	40	40	8895120	5577029	7.78	3.65	4.3%	2.02%
3	40	40	8896813	5382974	7.78	3.52	4.3%	1.95%
4	40	40	8785494	5413173	7.69	3.54	4.3%	1.96%
5	40	40	8967588	5514595	7.85	3.61	4.3%	2.0%
6	40	40	8738358	5462255	7.65	3.58	4.2%	1.98%
Mean	40		8843515	5451035	7.74	3.57	4.3%	1.97%
SD			89053	83789	0.08	0.05	0.0004	0.0003
RSD %			1.01%	1.54%	1.00%	1.53%	1.00%	1.53%

(*) calculated calculated with reference to the dried drug.

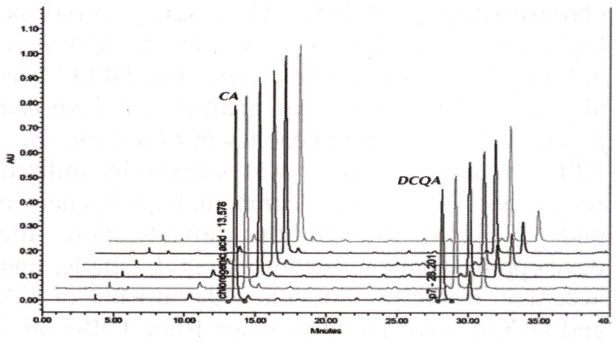

Fig. 8. Chromatogram of precision test in HPLC method

The method also demonstrated acceptable precision, with RSD values at 1% and 1.53% for CA and DCQA, respectively (Table 5 and Fig. 8).

3.4.5. Recovery: (Table 6).

3.4.6. Simultaneous quantification of CA and DCQA in samples collected:

Simultaneous quantification of the two phenolic acids from four samples ID A, B, C, and D was carried out using the established analytical method. The results are as indicated in table 7. The contents of CA were 4.30%, 5.60%, 5.50%, and 4.60% (w/w) for A, B, C,

and D of dried powder, respectively. The contents of DCQA were 1.97%, 2.60%, 2.93%, and 2.99% (w/w) for A, B, C, and D, respectively. All percentages were calculated with reference to the dried drug (Table 7 and Fig. 9).

Table 7. Recovery data of HPLC method

Refences	Peak area	Amount taken (mg)	Amount added (mg)	Amount (Theoretical) (mg)	Amount (found) (mg)	Recovery (%)	Average (%)
CA	10927393	1.8590	0.4	2.2590	2.2971	102%	101.2%
	10725741	1.8590	0.4	2.2590	2.2547	100%	
	11021060	1.8590	0.4	2.2590	2.3168	103%	
	12584273	1.8590	0.8	2.6590	2.6454	100%	
	12930702	1.8590	0.8	2.6590	2.7182	102%	
	13002165	1.8590	0.8	2.6590	2.7333	103%	
	14351709	1.8590	1.2	3.0590	3.0169	99%	
	15001560	1.8590	1.2	3.0590	3.1535	103%	
	14379620	1.8590	1.2	3.0590	3.0228	99%	
DCQA	6744148	0.8320	0.2	1.0320	1.0294	100%	100.8%
	7019006	0.8320	0.2	1.0320	1.0713	104%	
	6790449	0.8320	0.2	1.0320	1.0365	100%	
	8061473	0.8320	0.4	1.2320	1.2305	100%	
	8107344	0.8320	0.4	1.2320	1.2375	100%	
	8244164	0.8320	0.4	1.2320	1.2584	102%	
	9374213	0.8320	0.6	1.4320	1.4308	100%	
	9407614	0.8320	0.6	1.4320	1.4359	100%	
	9499169	0.8320	0.6	1.4320	1.4499	101%	

The recovery values of CA and DCQA ranged between 99 - 103% (average 101.20%) and 100 - 104 (average 100.78%), respectively (Table 6).

Table 8. Contents of CA and DCQA (%) in the different samples of *Flos Lonicerae*

Sample ID	CA	DCQA
A	4.30 ± 0.16	1.97 ± 0.11
B	5.60 ± 0.15	2.60 ± 0.17
C	5.50 ± 0.25	2.93 ± 0.12
D	4.60 ± 0.16	2.99 ± 0.15

Data are mean ± standard deviation obtained by triplicate analyses, comtent was calculated with reference to the dried drug

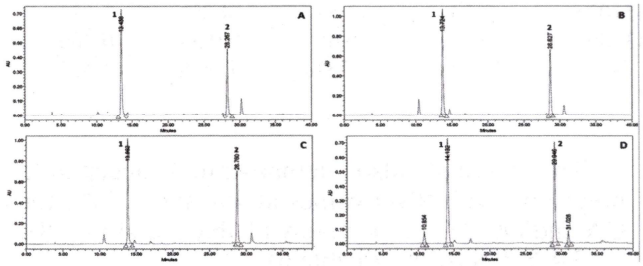

Fig. 9. HPLC chromatogram of 70% MeOH extracts of four *Flos Lonicerae* samples at 325 nm (1: CA, 2: DCQA, A, B, C, D: Samples collected)

4. Discussion

CA and DCQA are two of the most available phenolic acids that can be naturally found in herbs, fruits (e.g., apples, pears, many berries), vegetables, and especially green coffee beans [11],[12],13]. These compounds are biologically active polyphenols, playing important roles in therapeutic treatments such as antioxidant, antibacterial, hepatoprotective, cardioprotective, and anti-inflammatory activities [14]. Using High Speed Counter-Current Chromatography (HSCCC) and Prep-HPLC Guided by DPPH-HPLC Experiments, flavonoid glycosides and caffeoylquinic acid derivatives including 3,5-O-dicaffeoylquinic acid from leaves of *Lonicera japonica* were also isolated [15]. In this study, the conventional techniques were successfully used for isolation. Previously, CA and DCQA in *Lonicera japonica* collected from China were quantified by using high performance thin layer chromatography (HPTLC). The results showed the highest percentage content of CA and DQCA were 3.7 and 2.07%, respectively [16]. The HPLC was also used for quantitative analysis of *Lonicera japonica* strains or ten phenolics in *Flos Lonicerae*. HPLC with MS detector was sucessfully utilized for analysis of phenolic compounds in Lonicerae buds [17],[18],[19]. In this investigation, the developping analytic method is rapid, simple, and accuracy. In the present study, the amount of CA and DCQA were found to range from 4.30-5.60% and 1.97-2.99%, respectively, in various samples.

The amount of CA found in this study is higher than those in requirement of *Flos Lonicerae* meterial in both Chinese and Vietnamese Pharmacopoeia [6],[7]. The findings indicated that the yield of these active components of *Flos Lonicerae* varied with habitats.

5. Conclusion

Two main compounds were isolated and identified as CA and DCQA. A reliable and simple method was developed successfully for the quantitative analysis of two these major components using HPLC-DAD. The method was validated in terms of specificity, accuracy, precision, and system suitability of the main compounds. The results of this study might be beneficial for the standardization and developing the quality control profile of *Flos Lonicerae* by the phytopharmaceutical industries.

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QUANTITATIVE ANALYSIS OF TOTAL POLYPHENOLS IN THE LEAVES OF *TERMINALIA CATAPPA* L. BY UV-VIS ABSORPTION SPECTROSCOPY

Ha Van Oanh[a], Chu Thi Thanh Huyen[a], Nguyen Manh Tuyen, Nguyen Tien Dung, Do Quyen*

Ha Noi University of Pharmacy, Vietnam;

[a]These authors contributed equally to this manuscript as joint first authors

*Corresponding author: doquyen@hup.edu.vn

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Summary

Quantitative Analysis of total Polyphenols in the Leaves of *Terminalia catappa* L. by UV-Vis Absorption Spectroscopy

An UV-Vis absorption spectroscopy method had been developed and validated to quantify total polyphenols in the leaves of *Terminalia catappa* L. (TC). The TC leaf powder was extracted by ultrasonic method with 50% ethanol, followed by the reaction with Folin-Ciocalteu phenol reagent and photometric measurement at 730 nm. The developed method exhibited good linearity (R^2 = 0.9957) within a range of 1-8 µg/ml and LOD and LOQ were found to be 0.033 and 0.100 µg/ml, respectively. The developed method was also found accurate, and precise according to the regulatory guidelines. The developed method was applied to measure the total polyphenol content in samples collected from various provinces in Vietnam.

Keywords: *Terminalia catappa, Total polyphenols, UV-Vis.*