ISSN 1859 - 4735

JOURNAL OF MEDICINAL MATERIALS

No. 6 - Vol. 30
12/2025

NATIONAL INSTITUTE OF MEDICINAL MATERIALS

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JOURNAL OF MEDICINAL MATERIALS (Print and Electronic Journals)

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ISOLATION AND QUANTITATIVE ANALYSIS OF QUERCITRIN IN LEAVES OF SOME *POLYSCIAS* SPECIES COLLECTED IN VIETNAM

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Received October 22th, 2025 Accepted December 4th, 2025

Summary

From the leaves of *Polyscias fruticosa*, the major compound quercitrin, a flavonol glycoside, was isolated and elucidated. The contents of quercitrin in some *Polyscias* species leaves were quantitatively determined using an HPLC-DAD method, an Agilent C_{18} column, and an isocratic solvent system of acetonitrile and 0.1% phosphoric acid in water (84:16, v/v). The flow rate was kept at 0.8 mL/min. The injection volume was 10 µL. Detection was set at a wavelength of 257 nm. The method was validated for the system suitability (RSD of peak area = 0.43%; RSD of t_R = 0.06 %); linearity ranging from 12.5 to 100.0 µg/mL (R = 1); precision (RSD < 2.0 %), accuracy (recovery: 97.85%-102.63%). The limit detection (LOD) and quantification (LOQ) were 0.33 µg/mL and 0.99 µg/mL, respectively. The method was further applied to determine the quantity of quercitrin in some *Polyscias* species collected in Vietnam, and the results showed that all of these *Polyscias* leaves contain quercitrin. The leaves of *P. fruticosa* with the contents varying from 0.13 to 0.32 %, indicating that quercitrin could be regarded as a qualitative and quantitative marker compound for the leaves of *Polyscias* species. This is the first study to report a quantitative method for determining quercitrin in the *Polyscias* genus.

Keywords: *Polyscias species; Leaf; Quercitrin; Quantitative analysis.*

1. Introduction

Polyscias fruticosa (L.) Harms, belonging to the Araliaceae family, is mainly distributed in Vietnam, China, and other tropical countries [1],[2]. In Vietnam, *P. fruticosa* is widely used as a herbal medicine with the functions of ischemia, inflammation, and increasing blood flow in the brain [3]. Phytochemical studies on leaves of *P. fruticosa* resulted in the isolation of polyacetylenes [4], saponins [2], and flavonoids… [5] with a variety of bioactivities, such as antipyretic, anti-inflammatory, analgesic, and molluscicidal properties and antidiabetic activity [5].

Flavonoids are among the major bioactive constituents found in *Polyscias* species, with quercitrin being one of the key compounds reported to exhibit antioxidant, anti-inflammatory, antimicrobial, analgesic, wound-healing, immunomodulatory, and hepatoprotective effects [6],[7]. However, studies on the quantification of quercitrin within this genus remain limited. Therefore, in the present study, an HPLC/UV-based analytical method was established for the identification and quantification of quercitrin extracted from *P. fruticosa* leaves. This validated method is expected to provide a reliable basis for future evaluation and comparison of quercitrin content among other *Polyscias* species.

2. Materials and methods

2.1. Plant materials

Leaves of *P. fruticosa, P. filicifolia*, *P. guilfoylei*, *P. scutellaria* cv. *quinquefolia, P. scutellaria* were harvested in six provinces of Vietnam (Nam Dinh, Gia Lai, Phu Tho, Phu Yen, An Giang, and Thai Binh) in May 2022 and were identified by Dr. Tran Van On, Hanoi University of Pharmacy, and Dr. Phuong Thien Thuong, Division of Biotechnology, Vietnam-Korea Institute of Science and Technology (VKIST), Hanoi, Vietnam. Fresh samples were washed and dried at 50°C, and stored in sealed plastic bags before extraction. The sample labeled PFr, whose moisture content, identified with loss of drying, was 10.3% was selected for quantitative validation.

2.2. Isolation and structural elucidation of quercitrin from P. fruticosa leaves

Column chromatography (CC) was carried out on *silica gel* 60 (60 – 200 µm, Merck), and YMC RP-18 (120 Å, 150 µm), and thin layer chromatography (TLC) on *silica gel* 60 F_{254} and

RP-18 $F_{254}S$ (Merck). HPLC analysis was performed on a Shimadzu system, which consists of a vacuum degasser, a binary pump LC-20AD, a SIL-20A autosampler, a CTO-20A column oven, and a diode-array detector SPD-M20A. NMR spectra were measured on a Bruker Avance III 600 spectrometer (^{1}H at 600 MHz and ^{13}C at 150 MHz) with tetramethylsilane as the internal standard.

2.3. HPLC analysis of quercitrin in leaves of P. fruticosa

Standard preparation: Quercitrin (purity 98%, 5.0 mg) was purchased from Chengdu Biopurity (China). A standard stock solution of quercitrin was prepared by dissolving it in 75% methanol, then diluted to yield calibration curves in ranges of 12.5-100 µg/mL. The stock and working solutions were stored at 4 °C.

Sample preparation: Sample extraction factors were investigated including extraction solvents (ethanol, methanol, ethanol/water, and methanol/water), extraction method (ultrasonic and heat reflux methods), extraction time (30 – 120 min), the ratio of the mass of the sample to the volume of the solvent (1/25, 1/50, and 1/100 g/mL). The extraction parameters were chosen to obtain the highest content of quercitrin.

HPLC condition: Chromatographic separation was carried out on an Agilent C_{18} column (250 x 4.6 mm i.d., 5 µm). The column temperature was maintained at 30 °C. The mobile phase consisted of acetonitrile and 0.1% phosphoric acid in water (84 – 16, v/v). The flow rate was kept at 0.8 mL/min. The injection volume was 10 µL. Detection was set at a wavelength of 257 nm.

Method validation: Specification, system stability, linearity, accuracy, and precision were evaluated according to the AOAC [8] and ICH [9] guidelines.

The content of quercitrin in samples was calculated as follows:

$$X = \frac{C \times V}{m \times 100 \times (100 - a)}$$

C: The concentration of quercitrin in the sample solution, deduced from the calibration curve equation (µg/mL)

V: Volume of the sample solution (mL)

m: Weight of the test sample (g)

a: Moisture of the test sample (%)

3. Results

3.1. Extraction, isolation, and structural elucidation

The dried leaves of *P. fruticosa* (5.0 kg) were extracted with ethanol 70% (30 L) three times at room temperature, each time for 7 days, and then evaporated under reduced pressure. The EtOH extract (504.0 g) was suspended in water (1.5 L) and partitioned with *n*-hexane, methylene chloride (MC), ethyl acetate (EtOAc), and *n*-butanol (3L x 3, each) to give the residue of hexane-soluble extract (20.3 g), MC-soluble extract (178.0 g), EtOAc-soluble extract (71.0 g), *n*-butanol-soluble extract (113.0 g), and water layer. The EtOAc extract (71.0 g) was chromatographed on a *silica gel* chromatography column (CC) and eluted with a gradient solvent system of MC–MeOH (10:1→0:1, v/v) to obtain six fractions (PF-1A – PF-1F). Fraction 1C (8.3 g) was separated by *silica gel* CC eluting with MC–MeOH–H_2O (5:1:0.1, v/v) to provide four fractions (PF-2A – PF-2D). Fraction PF-2B (930.0 mg) was further purified by RP-18 CC using MeOH-H_2O (1:1, v/v) as an eluent to yield compound **1** (100.1 mg, quercitrin).

Quercitrin (**1**): Yellow powder; ^{1}H NMR(600 MHz, CD_3OD) δ_H (ppm): 6.16 (1H, d, J = 1.8 Hz, H-6), 6.32 (1H, d, J = 1.8 Hz, H-8), 7.32 (1H, d, J = 2.4 Hz, H-2'), 6.90 (1H, d, J = 8.4 Hz, H-5'), 7.29 (1H, dd, J = 2.4, 8.4 Hz, H-6'), 5.34 (1H, d, J = 1.2 Hz, H-1"), 0.94 (3H, d, J = 6.0 Hz, H-6"); ^{13}C NMR (150 MHz, CD_3OD) δ_C (ppm): 158.2 (C-2), 136.2 (C-3), 179.4 (C-4), 163.5 (C-5), 99.7 (C-6), 165.7 (C-7), 94.7 (C-8), 159.2 (C-9), 105.8 (C-10), 122.9 (C-1'), 116.3 (C-2'), 146.3 (C-3'), 149.7 (C-4'), 116.9 (C-5'), 122.9 (C-6'), 103.4 (C-1"), 72.0 (C-2"), 72.1 (C-3"), 73.2 (C-4"), 71.9 (C-5"), 17.6 (C-6").

Compound **1** was isolated as a yellow powder. The ^{1}H NMR spectrum of **1** showed signals of three aromatic protons for an ABX system at δ_H 7.32 (1H, d, J = 2.4 Hz, H-2'), 6.90 (1H, d, J = 8.4 Hz, H-5'), 7.29 (1H, d, J = 2.4, 8.4 Hz, H-6') in the B-ring, and two *meta*-coupling protons for a tetra-substitution in the ring A at δ_H 6.16 (1H, d, J = 1.8 Hz, H-6'), 6.32 (1H, d, J = 1.8 Hz, H-8'). In addition, an anomeric proton at δ_H 5.34 (1H, d, J = 1.6 Hz, H-1"), four oxygenated methine protons at δ_H 3.25 – 3.80 (4H, m, H-2" – H-5"), and a methyl proton at δ_H 0.94 (3H, d, J = 6.0 Hz, H-6") were

observed in the ^{1}H NMR spectrum, which were elucidated as α-L-rhamnose. The ^{13}C NMR spectrum showed 21 carbon signals, including a carbonyl carbon at δ_C 179.4 (C-4), seven oxygenated aromatic carbons at δ_C 158.2 (C-2), 136.2 (C-3), 163.5 (C-5), 165.7 (C-7), 159.2 (C-9), 146.3 (C-3'), 149.7 (C-4'), two quarternary carbons δ_C 105.8 (C-10), 122.9 (C-1') for the algycone part. Furthermore, the ^{13}C NMR data of **1** also revealed signals of an anomeric carbon at δ_C 103.4 (C-1"), four oxygenated methine carbons at δ_C 72.3 (C-2"), 72.2 (C-3"), 73.4 (C-4"), 72.0 (C-5"), and a methyl carbon at δ_C 17.8 (C-6") for the rhamnose moiety. Based on the analysis of ^{1}H and ^{13}C NMR data and comparison with those of previous publications, compound **1** was determined as quercitrin [10].

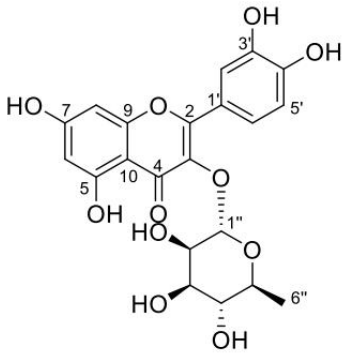

Fig. 1. Chemical structure of quercitrin (**1**) isolated from the leaves of *P. fruticosa*

3.2. HPLC analysis of quercitrin in leaves of P. fruticosa

3.2.1. Optimization of chromatographic conditions:

Based on the UV-Vis spectrum of quercitrin, the detection was set at 257 nm, which is the maximum absorption wavelength. Other analysis parameters, including mobile phase, flow rate, elution program, and column oven temperature, were examined to select the best separation conditions. The selected condition was mentioned in 2.3. Under the chosen condition, the peak of quercitrin in the sample solution is symmetric (tailing factor of 0.954) and well resolved (resolution of 2.886).

3.2.2. Optimization of extraction conditions:

Table 1 shows the results of investigating sample extraction. The selected procedure was that 2.0 g of leaf powder was refluxed with 50 mL of 75% methanol for 2 hours. The extract was filtered through a 0.45 µm membrane filter to give the sample solution.

Table 1. Investigation of the extraction condition

Extraction solvents (Fixed: m = 2.0 g, solvent volume 50 mL, heat reflux 60 min)			
Solvent	**Quercitrin (Mean ± SD, %)**	**Solvent**	**Quercitrin (Mean ± SD, %)**
Methanol	0.106 ± 0.004	Ethanol	0.081 ± 0.005
75% methanol	**0.127** ± 0.001	75% ethanol	0.115 ± 0.002
50% methanol	0.114 ± 0.003	50% ethanol	0.114 ± 0.001
Extraction methods and extraction time (Fixed: m = 2.0 g, solvent volume 50 mL of 75% methanol)			
Condition	**Quercitrin (Mean ± SD, %)**	**Condition**	**Quercitrin (Mean ± SD, %)**
Sonication, 30 min	0.057 ± 0.001	Reflux, 30 min	0.121 ± 0.005
Sonication, 60 min	0.067 ± 0.002	Reflux, 60 min	0.122 ± 0.006

Sonication, 120 min	0.069 ± 0.001	**Reflux, 120 min**	**0.125** ± 0.001
Sonication, 180 min	0.070 ± 0.001	Reflux, 180 min	0.126 ± 0.004
Ratio of the weight of the test sample to the volume of the solvent (g/mL) (Fixed: m = 2.0 g, solvent 75% methanol, heat reflux 2 hours)			
Condition	**Quercitrin (Mean ± SD, %)**	**Condition**	**Quercitrin (Mean ± SD, %)**
1 g/ 25 mL	**0.126** ± 0.002	1 g/ 100 mL	0.126 ± 0.001
1 g/ 50 mL	0.125 ± 0.001		

3.2.3. Validation of the analytical procedure:

Specification: The blank, standard, and sample solutions were injected into the HPLC system. The obtained chromatograms showed that there was no peak in the blank sample at the retention time of quercitrin in the standard solution. The chromatogram of the sample solution illustrated a clear separation of the quercitrin peak (Fig. 2). In addition, the match ratio of the UV spectrum of quercitrin in the standard and sample solution was 0.9995. Therefore, the method was specific for the determination of the targeted marker.

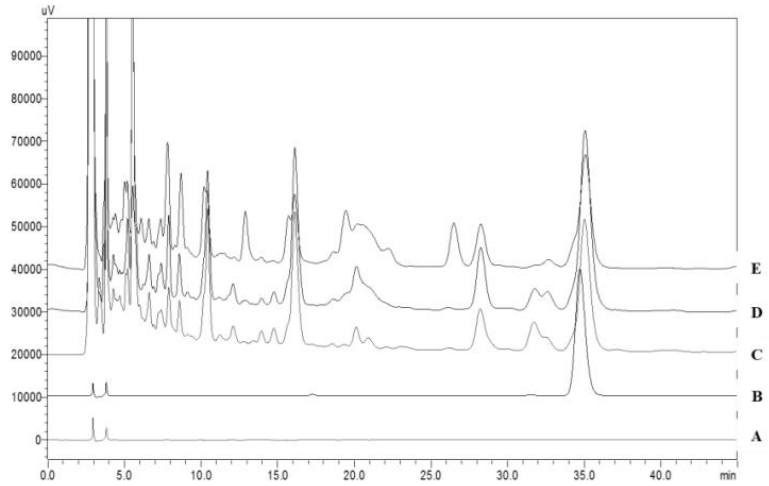

Fig. 2. Chromatograms of quercitrin and *Polyscias* spp. samples (A: Blank; B: Quercitrin; C: *P. fruticosa*; D: *P. scutellaria* cv. *quinquefolia*; E: *P. guilfoylei*)

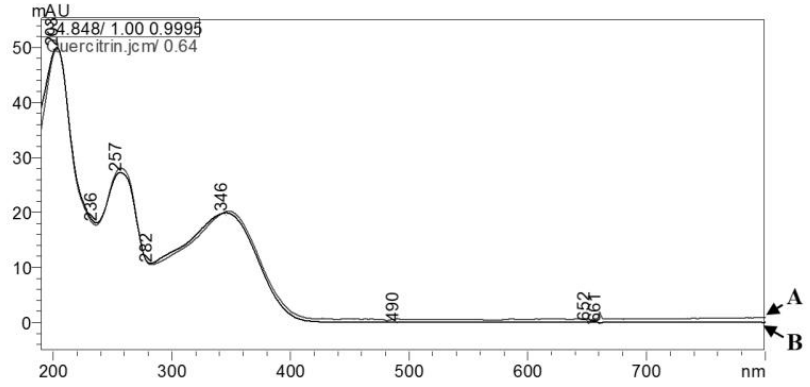

Fig. 3. UV spectrum of quercitrin in standard (A) and sample (B) solutions

Table 2. System suitability study

No.	Retention time (min)	Peak area (mAu.s)
1	34.896	1705578
2	34.888	1708384
3	34.861	1704547
4	34.834	1706555
5	34.867	1715822
6	34.883	1723349
RSD (%)	**0.06**	**0.43**

System suitability: As observed in Table 2, the RSD value of retention time and peak area of a standard solution (50.39 µg/mL) were both less than 2%. These results indicated that the analytical method conforms to the system suitability requirement.

Linearity: 10.07 mg quercitrin was diluted for the preparation of standard solutions. Calibration standards of five concentrations for quercitrin were assayed. Peak-area ratios of quercitrin obtained from chromatograms were utilized for the construction of a calibration curve, using weighted linear least squares regression. The result showed satisfactory linearity in the concentration ranges of 12.5-100 µg/mL. The calibration curve equation for quercitrin was y = 33873x – 16977, with linear correlation value R^2 =1 (Table 3 and Fig. 3).

Table 3. Linearity study Table

Concentration (µg/mL)	Peak area (mAu.s)
98.6860	3322368
74.0145	2494029
49.3430	1659008
24.6715	809036
12.3358	405400

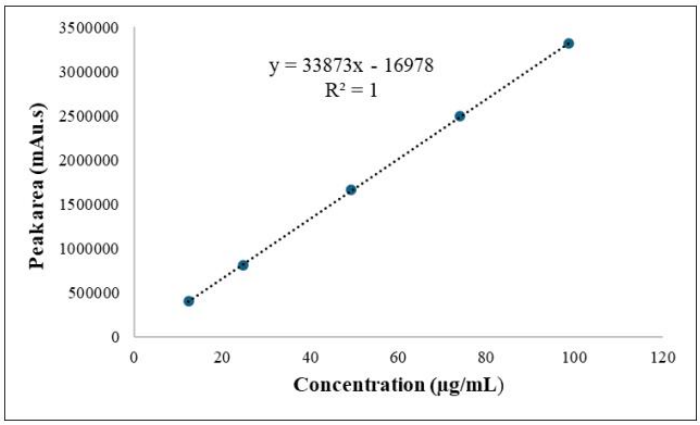

Fig. 4. Linearity study

Precision: Studies of repeatability and intermediate precision were carried out, and results were presented in Table 4. The average content of quercitrin is 0.130% and the RSD values are less than 2%. Therefore, the analytical method meets the requirement of precision.

Table 4. Precision study

No.	Day 1 Linearity: y = 33873x - 16978			Day 2 Linearity: y = 33464x-22612			Average: 0.129% RSD: 1.25%
	Sample weight (g)	Peak area (mAu.s)	Quercitrin content (%)	Sample weight (g)	Peak area (mAu.s)	Quercitrin content (%)	
1	2.00456	1555199	0.129	1.99919	1498940	0.127	
2	2.00784	1584493	0.131	2.00142	1493152	0.126	
3	2.01586	1573053	0.130	1.96789	1490934	0.128	
4	2.00014	1574207	0.131	2.02412	1556653	0.130	
5	2.00741	1574924	0.130	2.03476	1557483	0.129	
6	2.01247	1578840	0.130	2.01784	1559879	0.131	
Average			0.130			0.129	
RSD (%)			**0.61**			**1.39**	

Accuracy: 19.22 mg standard quercitrin (purity 98%) was dissolved in 200 mL of 75% methanol to get a standard solution (A). Diluted solutions from A are used to extract the leaf powder under the extraction conditions described in 3.2.2. The recovery was evaluated by comparing the theoretical concentration of quercitrin with the additional concentration identified in the chromatograms

The result presented in Table 5 indicated that the accuracy of the developed method was acceptable.

LOD and LOQ: The limit of detection and the limit of quantification were calculated by diluting sample solutions until signal-to-noise ratios reached 3 and 10, respectively. LOD and LOQ were estimated to be 0.45 and 1.49 µg/mL for quercitrin, equivalent to 0.001 and 0.004% in samples.

Table 5. Accuracy study

Linearity: y = 34067x + 16537
Concentration of solution A: 94.178 µg/ mL

Concentration level	Peak area (mAu.s)	Leaf powder weight (g)	Amount from leaf powder (µg/mL)	Dilution factor of solution A	Amount added (µg/mL)	Amount found (µg/mL)	Recovery (%)	Mean (%)	RSD (%)
25%	1995746	2.00178	46.4762	8	11.7723	11.6214	98.72	**98.58**	**0.69**
	2001690	2.00697	46.5967	8	11.7723	11.6753	99.18		
	1996700	2.00741	46.6069	8	11.7723	11.5187	97.85		
50%	2421875	2.01688	46.8268	4	23.5445	23.7793	101.00	**101.62**	**0.87**
	2417788	2.00935	46.6519	4	23.5445	23.8342	101.23		
	2428441	2.00863	46.6352	4	23.5445	24.1636	102.63		
70%	2687942	2.01806	46.8542	3	31.3927	31.5621	100.54	**101.53**	**1.03**
	2684607	2.00197	46.4806	3	31.3927	31.8377	101.42		
	2721629	2.09431	48.6245	3	31.3927	30.7806	98.05		
Mean (%) *(n=9)*									**100.07**
RSD (%) *(n=9)*									**1.67**

Sample analysis:

Table 6. Quercitrin content in leaves of some *Polyscias* species collected in Vietnam

Sample	Content (%) (Mean ± SD)					
	1	**2**	**3**	**4**	**5**	**6**
P. fruticosa	0.129 ± 0.002	0.318 ± 0.001	0.132 ± 0.001	0.307 ± 0.001	0.312 ± 0.001	0.290 ± 0.001
P. filicifolia	0.088 ± 0.002	0.062 ± 0.001	0.077 ± 0.001	0.055 ± 0.001	0.054 ± 0.001	0.055 ± 0.001
P. guilfoylei	0.111 ± 0.001	0.118 ± 0.001	-	-	0.097 ± 0.001	-
P. scutellaria cv. *quinquefolia*	0.215 ± 0.002	0.256 ± 0.001	-	0.200 ± 0.002	-	-
P. scutellaria	0.027 ± 0.004	-	0.015 ± 0.002	-	0.029 ± 0.004	-

(*1: Nam Dinh; 2: Gia Lai; 3: Phu Yen; 4: Phu Tho; 5: An Giang; 6: Thai Binh, - not collected)*

(To ensure that the concentrations fall within the linear range, 25 mL of extract from the P. scutellaria samples were concentrated and then made up to 10 mL).

The quantitative results of the quercitrin across *Polyscias* species collected from six provinces in Vietnam are summarized in Table 6. Overall, *P. fruticosa* and *P. scutellaria* cv. *quinquefolia* exhibited the highest contents, ranging from 0.129 – 0.318% and 0.200 – 0.256%, respectively. In contrast, *P. scutellaria* showed the lowest levels (0.015 – 0.029%), suggesting a limited capacity for biosynthesis or accumulation of the compound.

Geographical variations were evident among sampling sites. The highest content was detected in *P. fruticosa* from Gia Lai (0.318%), followed by samples from An Giang (0.312%) and Thai Binh (0.290%). In northern provinces such as Nam Dinh and Phu Tho, *P. scutellaria* cv. *quinquefolia* contained comparatively high levels (0.215 – 0.200%).

Collectively, *P. fruticosa* demonstrates consistently high and stable compound levels across diverse regions, making it a promising candidate for further phytochemical and pharmacological investigation. Meanwhile, the notably low concentrations in *P. scutellaria* and *P. filicifolia* indicate limited potential as primary sources of the compound. These results highlight the strong impact of species specificity and geographical origin on the chemical composition of *Polyscias* plants in Vietnam.

Quercitrin (quercetin-3-*O*-rhamnoside) is a naturally occurring flavonoid widely distributed in various fruits and plant-derived beverages such as grapes, lychees, and green tea. It exhibits notable anti-inflammatory and antioxidant activities, contributing to its therapeutic potential in multiple disease conditions [11]. Several studies have established and validated reversed-phase HPLC methods coupled with UV or DAD detection for its quantification, typically employing C_{18} columns and mobile phases consisting of acetonitrile and mildly acidic aqueous solutions. These analytical approaches have demonstrated excellent linearity, precision, and recovery across different plant matrices [12],[13]. To the best of our knowledge, this is the first study to develop and report a validated quantitative method for the determination of quercitrin in the *Polyscias* genus.

Acknowledgments: *This work was supported by the Ministry of Science and Technology* (Grand number NĐT.90.KR/20).

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