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CONTENTS VOL. 31(5), 2026
(Pages 355 - 450)

Page	SCIENTIFIC RESEARCH
357	**Saponins from the Leaves of *Panax vietnamensis* var. *fuscidiscus* Collected in Lai Chau Province, Vietnam -** *Vu Thi Diep, Do Thi Ha, Le Thi Xoan, Tran Minh Ngoc, Nguyen Thi Thanh Thanh, Nguyen Le Thuy Duong,*
365	**Benzyl, Flavonoid, and Iridoid Constituents from the Leaves of *Psychotria serpens* with Nitric Oxide Inhibitory Activity** - *Pham The Chinh, Vu Quang Tung, Pham Thi Tham, Phan Van Kiem, Nguyen Phuong Thao*
375	**Benzyl and Megastigmane Glucosides from the Leaves of *Zanthoxylum rhetsa* (Roxb.) DC. and their Inhibitory Effects on Nitric Oxide Production -** *Bui Thi Mai Anh, Nguyen Thi Mai, Nguyen Phuong Thao*
383	**Investigation of Flavonoid Constituents and Antioxidant Activity of Leaf Extracts from *Coccoloba uvifera* (L.) L.** - *Nguyen Thu Anh, Pham Van Son, Huynh Loi, Vo Van Leo*
391	**Triterpenoids and Acylglucosylsterol from *Galphimia glauca* Cav. Collected in Vietnam** - *Nguyen Thi Hue, Nguyen Thi Thu Trang, Tran Minh Ngoc, Nguyen Thi Hue, Do Thi Ha, Nguyen Van Tai*
398	**Phytochemical Constituents of the Branches and Leaves of *Alangium salviifolium* (L.f.) Wangerin Collected in Quang Ngai, Vietnam** - *Phan Thi Trang, Tran Minh Ngoc, Pham Van Cong, Dang Viet Hau, Do Thi Ha, Nguyen Van Tai*
405	**Chemical Constituents of *Bauhinia bracteata* Benth. Leaves and Their Antioxidant Activity -** *Nguyen Thi Trang, Le Minh Tuan, Pham Thi Thuy Trang, Tran Thanh Ha, Pham Thi Nguyet Hang*
412	**Isolation and Cytotoxic Evaluation of Compounds from the Twigs of *Millettia leptobotrya* Dunn** - *Ngo Van Cuong, Trinh Thi Thanh Van, Phi Thi Dao, Le Tran Nguyen Vu, Pham Van Cuong, Nguyen Thuy Linh, Doan Thi Mai Huong*
419	**Chemical Composition Analysis and Quantification of Perillaldehyde in *Perilla frutescens* Leaf Essential Oil Collected in Vietnam** - *Pham Thi Hien, Nguyen Thi Hai Yen, Do Thi Xinh, Nguyen Thi Thu, Phan Van Truong, Nguyen Thi Hang, Do Thi Ha, Tran Minh Ngoc, Nguyen Minh Khoi, Nguyen Thi Ha Ly*
428	**Preliminary Research on Establishing the Quality Standards for Wine-Processed *Radix Angelicae sinensis* -** *Do Thi Thuy Linh, Nguyen Thi Ha Ly, Phan Thanh Thuy, Nguyen Phu Quang, Pham Anh Minh, Tran Huu Khanh Tan, Bui Ngoc Tan, Nguyen Tuan Hiep*
438	***In vitro* Screening of Cytotoxic Activity of Vietnamese Medicinal Plants -** *Nguyen Thi Thu, Nguyen Tra My, Do Thi Ha, Vu Thi Diep, Nguyen Thi Hang, Tran Thi Hien, Hoang Thi Dieu Huong, Tran Thuy Giang*
446	**Evaluation of Biocompatibility and Preliminary Hemostatic Efficacy of a Chitosan-Based Hemostatic Sponge (Field Hemostatic Sponge) in Experimental Models -** *Vu Quang Thai, Le Thi Xoan, Nguyen Hoang Ngan, Do Thi Thuy Trang*

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CHEMICAL COMPOSITION ANALYSIS AND QUANTIFICATION OF PERILLALDEHYDE IN *PERILLA FRUTESCENS* LEAF ESSENTIAL OIL COLLECTED IN VIETNAM

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Summary

Perilla frutescens (L.) Britton (Lamiaceae) is a valuable medicinal and culinary plant widely grown in Vietnam. This study aimed to evaluate the quality, analyze the chemical composition, and quantify perillaldehyde in the essential oil of *Folium Perillae frutescensis* collected from different regions in Vietnam. The total essential oil content was extracted and determined according to the Vietnamese Pharmacopoeia V. Chemical profiling was carried out using Gas Chromatography-Mass Spectrometry (GC-MS). Additionally, a GC-MS method for quantifying perillaldehyde in the essential oil of *Folium Perillae frutescensis* was developed and validated in accordance with ICH guidelines. The results showed that the total essential oil content of the fourteen samples fluctuated significantly, ranging from 0.68% to 2.21% (mL/100 g on a dry basis). GC-MS analysis identified perillaldehyde as the primary constituent in all samples, accounting for 28.3% to 68.7% of the relative percentage composition. Other major volatile compounds included D-limonene, caryophyllene, α-bergamotene, myristicin, and elemicin. The quantitative GC-MS method demonstrated high specificity, excellent linearity ($R^2 = 0.9992$), good precision (RSD < 4%), and strong accuracy with a recovery rate between 96.31% and 102.30%. The concentration of perillaldehyde in the analyzed essential oils varied from 137.10 mg/mL to 833.48 mg/mL, equivalent to 0.09% to 1.42% in the crude herbal materials. These data reveal notable variations in the chemical profiles of Vietnamese *Perilla* leaf essential oils across geographic locations and provide a reliable scientific database for the quality standardization of this medicinal material.

Keywords: *Perilla frutescens*; *Essential oil; GC-MS; Chemical composition; Perillaldehyde.*

1. Introduction

Perilla frutescens (L.) Britton, a plant belonging to the Lamiaceae family, has long been recognized as a valuable "food and medicine homology" resource, playing a dual role in both culinary and traditional medicine [1],[2]. Its primary chemical components include phenols, flavonoids, terpenoids, and fatty acids–most notably Omega-3, which accounts for 54–64% of its seed oil content [3],[4]. These components provide potent biological effects, including antioxidant, anti-inflammatory, anti-allergic, neuroprotective, and anti-cancer activities. *Perilla* extracts are known to inhibit histamine release, thereby alleviating symptoms of asthma, allergic rhinitis, and atopic dermatitis [3],[5]. Modern studies have further confirmed the potential of *Perilla* in inhibiting various cancer cell lines (such as liver, lung, breast, and colorectal cancers) and managing metabolic disorders, including glycemic control, lipid-lowering, and uric acid reduction in patients with gout [4],[5],[6].

Alongside these non-volatile compounds, *Perilla* essential oil–a complex mixture containing approximately 150–200 characteristic volatile components such as perillaldehyde, limonene, and perilla ketone–contributes significantly to the plant's pharmacological properties [7]. Beyond its demonstrated antimicrobial, antifungal, and insecticidal properties, which suggest applications as a natural food preservative and biopesticide, the essential oil has achieved notable biomedical milestones [8],[9]. Recent research indicates that *Perilla* essential oil exhibits antidepressant effects by regulating neurotransmitter balances, such as serotonin and dopamine, while simultaneously promoting the expression of Brain-Derived Neurotrophic Factor (BDNF) [10]. Furthermore, the essential oil has demonstrated the ability to inhibit viral entry, including that of SARS-CoV-2, and effectively supports the treatment of respiratory inflammatory diseases

due to its superior antispasmodic and anti-inflammatory properties [11].

Despite these important potential benefits, the current Vietnamese Pharmacopoeia lacks specific parameters for evaluating the quality of *Folium Perillae frutescensis* [12]. In contrast, the Chinese Pharmacopoeia [13] and the Hong Kong Chinese Materia Medica Standards (HKCMMS) [14] mandate a total essential oil content of no less than 0.4%. Addressing this practical necessity, this study was conducted to evaluate the quality of *Perilla* essential oil, analyze variations in chemical composition using GC-MS, and perform quantitative determination of perillaldehyde in essential oils across samples collected from various ecological regions throughout Vietnam. This research aims to provide a reliable scientific database to support the standardization of this medicinal material.

2. Materials and Methods

2.1. Materials

Folium Perillae frutescensis samples were collected at several locations in eight different ecological regions in Vietnam. In addition, in May 2025, *Perilla frutescens* samples were collected from Tan Phong, Lai Chau Province, Vietnam, to obtain a sufficient quantity of essential oil (approximately 10 mL) for the development and validation of the GC–MS analytical method. Samples were collected and identified by the Center for Medicinal Resources, National Institute of Medicinal Materials (NIMM). After being harvested, perilla samples are picked for their leaves. The leaves are then washed, dried, ground, and stored. The sample is currently stored at the Department of Analytical Chemistry and Standardization, NIMM.

Table 1. Sample information used in this study

No.	Sample	Sampling time	Sampling location	No.	Sample	Sampling time	Sampling location
1	M5	01/05/2025	Trieu Co, Quang Tri	8	M33	21/07/2025	Cam Ranh, Khanh Hoa
2	M6	04/05/2025	Dong Tien Hung, Hung Yen	9	M34	20/07/2025	Dien Khanh, Khanh Hoa
3	M9	29/06/2025	Da Mai, Bac Ninh	10	M41	19/07/2025	An Khe, Gia Lai
4	M12	01/07/2025	Thanh Vinh, Thanh Hoa	11	M44	29/07/2025	Sin Ho, Lai Chau
5	M23	19/07/2025	Doan Ket, Lai Chau	12	M45	29/07/2025	Tua Sin Chai, Lai Chau
6	M25	19/07/2025	Dien Ban, Da Nang	13	M49	24/07/2025	Thanh An, Binh Duong
7	M29	20/07/2025	Chu Prong, Gia Lai	14	M61	15/08/2025	Tan Than, Dong Thap

2.2. Chemicals and standards

Perillaldehyde (CAS: 18031-40-8; Lot: CFS202501; purity of 98%) from Chemfaces, China.

All solvents used for mass spectrometry (GC-MS) analysis are from Merck and meet purity standards for mass spectrometry analysis**.**

2.3. Methods

2.3.1. Determination of total essential oil content

The essential oil content in the medicinal plant was determined according to the Vietnamese Pharmacopoeia V, Appendix 12.7, "Determination of essential oil in medicinal plants".

2.3.2. Qualitative analysis of essential oil composition and quantification of perillaldehyde in *Folium Perillae frutescensis* essential oil using the GC-MS method

- Standard perillaldehyde solutions preparation: accurately weigh approximately 20 mg of perillaldehyde CRS and dissolve it in 10 mL of *n*-hexane to obtain a stock standard solution. From this solution, perform dilutions at different ratios with *n*-hexane to obtain perillaldehyde standard solutions of various concentrations (20.75, 41.50, 83.00, 166.00, 207.50, 415.00 and 830.00 µg/mL) for constructing the calibration curve.
- Test sample solution preparation: the essential oil sample (previously dried over anhydrous Na_2SO_4) was processed as follows: accurately pipette 0.1 mL of pure essential oil, transfer it into a 50-mL volumetric flask, dilute to

volume with *n*-hexane, and mix well (solution A). Then, accurately pipette 1 mL of solution A into a 10-mL volumetric flask, dilute to volume with *n*-hexane, and mix well. All these solutions were filtered through a 0.22 µm membrane filter before GC-MS analysis.

- GC-MS analytical conditions: GC-MS analysis was performed on a GCMS-QP2020 system (Shimadzu, Japan) with a SH-Rxi-5Sil MS capillary column (30 m × 0.25 mm × 0.25 µm), temperature programmed as follows: 60°C (held for 2 minutes), 60−120°C (at 3°C/min, held for 5 minutes), 120−300°C (at 5°C/min, held for 3 minutes). The carrier gas was helium at a flow of 3.0 mL/min. The injection volume was 1 µL, and the split ratio was 1:20. Mass spectra were taken at 70 eV. The scan mass range was from 50−900 *m*/*z* at a sampling rate of 1.0 scan/s. The components of the oil were identified by their retention times, mass spectra, and computer-matched spectra against the NIST MS Version 2.2 Library. The percentage composition of the identified compounds was calculated from the GC peak areas and expressed as relative percentages.

Method validation: The GC-MS method for the quantification of perillaldehyde in the essential oil of *Folium Perillae frutescensis* was validated according to the ICH and AOAC guidelines [15],[16].

- Calculations:

Content of perillaldehyde in essential oil X_1 (mg/mL):

$$X_1 = \frac{C \times V \times K \times P}{0.1 \times 10^3}$$

Where: C is the analyte concentration in the test sample solution calculated from the calibration curve equation (µg/mL); V is the volume of the dilution solvent (mL); K is the dilution factor; P is the purity of the standard (P = 0.98).

Content of perillaldehyde in the crude material X_2 (%):

$$X_2\ (\%) = \frac{X_1 \times V_2 \times 100}{m \times 10^3}$$

Where: V_2 is the volume of the obtained essential oil (mL); m is the mass of the herbal material (g).

3. Results and Discussion

3.1. Determination of total essential oil content in Folium Perillae frutescensis samples

Determination of total essential oil content in *Folium Perillae frutescensis* samples was conducted according to the method presented in Section 2.3.1. The results of total essential oil content analysis in *Perilla* leaves samples are presented in Table 2.

Table 2. Total essential oil content in *Perilla* leaves samples collected in Vietnam (n=3)

No.	Sample ID	The content of total essential oil* (%, mL/100 g)	No.	Sample ID	The content of total essential oil* (%, mL/100 g)
1	**M5**	1.15 ± 0.00	8	**M33**	1.11 ± 0.00
2	**M6**	1.16 ± 0.00	9	**M34**	1.11 ± 0.00
3	**M9**	0.70 ± 0.00	10	**M41**	1.33 ± 0.00
4	**M12**	1.21 ± 0.00	11	**M44**	1.78 ± 0.00
5	**M23**	2.21 ± 0.01	12	**M45**	1.78 ± 0.00
6	**M25**	0.68 ± 0.00	13	**M49**	1.25 ± 0.00
7	**M29**	1.38 ± 0.00	14	**M61**	0.68 ± 0.01

**Content calculated on a d-----ry sample*

**These results were calculated on a dry sample.*

The experimental findings demonstrate a significant variation in both the total essential oil yield and the specific chemical composition of *Folium Perillae frutescensis* collected across different ecological regions in Vietnam. Regarding the total volatile oil content, all 14 samples successfully exceeded the minimum threshold of 0.4% mandated by both the Chinese Pharmacopoeia and the HKCMMS. Notably, sample M23 from Lai Chau exhibited the highest yield at 2.21%, whereas samples M25 (Da Nang) and M61 (Dong Thap) yielded the lowest

baseline at 0.68%. This wide fluctuation underscores the profound impact of localized climate conditions, soil attributes, and specific geographical altitudes on the secondary metabolic pathways regulating volatile oil biosynthesis in *Folium Perillae frutescensis*.

3.2. GC-MS analysis of the essential oil of Folium Perillae frutescensis

Chemical components of *Folium Perillae frutescensis* essential oil samples collected from different regions were analyzed using the GC-MS method. The results are presented in Table 3.

Table 3. The composition of the essential oil from *Folium Perillae frutescensis (Relative percentages, %)*

Compound	RT (min)	M5	M6	M9	M12	M23	M25	M29	M33	M34	M41	M44	M45	M49	M61
(*R*)-*α*-Pinene	5.32	0.2	0.4	-	-	0.3	-	0.1	-	-	-	0.1	-	-	-
Benzaldehyde	6.14	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-
(-)-*β*-Pinene	6.73	0.2	0.5	-	0.2	0.4	-	0.2	0.4	0.3	-	0.2	0.3	-	0.4
1-Octen-3-ol	6.81	0.1	-	0.2	-	-	0.8	0.2	-	-	-	0.2	0.2	-	-
D-Limonene	8.80	8.7	11.8	9.1	9.2	13.2	11.3	9.3	16.2	13.2	4.2	11.4	11.9	4.7	10.0
Eucalyptol	8.90	0.1	-	-	-	-	-	-	-	-	-	-	0.2	-	-
Linalool	12.22	0.9	1.3	3.3	0.9	1.5	1.8	1.1	1.7	0.9	0.9	1.3	1.3	0.5	1.2
L-*α*-Terpineol	17.46	0.2	0.4	0.4	0.3	0.4	0.4	0.5	0.3	-	-	0.5	0.5	0.3	0.4
Perillaldehyde	**22.38**	**28.3**	**49.1**	**48.6**	**33.9**	**50.8**	**54.6**	**56.7**	**48.7**	**45.4**	**58.8**	**68.7**	**66.9**	**50.7**	**63.5**
Anethole	22.95	1.1	-	-	-	-	-	-	-	-	-	-	-	-	-
trans-Shisool	23.01	-	-	-	-	0.6	-	-	-	-	-	-	-	-	-
Perillol	23.84	0.4	2.3	1.9	1.3	6.9	2.5	2.3	1.3	-	5.4	1.1	2.4	0.4	1.7
m-Eugenol	27.04	0.3	0.3	1.1	0.4	-	1.1	1.0	-	-	0.4	0.4	0.6	-	-
α-Copaene	28.38	0.2	-	-	-	0.3	-	-	-	-	-	-	-	-	-
(-)-*β*-Elemene	29.32	0.3	-	0.3	-	0.2	-	-	-	-	-	-	-	-	-
Caryophyllene	30.97	13.1	12.1	12.4	12.1	10.7	11.6	10.1	11.9	21.1	11.5	9.5	9.6	10.5	9.3
1,5,9,9-Tetramethyl-1,4,7-cycloundecatriene	33.10	2.1	2.1	2.2	1.7	1.4	1.9	1.8	1.6	3.3	1.7	1.0	1.0	1.2	1.1
(*E*)-*β*-Farnesene	33.56	0.5	-	-	0.2	-	-	-	-	-	-	4.8	4.3	-	-
β-Copaene	34.93	3.7	3.0	5.3	1.7	3.9	4.6	1.6	3.6	4.1	2.2	0.7	0.8	0.7	1.3
Bicyclogermacrene	36.04	0.5	0.4	0.8	0.3	-	-	-	-	-	-	-	-	-	-
α-Bergamotene	36.49	6.9	9.5	6.4	3.6	5.7	7.2	10.1	6.6	7.0	9.0	-	-	6.2	9.4
α-Farnesene	37.53	0.2	0.4	-	-	-	-	-	-	-	-	-	-	-	0.4
β-Cadinene	37.95	-	0.4	-	-	0.5	-	-	-	-	-	-	-	-	-
Myristicin	38.44	13.1	1.1	3.4	6.2	1.7	-	3.0	4.6	-	1.6	-	-	22.9	-
Elemicin	40.30	17.3	0.7	3.2	27.0	-	-	0.4	-	1.1	-	-	-	-	-
(6*E*)-Nerolidol	40.90	0.5	0.9	0.6	0.5	0.4	1.0	0.6	0.4	0.8	0.6	-	-	0.4	0.5
Caryophyllene oxide	41.36	0.9	2.5	0.3	0.5	0.6	0.9	0.6	1.1	2.3	2.0	-	-	1.5	0.8
tau-Cadinol	44.01	-	-	-	-	-	0.3	0.4	-	-	-	-	-	-	-
α-Cadinol	44.47	-	-	-	-	0.2	-	-	-	0.5	0.38	-	-	-	-
Methyl linolenate	55.29	-	-	-	-	-	-	-	0.3	-	0.79	-	-	-	-
Phytol	55.52	-	-	-	-	-	-	-	1.3	-	0.55	-	-	-	-
Total		**99.9**	**99.2**	**99.5**	**100.0**	**99.7**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**99.9**	**100.0**	**100.0**	**100.0**

-: Not detected.

The chemical profiles of the essential oils extracted from 14 samples of *Folium Perillae frutescensis* were comprehensively established by GC-MS. The results revealed that perillaldehyde was consistently the most dominant constituent across all investigated locations, with relative

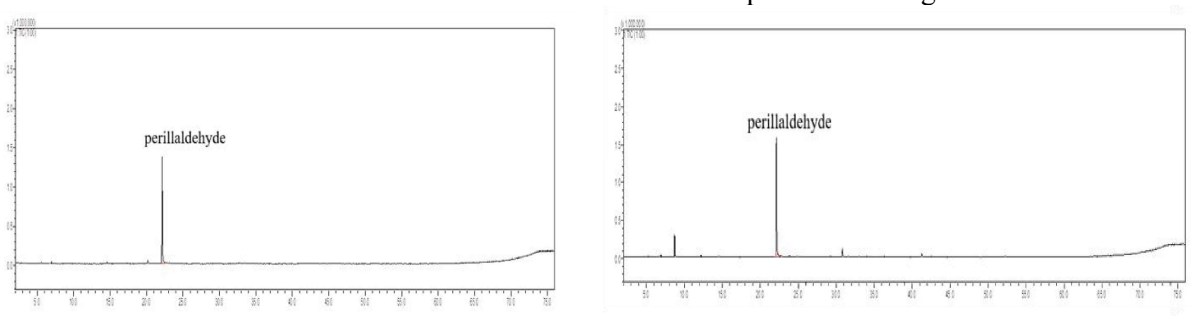
percentages ranging from 28.3% (sample M5 from Quang Tri) to 68.7% (sample M44 from Lai Chau). Some major volatile compounds were ubiquitously detected in all samples: caryophyllene (fluctuating from 9.3% to 21.1%), D-limonene (ranging from 4.2% to 16.2%). Additionally, *α*-bergamotene was verified in 12 out of 14 samples (absent only in Lai Chau samples M44 and M45), peaking at 10.1% in sample M29 (Gia Lai). These findings align with previous studies [17],[18],[19],[20] that have also identified perillaldehyde as the principal component in Perilla essential oils, albeit with varying dominance and concentration ranges depending on geographic origin and cultivar.

3.3. Quantification of perillaldehyde in Perilla essential oil using GC-MS

3.3.1. Method validation

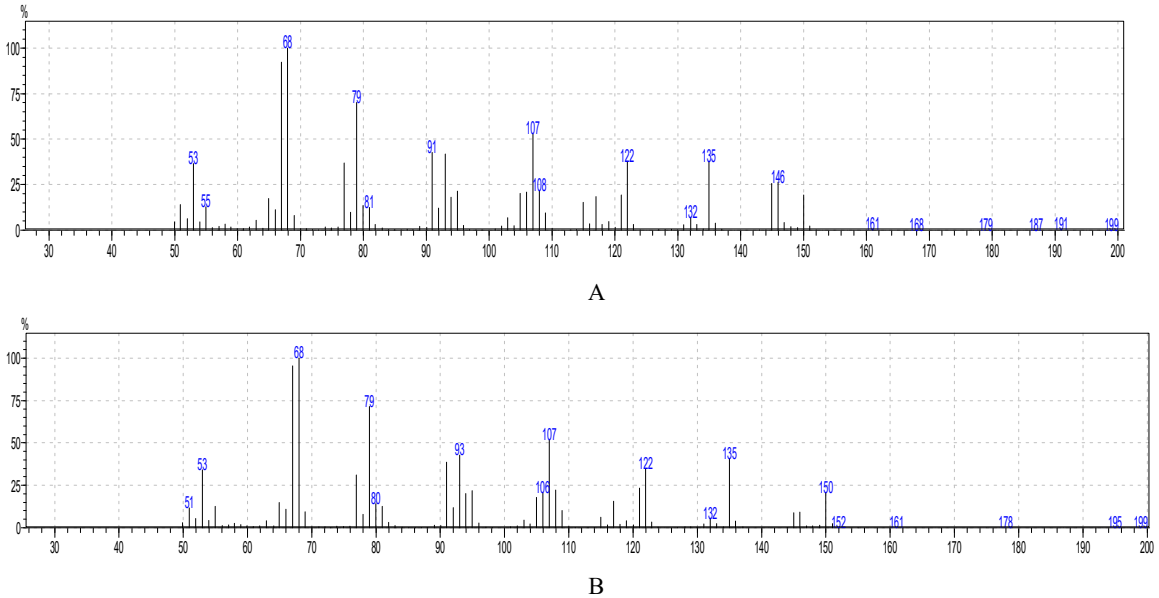
- **Specificity**

The perillaldehyde standard samples and *Folium Perillae frutescensis* essential oil test samples were analyzed using the selected GC-MS method and operating conditions. The obtained results are shown in Fig. 1. A comparison of the MS spectra of the perillaldehyde peak signals between the test sample and the reference standard is presented in Fig. 2.



A B

Fig. 1. GC-MS chromatograms of perillaldehyde standard (A) and *Folium Perillae frutescensis* essential oil (B)



A

B

Fig. 2. MS spectra of the perillaldehyde peak signal in the standard sample (A) and the test sample (B)

Table 4. Peak parameters

No.	Parameters	Mean
1	Retention time (Min)	22.108
2	Resolution	54.86
3	Tailing factor	1.445
4	Number of theoretical plates	387478

The obtained results indicated that, under the selected GC-MS conditions, the retention time of the perillaldehyde peak was 22.1 min. Correspondingly, a peak signal for perillaldehyde appeared at the identical retention time in the test sample. Comparison of the MS spectra of these two signals revealed high similarity (99% similarity score), demonstrating that the selected GC-MS method meets the specificity requirements.

- **System suitability**

The system suitability was evaluated by injecting the same perillaldehyde standard solution (207.5 µg/mL) six consecutive times for GC-MS analysis. The retention time and peak area of the perillaldehyde standard signal were recorded to calculate the mean and RSD% values of the six replicates. The results are presented in Table 5. The obtained data showed that all RSD% values were below 2.0%, demonstrating that the instrument is stable and suitable for perillaldehyde analysis.

Table 5. Results of the system suitability evaluation

No.	Retention time (minute)	Peak area
1	22.10	11077643
2	22.11	11516450
3	22.12	11601071
4	22.12	11749881
5	22.12	11519977
6	22.13	11450701
Mean	22.12	11485954
RSD (%)	0.04	1.79

- **Limit of Detection (LOD) and Limit of Quantitation (LOQ)**

The test sample with a predetermined perillaldehyde concentration was sequentially diluted until a signal-to-noise ratio (S/N) of 3–4 and 10 was obtained at the retention time of perillaldehyde on the chromatogram. Based on this method, the LOD and LOQ for perillaldehyde were determined to be 4.9 µg/mL and 16.32 µg/mL, respectively.

Table 6. Results of the perillaldehyde calibration curve construction

No.	Concentration of perillaldehyde (µg/mL)	Peak area
1	830.00	42687834
2	415.00	20109968
3	207.50	10077643
4	166.00	6929936
5	83.00	3528434
6	41.50	1273751
7	20.75	88110

- **Calibration Curve**

The results of the perillaldehyde calibration curve are presented in Table 6 and Fig. 3. The constructed perillaldehyde calibration curve showed an R^2 value of 0.9992 (> 0.99), demonstrating a good linear relationship between the peak area of the reference standard and its concentration in the analyzed solution.

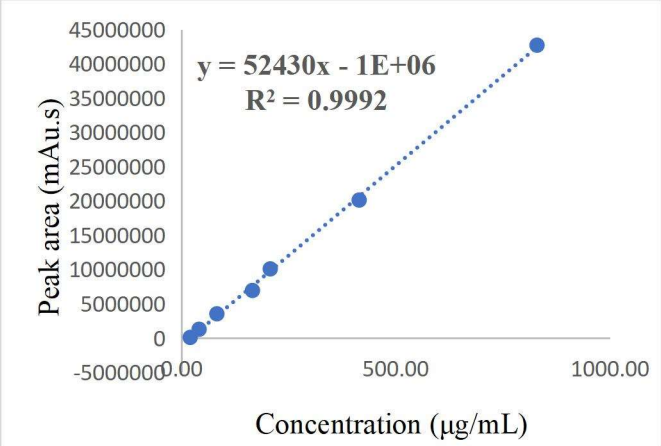


Fig. 3. The calibration curve of perillaldehyde

- **Repeatability**

Repeated analysis of the same test sample 6 times to obtain results evaluating the intraday repeatability of the method. The analysis was repeated 6 times on the same test sample on another day to collect the results of 12 analyses for repeatability assessment on other days. The results of the intra-day and inter-day repeatability evaluations are presented in Table 7.

Table 7. Results of the method repeatability evaluation

No.	Peak area	Content of perillaldehyde in essential oil (mg/mL)	Content of perillaldehyde in the crude material (%)
1	7555151	799.55	1.92
2	7805828	822.97	1.97
3	7436864	788.49	1.89
4	7500712	794.46	1.90
5	7853340	827.41	1.98
6	7717010	814.67	1.95
Mean (n=6)		**807.93**	1.93
RSD (%)		**1.67**	**1.67**
7	8143489	854.53	2.05
8	8073694	848.01	2.03
9	8046479	845.47	2.03
10	8127708	853.06	2.04
11	8177741	857.73	2.06
12	8122160	852.54	2.04
Mean (n=12)		**829.91**	**1.99**
RSDr (%)		**3.14**	**3.14**

The obtained results showed that the average content of perillaldehyde in the *Folium Perillae frutescensis* essential oil sample was 829.91 mg/mL, corresponding to 1.99% in the herbal material. The intra-day (n = 6) and inter-day (n = 12) RSD% values were 1.67% and 3.14%, respectively. Both values were below 4% [15], demonstrating that the method possesses good repeatability.

- **Accuracy**

The analytical samples for determining the recovery rate were prepared as follows: unspiked samples and spiked samples at three concentration levels of 50%, 100%, and 150% relative to the predetermined perillaldehyde content in the test sample. Detailed spiking concentrations and the evaluated recovery results are presented in Table 8.

Table 8. Results of the method recovery rate evaluation

	Theoretically added concentration (µg/mL)	Peak area	Recalculated actual concentration added (µg/mL)	Recovery rate (%)
50% spiked	83.00	12044546	79.94	96.3
	83.00	12222370	83.33	100.4
	83.00	12156795	82.08	98.9
100% spiked	166.00	16362746	162.30	97.8
	166.00	16553174	165.93	100.0
	166.00	16660864	167.99	101.2
150% spiked	249.00	21006095	250.86	100.7
	249.00	20759958	246.17	98.9
	249.00	21208508	254.72	102.3

The obtained results showed that the recovery rate for the quantitative method of perillaldehyde in *Folium Perillae frutescensis* essential oil ranged from 96.3% to 102.3%, demonstrating that the method possesses high accuracy.

3.3.2. Quantification of perillaldehyde in *Folium Perillae frutescensis* essential oil samples

The developed GC-MS method was applied to quantify perillaldehyde in *Folium Perillae frutescensis* essential oil samples. Each sample was analyzed in triplicate, and the results were calculated. The mean values determined for each sample are presented in Table 9.

Table 9. Results of perillaldehyde quantification in several *Folium Perillae frutescensis* essential oil samples (n = 3)

Sample ID	Content of perillaldehyde in essential oil* (mg/mL)	Content of perillaldehyde in the crude material* (%)
M5	394.55 ± 3.75	0.39 ± 0.00
M6	137.10 ± 2.60	0.13 ± 0.00
M9	306.97 ± 3.74	0.18 ± 0.00
M12	617.97 ± 3.62	0.61 ± 0.00
M23	715.85 ± 0.63	1.42 ± 0.00
M25	164.45 ± 1.58	0.09 ± 0.00
M29	720.20 ± 24.40	0.86 ± 0.02
M33	524.85 ± 16.00	0.52 ± 0.01
M34	463.41 ± 5.96	0.46 ± 0.00
M41	576.21 ± 6.63	0.69 ± 0.00
M44	734.07 ± 3.17	1.16 ± 0.00
M45	817.03 ± 10.70	1.30 ± 0.01
M49	833.48 ± 2.81	0.92 ± 0.00
M61	732.63 ± 14.90	0.43 ± 0.01

**Content calculated on an as-is basis*

The absolute concentration of perillaldehyde within the pure extracted essential oils of *Folium Perillae frutescensis* varied markedly from 137.10 ± 2.60 mg/mL (M6) to 833.48 ± 2.81 mg/mL (M49). When translated to the actual yield within the crude herbal materials, the absolute perillaldehyde content ranged from a minor 0.09 ± 0.00% (M25) to a robust 1.42 ± 0.00% (M23). Interestingly, while sample M49 possessed the highest perillaldehyde concentration per milliliter of essential oil, sample M23 provided the highest total perillaldehyde yield relative to the crude drug mass due to its exceptionally superior overall essential oil accumulation (2.21%).

Given that the current edition of the Vietnamese Pharmacopoeia lacks specific quantitative markers and standardized quality metrics for *Folium Perillae frutescensis*, these findings address a critical regulatory gap. The validated GC-MS quantification method and the comprehensive chemical mapping provided across diverse geographical cohorts offer a precise, scientific, and reproducible technical framework. Ultimately, these insights can be leveraged to establish official monographs, improve quality control, and select elite high-yielding cultivars for targeted medicinal cultivation in Vietnam.

4. Conclusion

This study provides a comprehensive assessment of the essential oil from *Folium Perillae frutescensis* collected across eight ecological regions in Vietnam. The total essential oil content of all 14 samples exceeded the minimum threshold of 0.4% (Chinese Pharmacopoeia, HKCMMS), with sample M23 (Lai Chau) achieving the highest yield at 2.21%. Phytochemical profiling via GC-MS confirmed perillaldehyde as the predominant constituent (28.3–68.7%). In contrast to previous studies that were primarily limited to semi-quantitative analysis based on the relative peak area percentage of perillaldehyde, this study has successfully established and validated a precise quantitative method using GC-MS with an

external standard. The developed method demonstrated high specificity, linearity, precision, and accuracy for the determination of perillaldehyde in *Folium Perillae frutescensis* oil. These findings establish a solid, scientific foundation to address the current regulatory gap in the Vietnamese Pharmacopoeia, facilitating the quality standardization, industrial control, and cultivation of high-quality perilla medicinal materials in Vietnam.

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