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SIMULTANEOUS QUANTIFICATION OF SCHAFTOSIDE AND ISOSCHAFTOSIDE FROM *HERBA DESMODII STYRACIFOLII* BY UPLC-PDA

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Summary

Desmodium styracifolium (Osbeck) Merr. (Fabaceae) is an important traditional medicinal herb used in many folk medicines to treat various diseases such as pyrexia, stranguria, renal calculi, dysuria, and oliguria. More than 50 chemical compounds have been isolated from *D. styracifolium*, including flavonoids, terpenoids, glycosides, phenols, and phytosterols. This study aimed to develop an UPLC-PDA method for the simultaneous quantification of schaftoside and isoschaftoside in *Herba Desmodii styracifolii*, and to apply the validated method to analyze their contents in various collected samples. The developed method was validated for linearity, limit of detection (LOD), limit of quantitation (LOQ), system suitability, specificity, precision, and accuracy. This method was linear with r^2 = 0.9999 for both compounds in the range of 0.5-500 mg/mL. The limits of detection and quantification were 0.125 and 0.41 µg/mL for schaftoside, and 0.0625 and 0.20 µg/mL for schaftoside and isoschaftoside, respectively. The method was successfully applied for the analysis of schaftoside, and isoschaftoside in six samples of *Herba Desmodii styracifolii* collected from various regions in Vietnam. The contents in the stem and leaf were found to be 0.319-427% and 0.034-0,012% for schaftoside and isoschaftoside, respectively. Besides, this content in the stem and leaf was also determined. Our study may be a good suggestion for quality control and standardization of *Herba Desmodii styracifolii*.

Keywords: *Desmodium styracifolium; Schaftoside; Isoschaftoside; Simultaneous quantification; UPLC-PDA*

1. Introduction

Desmodium styracifolium, namely Vảy rồng, Đồng tiền lông, Mắt trâu, Bươm bướm, Kim tiền thảo, Dây sâm lông, Mắt nai in vernacular Vietnamese, belongs to the Fabaceae family (Leguminosae) and is preferably abundantly found worldwide [1],[2]. *D. styracifolium* (Osbeck) Merr is an important traditional medicinal herb used in many folk medicines to treat various diseases such as pyrexia, stranguria, renal calculi, dysuria, and oliguria. In traditional Vietnamese medicine, *D. styracifolium* is a medicinal herb used to treat urinary tract infections, fever, kidney stones, cardiovascular diseases, and hepatitis. In traditional medicines, this plant has the properties and flavor as cool, sweet, and bland. Its meridian tropism is liver, kidney, and bladder meridians. It induces diuresis and relieves strangury, eliminates dampness and anti-icteric, detoxicates, and alleviates edema. The usage is dampness-dispelling medicinal (dampnessdraining diuretic medicinal) [2],[3],[4]. More than 50 chemical compounds have been isolated from *D. styracifolium*, including flavonoids, terpenoids, glycosides, phenols, and phytosterols. [2],[3]. Many bioactivities of extracts from *D. styracifolium* have been reported, including anti-urolytic, cholelytic, cholagogic, hepatoprotective, antioxidant, anti-inflammatory, and anti-diabetic effects [3],[4],[5]. The high-performance liquid chromatography fingerprint combined with the similarity analysis, hierarchical cluster analysis, principal component analysis, discriminant analysis, and the quantitative analysis of multi-components by single marker method were investigated. Four peaks were identified as schaftoside, isoorientin, isoschaftoside, and isovitexin [6]. Twenty-two phytophenol isomers in *D. styracifolium* have been simultaneously distinguished under the MS spectra negative model by using ultra-high-performance liquid chromatography coupled with hybrid quadrupole-orbitrap tandem mass spectrometry (UHPLC-Q-orbitrap MS/MS) technology [7]. In the Taiwan Pharmacopoeia 4th, the dried aerial parts of *D. styracifolium* contain not less than 3.0% of dilute ethanol-soluble extractives, not less than 5.0% of water extractives, and not less than 0.13% of schaftoside. The assay is carried out by using the HPLC method [4]. Until now, in the Vietnamese Pharmacopoeia V, this herb has not yet been quantified by the HPLC method [8]. This study aimed to develop an UPLC-PDA method for the simultaneous quantification of schaftoside, and isoschaftoside in *Herba Desmodii styracifolii* and

to use the developed method for analysis of its content in various samples collected. The structures of schaftoside and isoschaftoside are presented in **Fig 1.**

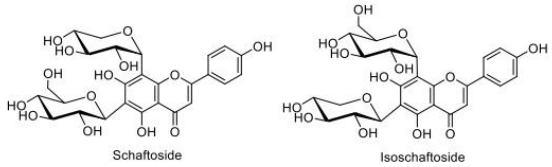

Fig. 1. Structures of schaftoside and isoschaftoside

2. Materials and methods

2.1. Plant materials

The aerial parts of *Desmodium styracifolium* (*Herba Desmodii styracifolii*) were collected in Bac Giang province in August 2017. The material was identified by Dr. Pham Dong Phuong, Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City. The herb was dried, cut, and powdered through a No. 355 sieve. The material was tested to met the quality standards specified in the monograph of Vietnamese Pharmacopoeia V. All saamples were collected in August, 2017. The materials are shown in **Table 1**.

Table 1. List of samples

No	Sample ID	Part and place of collection
1	BG	Herba, Bac Giang province, collected
2	BP	Herba, Binh Phuoc province, collected
3	ĐN	Herba, Đong Nai province, collected
4	TN	Herba, Tay Ninh province, collected
5	H1	Herba, Ho Chi Minh City, bought in store 1
6	H2	Herba, Ho Chi Minh City, bought in store 2
7	ST	Stem, Bac Giang province, collected
8	LF	Leaf, Bac Giang province, collected

2.2. Chemicals and reagents

The reference substances of schaftoside (ID 0710/0, 98.2% HPLC purity) and isoschaftoside (ID 1005/0; 98.2% HPLC purity) were purchased from Extrasynthese (France). HPLC grade solvents including formic acid 0,1% - methanol (86:14) and double distilled water were purchased from Merck (Merck KGaA, Darmstadt, Germany). The other solvents with analytical grades were purchased from Chemsol (Vietnam) or Xilong (China).

Stock solutions of schaftoside and isoschaftoside were initially prepared as 500 µg/mL in 50% MeOH. These stock solutions then were serially diluted to give nine working reference solutions with a concentration range of 0.1-500 µg/mL. All the dilutions were used for plotting the calibration curves of each compound.

2.3. Instruments

The UPLC analysis was performed using UPLC Acquity H-Class 2695 (Waters) equipped PDA 2996 detector (Waters). The column was Acquity UPLC®BEH C_{18} (1.7 µm; 2.1 × 50 mm) (Waters). The sonication was carried out by using Sonorex RK-1208H (Bandelin).

2.4. Methods

2.4.1. Investigation of UPLC chromatographic conditions:

The UPLC chromatographic conditions including wavelength, elution condition, and flow rate were investigated in this study. UPLC system operates at room temperature.

2.4.2. Investigation of preparation of sample solutions:

The preparation of sample solutions was studied.

2.4.3. Validation of the analytical method:

The developed method was validated for linearity, the limit of detection (LOD), the limit of quantitation (LOQ), system suitability, specificity, precision, and accuracy according to International Conference on Harmonization (ICH) guidelines [9].

Linearity was determined by using isoschaftoside (**1**) and schaftoside (**2**) reference solutions in 50% methanol at concentrations of 500, 250, 100, 50, 25, 10, 5, 2, 1, and 0.5 µg/mL. 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 60% methanol and were then analyzed using a UPLC method as investigated above. The Limit of Detection (LOD) of the method was determined at a signal-to-noise ratio (S/N) of 3 based on the standard sample and blank sample 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 a UPLC method as developed above. The retention time (Rt), peak areas (S), number of theoretical plates (N), capacity factor (k'), and tailing factor (T) were used as the system suitability parameters.

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

An accurate amount of standard solution at three different concentration levels was added to the sample materials and then analyzed in triplicate using a UPLC method as described above. The percentage of recovery was calculated as follows:

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

The yield (%) of schaftoside and isoschaftoside in samples was calculated as formula:

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

(Equation 1)

Where: X : yield (%); Sc: peak area of reference compound in standard solution; St: peak area of reference compound in sample solution; 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. The UPLC chromatographic conditions and solvent for extraction

3.1.1. Investigation detection wavelength

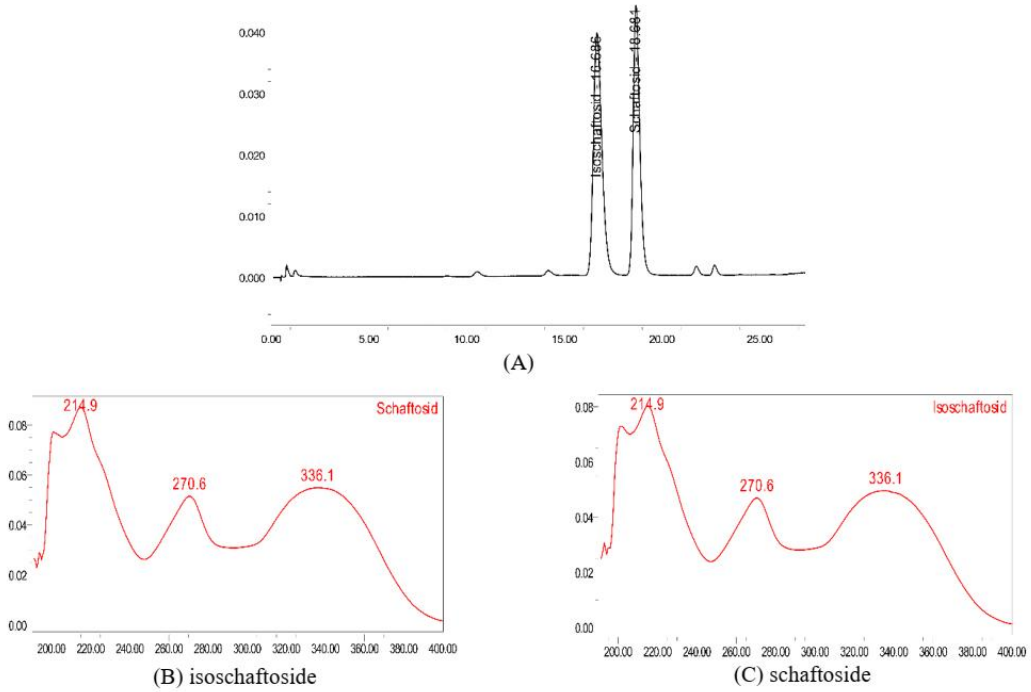

Fig. 2. Chromatogram of the standard mixture (A) and UV spectra of isoschaftoside (B) and schaftoside (C).

The UV spectrum of isoschaftoside and schaftoside showed maximum absorptions at λ max 214.9 nm; 270.6 nm; and 336.1 nm (**Fig. 2**). The maximum absorptions at λ max at 270.6 nm were set for quantitative analysis because of little noise on the chromatogram and its literature [10].

3.1.2. Investigation of elution:

Isocratic elution

Firstly, the elution condition was investigated by using a mixture of reference standards. The various solvent systems were studied, including MeOH-Formic acid 0.1% (14:86, 15:85; 16:84; 17:83) (**Fig. 4**). The result showed that MeOH-formic acid 0.1% (FA) (14:86) is the good condition for the separation

of two compounds with a flow rate of 0.25 mL/min (**Fig. 3**)

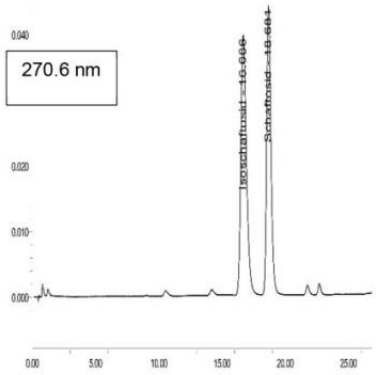

Fig. 3. Chromatogram of UPLC analysis of the mixture of isoschaftoside and schaftoside

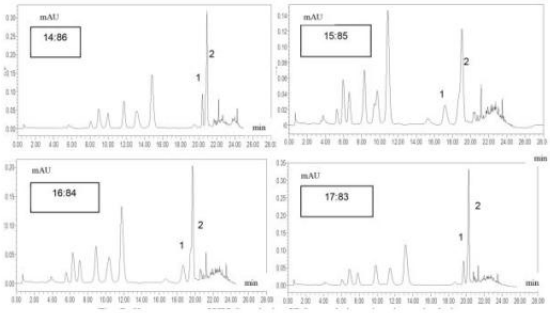

Fig. 4. Chromatogram of UPLC analysis of BG sample in various isocratic elutions

Note: All chromatograms were taken at wavelength of 270.6 nm, flow rate of 0.25 mL/min, Isochaftoside (1) and Schaftoside (2)

The two compounds were not completely separated, so the gradient elution was subsequently investigated.

Gradient elution

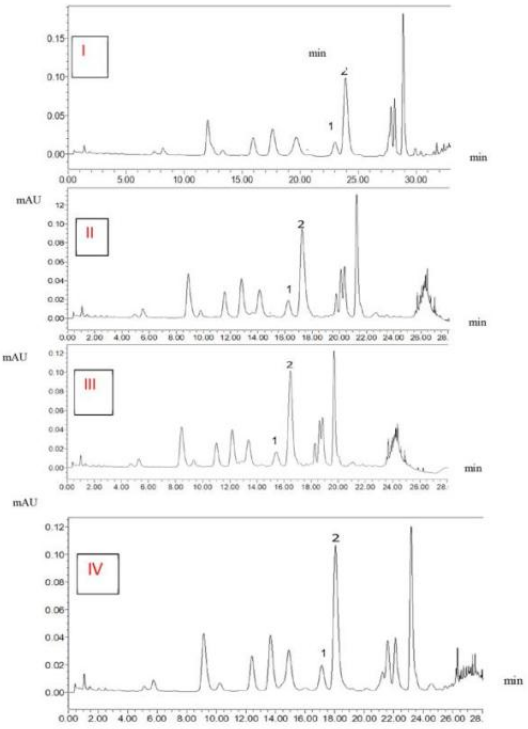

Fig. 5. Chromatogram of UPLC analysis of BG sample in various gradient elutions

Note: All chromatograms were taken at wavelength of 270.6 nm; Isochaftoside (1) and Schaftoside (2)

The chromatogram of UPLC analysis in various gradient elutions is shown in **Fig. 5**.

Table 2. Gradient elution conditions

Condition No.	Time (min)	MeOH (A) (%)	1% FA (B) (%)
I	0-5	14	86
	5-7	14 → 15	86 → 85
	7-20	15	85
	20-22	15 → 20	85 → 80
	22-25	20	80
	25-26	20 → 25	80 → 75
	26-32	25	75
II	0-7	14	86
	7-8	14 → 15	86 → 85
	8-14	15	85
	14-18	15 → 22	85 → 78
	18-24	22	78
	24-25	22-95	78-5
	25-28	95	5
III	0-9	14	86
	9-10	14 → 15	86 → 85
	10-15	15	85

Condition No.	Time (min)	MeOH (A) (%)	1% FA (B) (%)
	15-22	15 → 22	85 → 78
	22-23	22 → 95	78 → 5
	23-28	95	5
IV	0-9	14	86
	9-10	14 → 15	86 → 85
	10-15	15	85
	15-16	15 → 16	85 → 84
	16-22	16 → 22	84 → 78
	22-23	22-95	78-5
	23-28	95	5

Two compounds were well separated under elution condition No. 4 (Table 2) with a resolution of 1.75.

3.1.3. Investigation of solvents for sample extraction:

The sample (about 200 mg) was extracted with 50 mL of various solvents at 50°C for 30 min by sonication. The extract was filtered through a 0.22 mm membrane filter and subjected to a UPLC system to obtain peak area. The results showed that 50% MeOH was a suitable solvent for extraction because of the highest peak area observed (**Table 3**).

Table 3. Solvent and peak area in investigation of solvent for extraction

No	Solvent	Weight (mg)	Peak area (µV.S)
1	Distilled water	200,05	1176994
2	**50% MeOH**	**200,08**	**2762655**
3	70% MeOH	200,07	2324346
4	80% MeOH	200,05	2374282
5	90% MeOH	200,10	2264817
6	70% EtOH	200,15	2577200
7	90% EtOH	200,09	2412647

3.1.4. Investigation of column temperature:

The column temperature was studied at 30, 35, and 40°C by injecting similar volumes and extract. The separation of reference peaks was compared. The result demonstrated that the separation of reference peaks was good at 40°C (**Fig. 6**).

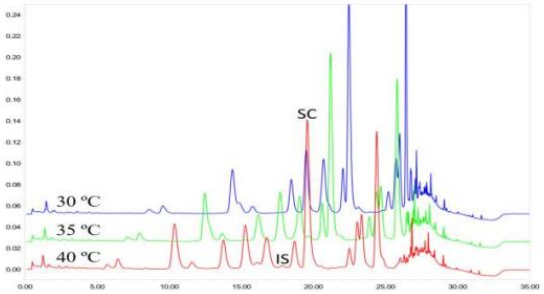

Fig.6. Chromatogram of sample run at various column temperatures.

3.2. Method validation and analysis

3.2.1. System suitability, linearity, limit of detection (LOD), and limit of quantification (LOQ)

Table 4. Results of system suitability tests of the UPLC method

No of injection	RT		S		k'		N (Theoretical plates)		T (tailing)	
	1	2	1	2	1	2	1	2	1	2
1	17.606	19.425	1357692	1136328	13.672	15.188	12505	25607	1.500	1.502
2	17.408	19.083	1356430	1130966	13.507	14.903	11297	22571	1.439	1.421
3	17.201	19.087	1372370	1130474	13.334	14.906	10642	22634	1.431	1.460
4	17.197	19.260	1358065	1129817	13.331	15.050	10494	23857	1.451	1.478
5	17.460	19.124	1360743	1151448	13.550	14.937	11696	23100	1.469	1.494
6	17.248	19.308	1366385	1136552	13.373	15.090	10944	24170	1.481	1.478
Mean	**17.353**	**19.215**	**1361947**	**1135951**	**13.461**	**15.012**	**11263**	**23657**	**1.462**	**1.472**
RSD (%)	**0.96**	**0.72**	**0.46**	**0.72**						

The system is suitable for analysis with RSD values less than 2% (Table 4). Linear ranges, regression equations, LOD and LOQ data of isoschaftoside and schaftoside are shown in **Tables 5** and **6**.

Table 5. Correlation of concentration of standard references and its peak areas

C (µg/mL)	Peak area of schaftoside (µV*s)	Error calculated (%)	Peak area of isoschaftoside (µV*s)	Error calculated (%)
0.5	9793	13.1	9733	53.7
1	18933	11.3	23052	20.8
2	46540	12.3	50099	3.5
5	99155	2.7	113444	8.1
10	209678	3.4	245287	1.4
25	493849	2.3	580698	3.0
50	1026039	1.6	1212258	1.6
100	2083753	3.2	2455140	3.1
250	4974705	1.4	5871889	1.3
500	10108608	0.2	11918039	0.2

Note: The error in concentration from the standard curve does not exceed 15% except for concentration of 0.5 µg/mL of isoschaftoside.

Table 6. Linear ranges, regression equations, LOD, and LOQ data of isoschaftoside and schaftoside

Compounds	Linear range (µg/mL)	Regression equation*	r^2	LOD (µg/mL)	LOQ (µg/mL)
Isoschaftoside (**1**)	1 - 500	y = 23781x + 4222	0.9999	0.0625	0.20
Schaftoside (**2**)	1 - 500	y = 20180x - 1024.3	0.9999	0.125	0.41

x represents the concentrations of **1 and **2** in µg/mL; y represents the peak area; detected at UV 270.6 nm.*

The linearity of the method was assessed through regression analysis between the peak area and the concentrations of the reference compounds schaftoside and isoschaftoside within the range of 0.5 to 500 µg/mL. The results showed that the correlation coefficient (r^2) was 0.9999 for both analytes, indicating a high degree of linearity across the investigated range.

In addition, the accuracy of the method should be evaluated at a minimum of three representative concentration levels (low, medium, and high) within the linear range. The study conducted recovery tests and obtained results ranging from 93.9% to 101.1% for isoschaftoside and from 95.1% to 100.1% for schaftoside. Moreover, at certain low concentration points, the obtained values may deviate by more than 50% from the theoretical value; therefore, it is necessary to determine both the absolute and relative error to evaluate the lower limit of the method's accuracy (accuracy limit).

3.2.2. Specificity:

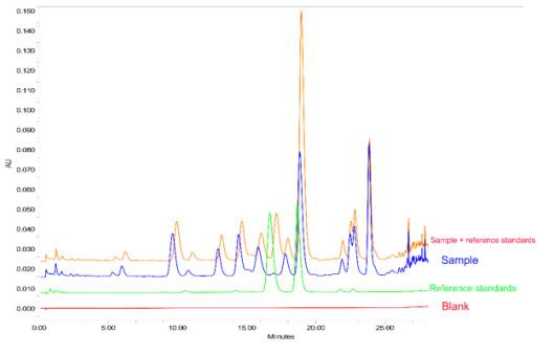

Fig. 7. Chromatogram of specificity test in UPLC method

In the control for sample testing, no peak was observed (data not shown), indicating the dissolving solvent (50% methanol, blank) did not interfere with the results for the separation of two compounds. The results indicated that the quantitative method was validated for specificity (**Fig. 7**).

3.2.3. Precision:

Table 7. Precision test of the UPLC method

Sample No	Sample weight (mg)	Peak area		Amount (mg)		Percent (%) (*)	
		1	2	1	2	1	2
1	200.2	21390	1479108	8.69	695.3	0.0043	0.347
2	200.1	21133	1514273	8.64	715.7	0.0043	0.358
3	200.4	20423	1516703	8.21	705.3	0.0041	0.352
4	200.0	20320	1497057	8.31	707.9	0.0042	0.354
5	200.2	20830	1490068	8.51	703.9	0.0042	0.352

Sample No	Sample weight (mg)	Peak area		Amount (mg)		Percent (%) (*)	
		1	2	1	2	1	2
6	200.9	19630	1487668	8.03	703.8	0.0040	0.350
Mean	**200.3**	**20621**	**1497480**	**8.40**	**705.3**	**0.0042**	**0.352**
SD		**634**	**15103**	**0.26**	**6.6**	**0.000001**	**0.000035**
RSD %		**3.1**	**1.0**	**3.1**	**0.9**	**3.2**	**0.99**

(*) calculated with reference to the dried drug.

The method also demonstrated acceptable precision, with RSD values at 3% and 1% for isoschaftoside and schaftoside, respectively (**Table 7**).

3.2.4. Recovery:

The recovery values of isoschaftoside (1) and schaftoside (2) ranged between 93.9% - 101.1% (average of 96%) and 95.10% - 100.1% (average of 98%), respectively (**Table 8**)

Table 8. Recovery data of UPLC method

References	Peak area	Amount taken (mcg)	Amount added (mcg)	Amount (Theoretical) (mcg)	Amount (found) (mcg)	Recovery (%)	Average (%)
1	37102	8.56	6.87	15.43	15.60	101.1	96.6
	35078	8.59	6.87	15.46	15.12	97.8	
	35831	8.50	6.87	15.37	15.33	99.7	
	38404	8.44	8.35	16.79	15.87	94.5	
	38231	8.46	8.35	16.81	15.79	93.9	
	38301	8.49	8.35	16.84	15.82	93.9	
	44032	8.41	10.31	18.72	18.19	97.2	
	43866	8.45	10.31	18.76	18.14	96.7	
	43041	8.54	10.31	18.85	17.77	94.3	
2	2631539	718.74	539.28	1258.02	1256.84	99.9	98
	2629537	721.21	539.28	1260.49	1256.12	99.7	
	2627007	713.81	539.28	1253.09	1254.92	100.1	
	1409.58	708.52	701.06	1409.58	1387.70	98.4	
	1411.69	710.63	701.06	1411.69	1386.44	98.2	
	1414.16	713.10	701.06	1414.16	1400.48	99.0	
	3097236	706.40	849.37	1555.77	1479.55	95.1	
	3166495	709.57	849.37	1558.94	1512.62	97.0	
	3225870	720.86	849.37	1570.23	1540.71	98.1	

3.2.5. Summary of the developed method:

System and stationary phase: UPLC Acquity H-Class 2695 (Waters) equipped PDA 2996 detector (Waters), UPLC®BEH C_{18} (1.7 µm; 2.1 × 50 mm) (Waters).

Column temperature: 40°C.

Mobile phase: A solution of methanol (A) and 1% FA (B) (**Table 9**).

Table 9. Mobile phase in UPLC analysis

Time (min)	MeOH (A) (%)	1% FA (B) (%)
0-9	14	86
9-10	14 → 15	86 → 85
10-15	15	85
15-16	15 → 16	85 → 84
16-22	16 → 22	84 → 78
22-23	22-95	78-5
23-28	95	5

Reference standard solution: Weigh accurately a quantity of isoschaftoside and schaftoside, and dissolve them in 50% methanol to produce a solution.

Sample solution: Weigh accurately 200 mg of the powdered sample (through sieve size number of 355) and place it in a conical flask with a stopper, accurately add 50 mL of 50% methanol, weigh, ultrasonicate for 30 minutes at 50°C, cool, weigh again, replenish the loss of the weight with

50% methanol, mix well, filter, and the filtrate was filtered through 0.22 mm membrane filter before injecting into the UPLC system.

Procedure: Inject accurately 2 µL of each of the reference standards solution and the sample solution into the liquid chromatography apparatus, and calculate the content by using equation 1.

3.3. Simultaneous quantification of schaftoside and isoschaftoside in samples collected

Simultaneous quantification of isoschaftoside and schaftoside from various samples ID including BG, BP, ĐN, TN, H1, H2, ST, and LF was carried out using the established analytical method. The results are as indicated in **Table 8**. The contents of schaftoside and isoschaftoside ranged 0.281 - 0.376% and 0.004 - 0.014% (w/w) for schaftoside and isoschaftoside, respectively. The contents of schaftoside and isoschaftoside in the leaf were higher than those in the stem and aerial parts. All percentages were calculated with reference to the dried drug. The data of quantitative analyses of various samples are shown in Table 10 and **Fig. 8**).

Table 10. Contents of schaftoside and isoschaftoside (%) in the different samples of herba, stem, and leaf of *D. styracifolii*

No.	Sample ID	Place of collection	Average of percentage (%) for Schaftoside	Average of percentage of isoschaftoside
1	BG	Bac Giang province	0.34602 ± 0.00004	0.004123 ± 0.000003
2	BP	Binh Phuoc province	0.37601 ± 0.00002	0.014113 ± 0.000003
3	ĐN	Dong Nai province	0.35704 ± 0.00001	0.0051 ± 0.000002
4	TN	Tay Ninh province	0.34908 ± 0.00003	0.00402 ± 0.000002
5	H1	Ho Chi Minh city, in store 1	0.28102 ± 0.00002	0.006034 ± 0.000001
6	H2	Ho Chi Minh city, in store 2	0.398 ± 0.00005	0.006012 ± 0.000002
7	ST	Stem, Bac Giang province	0.04801 ± 0.00001	Not detected (*)
8	LF	Leaf, Bac Giang province	0.52602 ± 0.00003	0.008100 ± 0.000001

* under the limit of detection at 0.06 mg/mL.

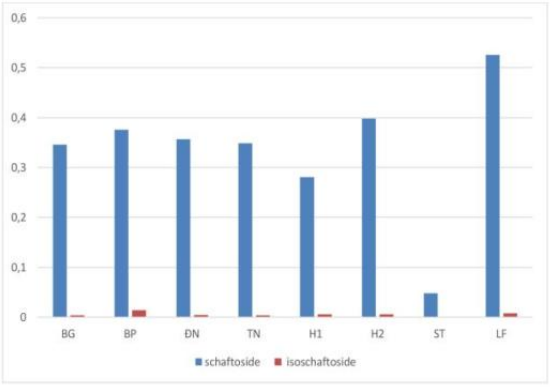

Fig. 8. Contents of schaftoside and isoschaftoside (%) in the different samples of herba, stem, and leaf of *D. styracifolii*

4. Discussion

While most flavonoid *C*-glycosides only occur in certain plant families, schaftoside and isoschaftoside, also *C*-glycosides, are popularly present in higher plants. They have been reported in, at least, 184 plant species of 39 families. Particularly, they are present in cereal crops, such as Rice, Maize, Wheat, and Sorghum, as well as in medicinal herbs, such as Licorice root (*G. uralensis*) and Baical Skullcap root (*S. baicalensis*), *Passiflora incarnata, Grona styracifolia*, They possess a variety of biological activities, including anti respiratory syncytial virus, antidiabetic, antihypertensive, hepatoprotective, anti-inflammatory, and antioxidant activities to mammals, indicating their potential applications as drugs or dietary supplements. Moreover, (iso)schaftosides are important plant defense compounds. The contents of (iso)schaftosides in rice shoots increased by around 40% during 6 and 24 h after penetration of the rice blast fungus *Magnaporthe oryzae* [11],[12],[13]. Schaftoside is the marker for quality control of *Herba Desmodii styracifolii* in Taiwan Herbal Pharmacopoeia 4th. *Herba Desmodii styracifolii* contains not less than 3.0% of dilute ethanol-soluble extractives, not less than 5.0% of water extractives, and not less than 0.13% of schaftoside [4]. Quantitative and chemical fingerprint analysis of *D. styracifolium* by high-performance liquid chromatography combined with chemometrics were reported. Four peaks were identified as schaftoside, isoorientin, isoschaftoside, and isovitexin [14]. The quality

evaluation of *D. styracifolium* using HPLC with photodiode array detection and electrospray ionisation tandem mass spectrometry was studied. Among the constituents analysed, schaftoside was determined as the main component, this contents varying from 0.77 to 4.89 mg/g (0.00077-0.00489%) [10]. When the bias calculated from a standard curve is more than 20%, it indicates that the method is not accurate or reliable enough at that concentration level, and further investigation and correction may be needed. While a 20% bias might be acceptable for the LLOQ (Lower Limit Of Quantification) in some bioanalytical method validation guidelines, according to the European Medicines Agency, for other concentrations, bias should generally be within 15% [15]. In this study, the bias exceeding 20% at concentration of 1 mg/mL for isoschaftoside can be caused by low concentrations. So, the result of quantification for isoschaftoside in Table 10 can be not accurate because of low concentration.

In this study, the results showed that the content of schaftoside in the leaf was higher than that in the stem. Moreover, the schaftoside content in the sample collected in Binh Phuoc province was higher than that in others collected. Particularly, the schaftoside content in the sample bought in store 1 in Ho Chi Minh City is slightly higher than in others, but the origin of this sample was not known.

5. Conclusion

A reliable and simple method was developed successfully for the quantitative analysis of two of these components using UPLC-DAD. The method was validated in terms of the specificity, accuracy, precision, and system suitability of these compounds. The results of this study might be beneficial for the standardization and development of the quality control profile of *Herba Desmodii styracifolii* by the phytopharmaceutical industries.

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