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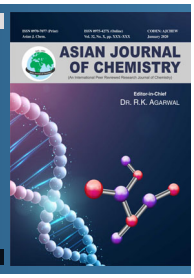

Compositional Comparison of Essential Oils Extracted from Flowers and Aerial Parts of *Elsholtzia winitiana* var. *dongvanensis* Phuong Harvested in Ha Giang Province, Vietnam

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Elsholtzia winitiana var. *dongvanensis* Phuong is a plant contains an essential oil characterized by an important chemical transformation. Essential oils were isolated from *Elsholtzia winitiana* var. *dongvanensis* Phuong flowers and aerial parts by hydrodistillation and analyzed for chemical constituents by GC-MS. Twenty-two components accounting for 99.99% of the total oil of flowers and twenty-three components accounting for 98.72 % of the total oil of aerial parts were identified. The composition of the essential oil contains aldehyde, aromatic ketone, monoterpenoid and secquiterpenoid. Major components found in two essential oil samples were andehyde, terpenoid, ketone benzaldehyde, germacrene D, E-caryphyllene and caryophyllene oxide. Moreover, essential oil sample isolated from flowers and aerial parts of *E. winitiana* var. *dongvannensis* was dominated by the high content of rosefuran (75.67%) and rosefuran epoxide (71.33%), respectively.

Keywords: *Elsholtzia winitiana*, Essential oils, Hydrodistillation, GC-MS, Rosefuran, Rosefuran epoxide.

INTRODUCTION

The importance of aromatic plants in Vietnam is significant, especially in Southeast Asia, due to their applications in folk remedies and their potential for diverse commercial exploitation. Such fields as pharmaceuticals, perfumes and cosmetics, distilleries, flavors and aroma enhancers, and food industry. In recent years, people have tended to use products derived from nature. In particular, essential oils with many useful uses have been applied in many production areas and life such as medicine, food, personal care products [1]. Essential oils are a potential source of raw materials, the prospects for socio-economic development of Vietnam in the present as well as in the future [2-5]. Essential oils can be extracted from plants by many methods in which the hydrodistillation method has been widely used and can be applied on a commercial scale. Essential oils are a highly refined liquid (most often by distillation by steam or water) from leaves, stems, flowers, bark, roots or other plant components [6-8]. It is a mixture of organic substances mixed together, has a characteristic odour. At room temperature, essential oils are mostly liquid or insoluble in water but soluble in organic solvents such as alcohols and fats. Essential oils evaporate with steam, have a spicy, sweet taste and have antibacterial and antioxidant properties against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* but did not show that activity against *Bacillus subtilis*, *Lactobacillus fermentum*, *Salmonella enterica* and *Pseudomonas aeruginosa* [9-13]. Essential oils are widely used in the fields of manufacturing and life caring products, especially in the cosmetics, food and pharmaceutical industries [14-16].

Plant essential oil are mixtures of plant secondary compounds, consisting mainly of terpenoids aromatic phenol, oxides,

ethers, alcohols, esters, aldehydes and ketone [17-21]. *Elsholtzia* (Lamiaceae) is a genus of ornamental and aromatic herbs that are distributed mainly in China, Myanmarr, Thailand, Vietnam and a few African and European countries. However, most studies only focus on ten *Elsholtzia* species which are *E. blanda*, *E. bodinieri*, *E. ciliata*, *E. cristata*, *E. densa*, *E. eriostachya*, *E. ianthina*, *E. rugulosa*, *E. splendens* and *E. stauntonil*, leaving many other species unexplored [22-24]. *Elsholtzia winitiana* var. *dongvanensis* Phuong is an endemic species of the *Elsholtzia* genus that is distributed in Ha Giang province, Vietnam and used as an ingredient in traditional medicine for treatment of colds, headaches, coughs, pharyngitis and fever [25]. The species is one of the seven endemic plant species in Vietnam and mostly found in Dong Van highland plateau, Ha Giang province [26-29]. The tree is about 1-2 m high. Body square, hairy. Leaves opposite, with short stalks, wedge-shaped stems, pointed tips, 3-8 cm broad, 1.5-2 cm wide, smooth upper surface, soft underparts, uneven teeth edges and prominent veins clear. Inflorescence in the middle of leaves or branches into thick cotton, slightly lateral, 6-12 cm long; bracts are shorter than flowers, have thick hairs; small white flowers. The fruits are oval flat, smooth, when ripe dark brown and the whole plant smells like eucalyptus.

Lesueur *et al*. [29] showed that this essential oil mainly contained 1.8-cineole as the main ingredient (65.6%); Other compounds present at significant levels are α-pinene (5%), *p*-cymene (4.7%), β-terpinene (3.9%), sabinene (2.5%), α-pinene (1.8%), myrcene (1.7%) and α-terpinene (1.7%). The significance of the species lies in the fact that the bile of bloomed *Elsholtzia winitiana* flower allows the production of mint-honey, a local specialty that has a special and distinctive odour [30-32]. In this study, essential oils extracted from *E. winitiana* var. *dongvanensis* flowers and aerial parts were obtained by hydrodistillation and compared for composition. Determination of volatile composition of the extracted essential oils was performed by GC-MS method.

EXPERIMENTAL

Plant material: *Elsholtzia winitiana* var.. *dongvanensis* Phuong plants were collected in November 2019, at Dong Van district of Ha Giang province, Vietnam. The specimen of the plant was botanically identified by Dr. Nguyen Quoc Binh, Vietnam National Museum of Nature, Vietnam Academy of Science and Technology.

The herb grows into dust of 1-1.7 m high with thick white fluffies. Square body has 4 grooves and the grown leaves. were aligned with slender, sharp and two-pointed blades. Leaves were 4-9 cm long, 1.5-3.5 cm wide with fluffies on both sides and the edge of leaves was serrated with stalk of 6-8 mm long. After harvesting, the flowers and the aerial parts were separated and then essential oils were separately extracted. Used materials were ensured of uniform quality, fresh and free from pests and diseases.

Extraction of *E. winitiana* var. *dongvanensis* essential oils: A clevenger-type apparatus (Fig. 1) was employed to isolate the essential oils from the flowers and aerial parts of *E. winitiana* var. *dongvanensis*. The hydrodistillation process commenced

Fig. 1. Process of *E. winitiana* var. *dongvannensis* essential oil extraction by hydro-distillation

by adding 400 g of fresh plant materials into distilled water at a ratio of 1:2, followed by boiling for 4 h, starting from the appearance of the first essential oil drop in the collector. The products were dehydrated with Na_2SO_4 to afford essential oils. The essential oil was then stored in a dark bottle until analyzed with gas chromatography.

Gas chromatography (GC) analysis: An Agilent Technologies HP7890A GC instrument was employed to perform GC-MS analysis of the essential oils. The instrument was equipped with Agilent Technologies HP5975C mass spectrum detector and DB-XLB column (60 m × 0.25 mm, film thickness 0.25 µm, Agilent Technologies). Temperature of the injector and detector was set at 250 and 280 ºC, respectively. The progress of the column temperature initiated at 40 ºC, followed by a 20 ºC/min rise to 140 ºC and a subsequent rise to 270 ºC at 4 ºC/min. Helium with the flow rate of 1 mL/min was used as the carrier gas. Samples were injected by splitting with the volume of 1 µL and the split ratio of 100:1. The MSD conditions were as follows: ionization voltage 70 eV, emission current 40 mA, acquisitions scan mass range 35-450 amu under full scan. Homologous *n*-alkane series were used as the reference in which their retention times were used as a base for calculation of retention time indices of constituents in the sample. The relative amounts of individual components were calculated based on the GC peak area (MSD response) without correction.

RESULTS AND DISCUSSION

Fresh flowers materials (400 g) afforded 5.1 mL (g) of essential oil, while the fresh aerial parts sample (400 g) afforded 3.5 mL (g) of essential oil. Approximated average essential oil yield was 0.75-0.82%. Visually, the colour of the obtained essential oil was light yellow and the density was lighter than water. The odour was light and similar to rose oil. The yellow colour of the essential oil obtained from flowers was lighter than that obtained from aerial parts. A previous report [30] indicated that the extraction of essential oils from aerial parts of *E. witiania* (dry sample) harvested in Moc Chau district, Son La province afforded the yield of 0.58%. Another dry

sample in Sa Pa district, Lao Cai province gave the yield of 1.28% (dry weight).

Composition of *E. winitiana* var. *dongvanensis* essential oils are shown in Tables 1 and 2. In essential oil of aerial parts, a total of 22 different compounds were detected, corresponding to the chromatogram (Fig. 2) indicating 22 different peaks at different concentrations, accounting for 99.99% of total essential oil content. Major constituents in the essential oil of aerial parts contained rosefuran (1.77%), E-caryophyllene (11.12%) and rosefuran epoxide (69.56%). In essential oil

TABLE-1
RETENTION TIME (min) AND PEAK AREA (%) OF THE DIFFERENT COMPOUNDS FOUND IN ESSENTIAL OIL EXTRACTED FROM AERIAL PARTS OF *E. winitiana* var. *dongvanensis* ANALYZED BY GC-MS

Peak	RT	RI	Hit (%)	Chemical name	Integral	%
1	10.97	967	76	Benzaldehyde	6652442	0.97
2	11.33	979	89	Sabinene	767573	0.11
3	11.51	985	96	β-Pinene	1374597	0.20
4	13.15	1034	87	Limonene	4606966	0.70
5	13.27	1038	69	1,8-Cineole	2051716	0.36
6	14.51	1074	79	Acetophenone	2496819	0.36
7	15.38	1100	51	Rosefuran	83938625	12.47
8	15.51	1103	79	Linalool	2111572	0.39
9	17.53	1161	0	Unknown (95, 166, RI 1161)	7509417	1.10
10	18.06	1176	0	Unknown (95, 166, RI 1176)	23742715	3.50
11	18.25	1181	59	Rosefuran epoxide	416900065	63.17
12	18.92	1200	54	α-Terpineol	1058438	0.15
13	19.13	1206	57	8,9-Dehydrothymol	1276940	0.17
14	19.39	1214	0	Unknown (96, 168, RI 1214)	31146084	4.61
15	19.68	1222	0	Unknown (95, 166, RI 1222)	14016308	2.06
16	21.15	1265	53	Piperitone	1237302	0.18
17	22.70	1310	79	Dehydroelsholtzia ketone	1767180	0.26
18	26.84	1437	84	E-Caryophylene (=β-Caryophylene)	45630585	6.74
19	27.92	1471	97	α-Humulene	5459896	0.80
20	28.75	1498	90	Germacrene D	8026621	1.19
21	29.23	1514	47	Bicyclogermacrene	1307826	0.19
22	31.94	1605	8	Caryophyllene oxide	1691332	0.31
				Total		99.99

TABLE-2
RETENTION TIME (min) AND PEAK AREA (%) OF THE DIFFERENT COMPOUNDS FOUND IN ESSENTIAL OIL EXTRACTED FROM FLOWER PARTS OF *E. winitiana* var. *dongvanensis* ANALYZED BY GC-MS

Peak	RT	RI	Hit (%)	Chemical name	Integral	%
1	10.96	966	75	Benzaldehyde	9676362	2.10
2	11.33	979	84	Sabinene	735945	0.16
3	11.50	984	94	β-Pinene	1110442	0.24
4	13.14	1034	86	Limonene	2215065	0.52
5	13.26	1038	71	1,8-Cineole	987859	0.24
6	14.50	1074	67	Acetophenone	1853542	0.40
7	15.36	1099	55	Rosefuran	8123877	1.77
8	15.51	1103	72	Linalool	1813703	0.40
9	18.05	1175	0	Unknown (95, 166, RI 1175)	8198505	1.75
10	18.24	1181	61	Rosefuran epoxide	314457713	69.56
11	18.92	1200	71	α-Terpineol	1019881	0.22
12	19.37	1213	0	Unknown (96, 168, RI 1213)	21243532	4.60
13	21.14	1265	49	Piperitone	797482	0.17
14	22.70	1310	66	Dehydroelsholtzia ketone	997902	0.21
15	25.31	1389	89	α-Copaene	775261	0.17
16	25.66	1400	75	β-Buorbonene	482496	0.12
17	26.83	1437	81	E-Caryophylene (=β-Caryophylene)	51035392	11.12
18	27.91	1471	98	α-Humulene	5956350	1.29
19	28.75	1498	94	Germacrene D	7959744	1.74
20	29.22	1513	51	Bicyclogermacrene	1152810	0.28
21	29.90	1536	44	δ-Cadinene	463743	0.10
22	31.93	1605	74	Caryophylene oxide	6198046	1.43
23	32.70	1631	0	Humulene epoxide II	607601	0.13
				Total		98.72

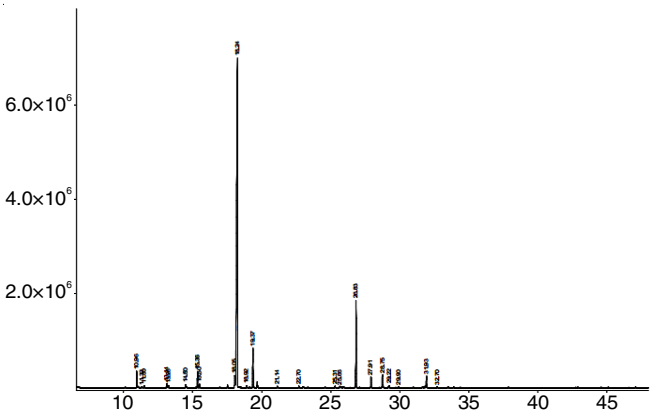

Fig. 2. Chromatogram showing the chemical composition of essential oils extracted from aerial parts analyzed by GC-MS

extracted from flowers, there were 22 different compounds, corresponds to the chromatogram in Fig. 3. Around 22 different (accounting for 99% of total essential oil content) peaks at different concentrations were detected with major constituents being E-caryophyllene (6.74%), rosefuran (12.47%) and rosefuran epoxide (63.17%). Comparing these results, one could observe some compositional similarities between essential oil obtained from flowers and from aerial parts. However, differences were recognized in quantities of some main compounds. To be specific, for benzaldehye compound, the contents were 0.97 and 2.10%, respectively for samples obtained from flowers and aerial parts while caryophyllen compounds accounted 6.74% and 11.12%. Interestingly, the proportion of rosefuran compound in the essential oil in the aerial parts and flowers accounted only 1.77% and 12.47%, respectively. This results in a total ratio of two rosefuran and rosefuran epoxide compounds of 71.33% and 75.64%, respectively corresponding to essential oils obtained from aerial parts and flowers. Rosefuran and rosefuran epoxide are a desirable component of rose oil, often utilized as a natural fragrance material.

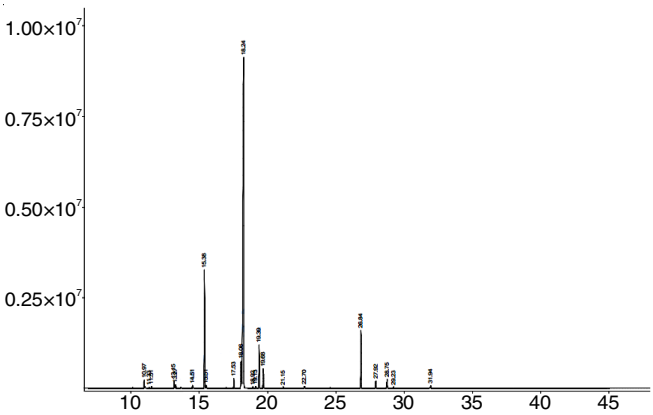

Fig. 3. Chromatogram showing the chemical composition of essential oils extracted from flowers analyzed by GC-MS

Essential oils obtained from aerial parts of geographically different Vietnamese *E. winitiana* samples were previously analyzed, exhibiting considerably different compositions. To be specific, the plant harvested in Moc Chau district of Son La province was characterized by the abundance of elsholtzia ketone (87.5%), The essential oil obtained from plant harvested in Sa Pa district of Lao Cai province contained mostly rosefuran (56.0%) [29]. Interestingly, the former essential oil sample did not exhibit the signals of rosefuran and rosefuran epoxide. In addition, dehydroelsholtzia ketone, second major component in the second sample (22.8%), amounted only to 3.9% in the first sample. In general, current results of aerial part essential oil share some compositional similarities with the latter sample in terms of minor components, especially several monoterpenes [33]. This suggests that *E. winitiana* and *E. winitiana* var. *dongvanensis* can be distinguished by determining their chemical composition of essential oils. The chemical composition of eugenol varies from region to region, possibly due to a number of environmental factors, a portion of the tree being used, the age of the tree and the period of the growing season or even genetic factors.

Conclusion

This study was conducted to determine the chemical composition of essential oils extracted from *Elsholtzia winitiana* var. *dongvanensis* harvested in Ha Giang province of Vietnam. The hydrodistillation process of flowers and aerial parts of *E. winitiana* var. *dongvanensis* under the respective conditions achieved 0.75% and 0.82%, respectively. The GC-MS analysis identified 22-23 components that contribute to the formation of essential oils from aerial parts and flowers of *E. winitiana* var. *dongvannensis* accounted 98-99.99% of the total essential oil composition. Chromatographic analysis of *E. winitiana* var. *dongvannensis* essential oils indicates the the presence of rosefuran and rosefuran epoxide in abundance, which are valuable compounds in fragrance industries. Thus, efforts should be attempted for the optimization of extraction process of essential oil from flowers and aerial parts of *Elsholtzia winitiana* var. *dongvannensis*.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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