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The chemotypes of Elsholtzia ciliata (Thunb.) Hyl. germplasms in Vietnam

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ABSTRACT

Elsholtzia ciliata (Thunb.) Hyl., a plant from the *Lamiaceae* family, is an aromatic plant used in both culinary and traditional medicine in Vietnam and other Asian countries. The aim of this study is to classify chemotypes of *E. ciliata* from various ecological regions in Vietnam based on the essential oil compositions. A total of 11 accessions, including both cultivated and wild-grown plants, were collected. Their essential oil compositions were analyzed by using GC-MS. The results identified 61 compounds, with 15 major components of the essential oils. Hierarchical cluster analysis (HCA) based on 15 major components revealed two distinct chemotypes: citral and limonene. The cultivated accessions were primarily classified as the citral chemotype, characterized by high contents of *(α+β)*-citral. These compounds are commonly found in culinary herbs. On the other hand, the wild-grown accessions were classified as the limonene chemotype, dominated by limonene. This study contributes to the understanding of *Elsholtzia ciliata* (Thunb.) Hyl. chemotypes in Vietnam, suggesting potential applications based on chemotype classification.

INTRODUCTION

Elsholtzia ciliata (Thunb.) Hyl., a member of the *Lamiaceae* family, is an aromatic plant which used as significant culinary and traditional folk medicinal in Vietnam [1]. While it is extensively cultivated in Asian countries, it grows naturally in Europe and North America, where it is collected for essential oil component studies. This research aims to collect and analyse both cultivated

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and wild-grown *E. ciliata* accessions from diverse ecological regions in Vietnam to identify and classify the chemotypes based on the composition and concentration of specific constituents in their essential oils. The outcomes of this study will provide valuable insights for the identification and economic assessment of *E. ciliata* species in Vietnam.

MATERIALS AND METHODS

MATERIALS

Materials consist of branches bearing leaves and flowers of *Elsholtzia ciliata* (Thunb.) Hyl., collected from August to November in five different ecological regions in Vietnam (a total of 11 samples). Among these, three samples (EC-W1, EC-W2, EC-W3: Lao Cai) were collected from wild-growing plants in Northern Midlands and Mountainous Region, while the remaining eight samples were collected from cultivated plants at local markets: Red River Delta Region (EC-C3, EC-C4, EC-C7: Hanoi, Ninh Binh); Northern Midlands and Mountainous Region (EC-C1, EC-C5: Lao Cai, Hoa Binh); North Central Coast Region (EC-C6, Thua Thien Hue); Southeast Region (EC-C2: Hochiminh City); Central Highlands Region (EC-C8: Phu Yen).

The samples were identified by Msc. Nghiem Duc Trong (Hanoi University of Pharmacy). The voucher specimens were deposited at the herbarium HNIP (HUP).

METHODS

Isolation of essential oil

The shade-dried branches bearing leaves and flowers were chopped into small pieces (5-10 mm). After that, the materials (200 g for each sample) were immersed in water (2L) and submitted to distillation of essetial oil. The essential oils (EOs) were isolated by hydro-distillation using an apparatus according to the Vietnamese Pharmacopoeia [2] for 2-3 hours until the oil level was constant. The essential oil yield of 11 samples was calculated based on dry weight (0.6% - 0.9% yields). Anhydrous sodium sulfate was added for removal of the water if any (~2-5% w/w). After that, the oil was stored in a vial at 4-5°C in the dark prior to the GC analysis.

GC-MS analysis of essential oil

The essential oil was diluted in n-hexane to the concentration of 1:1000 (v/v). Gas chromatography analysis was performed using the GC Intuvo 9000 equipped with mass spectrometer detector MSD 5977B (Agilent Technologies, USA), using a non-polar DB-5MS fused silica capillary column (30 m × 250 µm × 0.25 µm). The oven temperature was programmed from 50°C, kept in 3 minutes; then the temperature was increased to 280°C at a rate of 5°C min-1. The inlet temperature was set at 250°C, the split ratio was 300:1. Helium with the flow rate of 1 ml. min-1 was applied as the carrier gas. The transfer line temperature was set at 250°C. Electron Ionization (EI) energy was 70 eV with a scan range from 35-450 amu. The solvent delay time was 4 min and took 50 min for the total run.

The retention indices (RI) of components were determined using the n-alkanes (C8-C20) analysed using the same condition. The volatile components were identified by comparing their mass spectra and RI values to those from reference compounds and mass spectral library NIST 2014, the NIST Chemistry Web Book, and the Adams book [3].

Data analysis

To assess the chemical relationships among the analysed *E. ciliata* accessions, a hierarchical cluster analysis (HCA) was conducted, and the results were presented in the form of a dendrogram. The HCA was

conducted using Rstudio (2014.12.1) with the Euclidean distance measure and incorporated two components: MS data and relative abundances of the test materials. This approach helped reveal the interrelationships among the accessions and provided insights into their chemical similarities or differences.

RESULTS AND DISCUSSIONS

RESULTS

Chemical composition of E. ciliata essential oils

The GC-MS analysis of essential oil of 11 *E. ciliata* samples resulted in identification of 61 compounds, of which 15 are major essential oil compounds. These compounds were: 1-octen-3-ol (0-7.10%), 3-octanone (0-3.64%), limonene (0-71.0%), 1,8-cineole (0-9.73%), *trans-β*-ocimene (0-36.2%), verbenone (0-17.2%), nerol (0-3.50%), *β*-citral (0-18.8%), D- carvone (0-24.9%), *α*-citral (0-23.4%), geraniol (0-3.10%), geranyl acetate (0-6.98%), caryophyllene (0-1.32%), *trans-β*-farnesene (0-22.7%), humulene (0-6.49%) (see Table 1). From the 15 major compounds, 8 of them (see Figure 1) occur in more than 10%. The 8 compounds were: limonene, *trans-β*-ocimene, D-carvone, *α*-citral, *trans-β*-farnesene, verbenone, *β*-citral, caryophyllene.

Limonene was the dominant constituent occurring up to 71.0% (EC-W3) and over 36% with the two accessions EC-W2 (36.7%) and EC-W1 (36.1%). Limonene was not found or recorded at less than 3.5% in the remaining eight accessions (EC-C1 to EC-C8) (see Table 1).

Trans-β-ocimene was the second most abundant constituent ranging from absent to 36.2%. Eight accessions recorded above 12%: EC-C4 (36.2%), EC-C5 (21.5%), EC-C3 (21.0%), EC-C8 (18.0%), EC-C1 (16.6%), EC-C2 (15.7%), EC-C7 (13.3%), EC-C6 (12.5%), and two accessions recorded at less than 1.5%: EC-W1 (1.40%), EC-W2 (1.36%). Meanwhile, it was not found in EC-W3 (see Table 1).

D-carvone was one of the dominant constituents in this study with 24.9% (EC-W1), followed by 23.8% (EC-W2), and 15.5% (EC-W3) across accessions with an average of 21.4%, was not found in the remaining eight accessions (EC-C1 to EC-C8) (see Table 1).

α-citral, *β*-citral, *trans-β*-farnesene, caryophyllene were also dominant constituents ranging: *α*-citral from absent to 23.4%, *β*-citral from absent to 18.8%, *trans-β*-farnesene from absent to 22.7%, caryophyllene from absent to 13.2%. These four chemical compositions were the dominant compound in all eight accessions analyzed (EC-C1 to EC-C8) with more than 13.5% *α*-citral, 10.0% *β*-citral, 7.5% *trans-β*-farnesene, 5.0% caryophyllene and they were not exhibited in three accessions EC-W1, EC-W2, EC-W3 (see Table 1).

Vebenone was one of abundant constituent, its content ranging from 16.9% (EC-W2) to 17.2% (EC-W1), and it was not absent in the remaining accessions (see Table 1).

Cluster analysis

The hierarchical cluster analysis based on 15 major compounds resulted in the segregation of the *E. ciliata* accessions into two distinct clusters (see Figure 2).

Cluster I: This cluster comprised eight accessions, accounting for 72.7% of the total accessions (see Figure 2). The distinguishing characteristic of this cluster is the high content of specific compounds, namely *(α+β)*-citral, *trans-β*-ocimene, *trans-β*-farnesene, and caryophyllene, leading to its classification as the "citral" chemotype (the

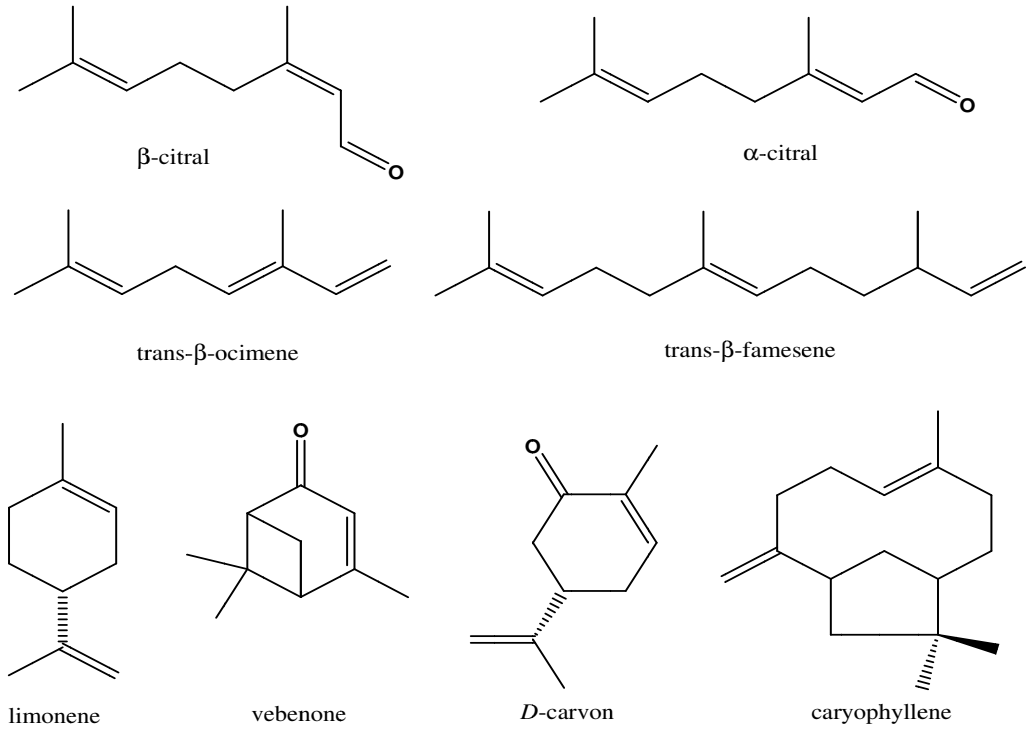

Figure 1. Chemical structures of the 8 major compounds of E. ciliata essential oil

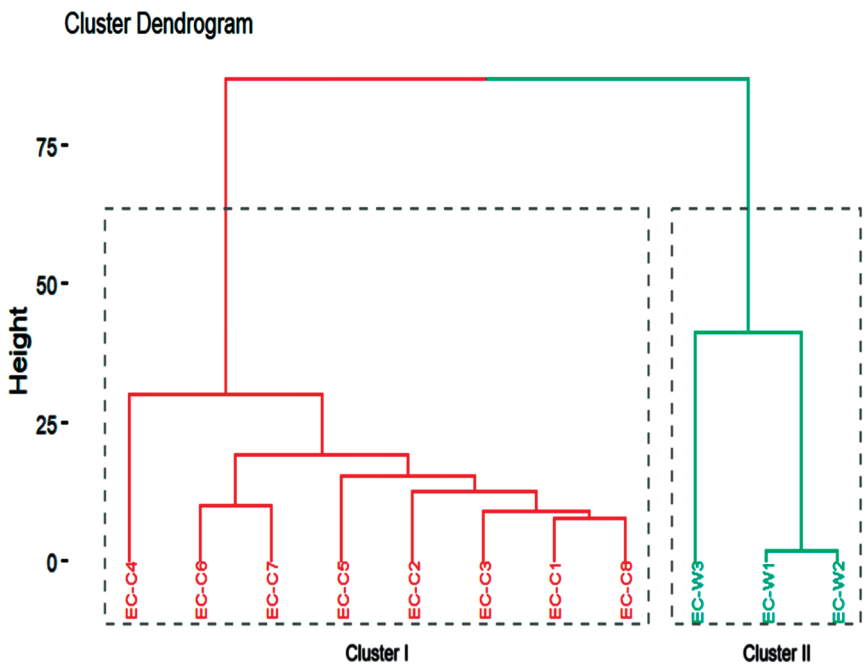

Figure 2. Dendrogram illustrating the clustering patterns of the 11 E. ciliata accessions based on different chemical compounds.

chemotype name is derived from the predominant essential oil ingredient). All eight accessions in this cluster recorded *(α+β)*-citral content above 24%: EC-C6 (42.1%), EC-C2 (40.2%), EC-C3 (39.5%), EC-C1 (38.1%), EC-C4 (37.5%), EC-C8 (31.8%), EC-C7 (31.3%), and EC-C5 (24.1%). The content of *trans-β*-ocimene ranged from 12.5% to 36.2%, while *trans-β*-farnesene content varied from 7.68% to 22.7%, and caryophyllene content ranged from 5.33% to 13.2%. These compound profiles collectively characterized the accessions within Cluster I.

Cluster II: This cluster consists of three accessions, accounting for 27.3% of the total accessions (see Figure 2). The distinguishing feature of this cluster is the high content of specific compounds, namely limonene, D-carvone, and vebenone, which classify it as the "limonene" chemotype. One accession (EC-W3) exhibited a significantly high limonene content (71.0%). Similarly, the other two accessions (EC-W2 and EC-W1) in this cluster also displayed elevated limonene contents (36.7% and 36.1%, respectively). Furthermore, all three accessions exhibited D-carvone contents above 15.0%: EC-W1 (24.9%), EC-W2 (23.8%), and EC-W3 (15.5%).

DISCUSSION

Our investigation of the essential oil composition of *Elsholtzia ciliata* was carried out using plant materials collected from five

Table 1. Major chemical compositions of the essential oils of 11 Elsholtzia ciliata samples

Samples / CC (%)	EC-W1	EC-W2	EC-W3	EC-C1	EC-C2	EC-C3	EC-C4	EC-C5	EC-C6	EC-C7	EC-C8	Range
1-octen-3-ol	-	-	-	3.42	7.10	4.22	6.30	4.38	-	4.15	-	0-7.10
3-octanone	3.56	3.64	-	-	-	-	-	1.19	-	-	-	0-3.64
limonene	36.1	36.7	71.0	1.02	3.10	2.87	-	2.58	-	1.68	-	0-71.0
1,8-cineole	1.76	1.33	9.73	0.85	-	-	-	-	-	-	-	0-9.73
trans-β-ocimene	1.40	1.36	-	16.6	15.7	21.0	36.2	21.5	12.5	13.3	18.0	0-36.2
verbenone	16.9	17.2	-	-	-	-	-	-	-	-	-	0-17.2
nerol	-	-	-	0.86	3.50	-	-	1.41	-	1.37	-	0-3.50
β-citral	-	-	-	17.4	16.8	18.8	17.0	10.3	18.7	15.7	14.8	0-18.8
D-carvone	24.9	23.8	15.5	-	-	-	-	-	-	-	-	0-24.9
α-citral	-	-	-	20.7	23.4	20.7	20.5	13.8	23.4	15.6	17.0	0-23.4
geraniol	-	-	-	-	3.10	-	-	1.37	-	0.67	-	0-3.10
geranyl acetate	-	-	-	-	0.70	1.85	6.98	0.98	0.53	1.36	-	0-6.98
caryophyllene	-	-	-	13.2	9.20	7.7	5.33	6.08	11.0	9.57	9.76	0-13.2
trans β-farnesene	-	-	-	13.3	8.30	14.9	7.68	14.2	22.1	22.7	13.2	0-22.7
humulene	5.42	6.49	-	2.85	-	1.39	-	0.64	-	1.25	-	0-6.49

ecological regions of Vietnam, including both cultivated and wild-growing populations. Notably, wild specimens were encountered only in Bat Xat District (Lao Cai Province). Based on GC–MS profiling, the samples could be clearly divided into two chemotype clusters. Cluster I (citral chemotype) comprised all cultivated accessions (EC-C1→EC-C8), whereas Cluster II (limonene chemotype) included exclusively the wild-growing samples (EC-W1→EC-W3). These findings are in agreement with previous studies conducted in Vietnam, which consistently reported that cultivated E. ciliata used as a culinary herb across similar ecological regions predominantly belongs to the citral chemotype [4–9]. A defining feature of cultivated Vietnamese *E. ciliata* essential oils is the predominance of four major constituents: *(α+β)*-citral, *trans-β*-ocimene, *trans-β*-farnesene, and caryophyllene supporting their classification within this chemotype.

To date, no studies from Vietnam have described an essential oil profile of *E. ciliata* dominated by limonene, *D*-carvone, or verbenone. The present work therefore provides the first evidence of a limonene chemotype occurring in wild-growing *E. ciliata* populations in the country. Ethnobotanical surveys of Lamiaceae medicinal plants conducted across several northern mountainous provinces further revealed a highly restricted distribution of wild *E. ciliata,* which was observed exclusively in Y Ty (Bat Xat District) and not in Sa Pa, despite both areas belonging to the same northern mountainous ecological zone of Lao Cai Province. This localized occurrence may suggest ecological or genetic differentiation between cultivated and wild populations.

At a broader geographical scale, previous investigations from Lithuania and Russia have identified another distinct chemotype characterized by high levels of acylfuran derivatives, including rosefuran, elsholtzia ketone, and dehydroelsholtzia ketone [10,11]. Similarly, studies conducted in Korea reported the presence of three chemotypes: citral, limonene, and acylfuran although the origin of the analyzed materials (cultivated versus wild) was not specified [12]. Taken together, these comparisons highlight the considerable chemical polymorphism of *E. ciliata* essential oils across different regions and emphasize the importance of documenting local chemotypic diversity for both ethnobotanical and phytochemical studies.

CONCLUSION

The collection of 11 *Elsholtzia ciliata* accessions from 5 ecological regions in Vietnam, when analysed for their essential oil compositions, revealed the presence of two distinct chemotypes: citral and limonene. The cultivated accessions were classified as belonging to the citral chemotype. These cultivated accessions are commonly utilized as a culinary herb, which is dominated by citral compounds. In contrast, the wild-grown accessions were identified as belonging to the limonene chemotype. These accessions, collected from their natural habitats, are primarily used in traditional folk medicine. This distinction in chemotypes based on essential oil compositions highlights the variability in the chemical profiles of *Elsholtzia ciliata* accessions in difference ecological regions of Vietnam and their associated uses in culinary and medicinal practices.

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CONFLICTS OF INTEREST

None.

REFERENCES

1. National Center for Natural Science and Technology. [Flora of Vietnam: *Lamiaceae* Lindl. (Labiatae Juss.), Vol. 2]. Hanoi, Vietnam: Science & Technics Publishing House. 2000, p. 225- 237. (in Vietnamese).
2. Ministry of Health of Vietnam. [Vietnamese Pharmacopoeia V. Vol. 2]. Hanoi, Vietnam: Medical Publishing House. 2017. (in Vietnamese).
3. Adams Robert P. Identification of essential oil components by gas chromatography-mass spectrometry, ed. 4.1. Carol Stream, USA: Allured Publishing Corp. 2017.
4. Nguyen XD, Len VH, Le HH, Leclercq PA. Composition of the essential oils from the aerial parts of *Elsholtzia ciliata* (Thunb.) Hyland. from Vietnam. *J. Essent. Oil Res.* 1996, 8(1):107-109.
5. Le TB, Beaufay C, Nghiem DT, Marie-Paule Mingeot-Leclercq, Joëlle Quetin-Leclercq. In Vitro Anti-Leishmanial Activity of Essential Oils Extracted from Vietnamese Plants. *Mol.* 2017, 22(7):1071.
6. Nguyen DD, Nguyen TKT, Mai TNT, Ninh KB, Quach TTV, Quan CT *et al.* Chemical composition of the essential oil of *Elsholtzia ciliata* grown in Phu Tho province. *J Sci Technol Hung Vuong Univ.* 2022, 27(2): 80-84.
7. Tran TQA, Vo TTH. Reseach on chemical composition and antibacterial of *Elsholtzia ciliata* essential oil. *JASAT.* 2022, 6(1): 2881-2890. (in Vietnamese)
8. Luu Tang PK, Nguyen HT, Cao CL, Nguyen Ngoc TP, Nguyen HV, Truong V et al. Chemical composition of Elsholtzia ciliata (Thunb.) Hyland essential oil in Vietnam with multiple biological utilities: a survey on antioxidant, antimicrobial, anticancer activities. *Acad. J. Biol.* 2023, 45(3): 99-110.
9. Phan VT, Dam TBH, Phan Hoang TB, Tran TMT, Vu TH, Nguyen TKA. Factors influencing content and composition of essential oil of Elsholtzia ciliata (Thunb.) Hyland collected in Dak Lak and antibacterial activity of the obtained essential oil. *J. Sci. Technol. Hanoi Univ. Ind.* 2024, 60(3): 68–76.
10. Pudziuvelyte L, Stankevicius M, Maruska A, Petrikaite V, Ragazinskiene O, Draksiene G *et al.* Chemical composition and anticancer activity of *Elsholtzia ciliata* essential oils and extracts prepared by different methods. *Ind. Crops Prod.* 2017, (107):90-96.
11. Elena A. Korolyuk, Wilfried König, Alexey V. Tkachev. Composition of essential oil of Elsholtzia ciliata (Thunb.) Hyland from the Novosibirsk region, Russia. *Journal.asu.ru.* 2002, 1: 31-36.
12. Song JS. [Chemotaxonomy based on essential oil composition and characteristics of native *Elsholtzia ciliata* (Thunb.) Hylander]. Seoul, Korea: Seoul National University. 2000. (In Korean).