Volatile composition of *Perilla frutescens* (L.) essential oil in Thai Binh Province, Vietnam extracted by microwave-assisted distillation method

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**Abstract.** The chemical composition of essential oils (EOs) obtained from *Perilla frutescens* (L.) (Lamiaceae) was determined. EOs of perilla was obtained by microwave-assisted extraction, giving the optimum content of 0.0941%. The composition of volatile constituents in the extracted oil was analyzed by mass chromatography (GC - MS), showing that the content of volatiles accounted for 97.58% of the total EOs content. Some major components in the obtained EOs were Perilla aldehyde 62.13%, Limonene (4.99%), Linalool (1.41%), Caryophyllene <E>- (6.72%), Humulene <a> (1.11%), Caryophyllene oxide (2.45%), and Perilla alcohol (0.8%). Obtained perilla EOs may find the use as an alternative natural supplements in food, pharmaceutical and cosmetic products.

1. Introduction

Essential oil (EOs) plays an important role in the production of chemical raw materials and for various industries including flavor industry, food and cosmetic [1-8]. EOs are mixtures of organic substances that are mixed together and has a characteristic odor. At room temperature, EOs mostly liquid or insoluble in water, but very soluble in organic solvents such as alcohols and fats. The EOs evaporate with steam, has a specific aroma and strong antiseptic properties [9-10]. In recent years, people have tended to consume natural, non-toxic food, pharmaceutical and cosmetic products that can reduce the uptake of chemicals [11]. Therefore, the plants that produce EOs have been in social interest for their use in many fields of production and life. The tropical climate region with the average heavy rainfall of 1500-3000 mm and the relative high humidity in the summer of 85-95% of Vietnam favor the development of a broad range of vegetation [12-14].

Of the more than 550 types of plants containing rich and diverse essential oils in Vietnam, perilla (*Perilla frutescens* (L.)) is one of the species that attracts a great deal of attention. Perilla plants originated from the mountains of India and China, then were propagated everywhere on the continent. The tree also grows in temperate climates of Europe. In the United States and the UK, wild perilla is also observed. Currently, perilla leaf essential oil is widely produced in Japan, China and South Korea. Perilla belongs to
the genus Perilla and Lamiaeaceae family [15]. Perilla leaves contain 0.3-1.3% of the essential oil in dry basis [16]. The composition that is diverse and abundant in useful component has enabled the use of perilla essential oil in a wide array of applications including medicine, perfume production, cosmetics and food additives. To be specific, it was found that the major components in the perilla essential oil were perilla aldehyde (55%), limonene (20-30%), α-pinene, β-caryophyllene, fimalool and perilla alcohol [17]. In addition, previous evidence also pointed out that essential oil extracted from the perilla leaves exerted antibacterial, anti-poisoning effect for crabs and fish and could reduce symptoms of depression. On the other hand, perilla EOs is used as a flavoring and artificial sweetener in soft drinks, sauces, and cigarettes [18]. Perilla essential oil is usually extracted from perilla leaves by steam distillation or extraction with organic solvents [19; 20].

Microwaves are electromagnetic waves that travel at the speed of light. Electromagnetic waves in the 300 MHz to 300 GHz frequency range can generate heat when they penetrate and interact with molecules like water in the material. Most household microwaves use a frequency of 2450 MHz, at this frequency $\lambda = 12.24$ cm [21]. The microwave oven is connected to a Clevenger type device to operate the distillation process as shown in Fig. 1. Raw materials and distilled water are put into a 1000 mL container, installed in the microwave compartment. Under the action of microwaves, the water in the plant cells is heated up, the internal pressure increases suddenly causing the essential oil-bearing tissues to rupture. Essential oil escapes, attracts steam to a condensing system (steam distillation method) or dissolves into an organic solvent covering the material (impregnation method) [22]. In this study, we explored the possibility of using microwave-assisted distillation in extraction of perilla EOs. The assistance of microwave could bring about numerous advantages including warm time efficiency, faster energy transfer, response to heat control process, lower cost, reduced waste water and simple operation. The study results are expected to contribute to process intensification involving distillation of perilla essential oil and valorization of aromatic compounds obtained from plants in general in from perilla in particular.

2. Materials and method

2.1. Plant materials

Fresh and healthy Perilla plants used for essential oil extraction were collected under dry conditions in Dong Chau - Tien Hai - Thai Binh province, Vietnam in July 2019. Perilla trees are harvested when the tree started to bud. The collected plants were washed thoroughly with distilled water, let dry at room temperature and ground finely to the size of 1-3 mm. The following chemicals were used at an analytical grade: n-hexane, anhydrous sodium sulfate (Na$_2$SO$_4$) and distilled water.

2.2. Isolation of essential oil

Perilla essential oil was obtained by microwave distillation [23]. First, 100 g of finely ground raw material was put into a 1000 mL flask and then extracted at 450 W for 40 min using a microwave distillation device. The apparatus included a microwave oven connected to a Clevenger-style extraction instrument. During the extraction process, the steam carrying essential oils continuously flows through the condenser located outside the microwave cavity where it was condensed. After dissociation, the collected essential oil still contains a certain amount of water. This mixture was dehydrated with Na$_2$SO$_4$ until all the water has been removed. The essential oil was then stored in dark vials until GC-MS analysis. The yield of perilla oil obtained (%) is calculated by following equation:

$$\text{Yield of perilla oil (\%) = \frac{\text{the volume of Perilla oil obtained (mL)}}{\text{the amount of turmeric originally used for or the experiment (g)}}$$  \hspace{1cm} (1)
2.3. GC-MS analyses

Chemical composition of the perilla EOs was determined by GC-MS analysis [24]. A GC Agilent 6890 N instrument was coupled with HP5-MS column and MS 5973 inert. The head column was 9.3 psi of pressure. The EOs was added with n-hexane and dehydrated with Na<sub>2</sub>SO<sub>4</sub>. The flow rate was kept constant at 1 mL/min. The temperature of injector was set at 250°C and the rate of division was 30. Thermal program for samples began at 50 °C for 2 min, then continued to rise from 80 °C, 150 °C, 200 °C and finally reached 300 °C.

3. Results and discussion

After conducting the extraction of perilla EOs by microwave assisted steam distillation, the essential oil was obtained with the yield of 0.0941%. Perilla essential oil is a transparent, volatile liquid with clear and pale yellow color. The color of the obtained Perilla essential oil was clear and had pale yellow color. The essential oil had a strong and distinct aroma. Perilla essential oil is insoluble water or slightly soluble. But it dissolves well in organic solvents. Therefore, using microwave to heat the solvents and plant tissues during the extraction process contributed to improved kinetics of the extraction process, shorter extraction time, lower solvent consumption, higher extraction rates and lower cost in comparison with traditional extraction methods [25]. Liu et al. conducted a series of studies on the essential oil content of extracted from other parts of Perilla harvested from southern China region, showing that the yields were 0.53, 0.22, 0.11 and 0.10% in leaves, whole plants, stems and roots respectively [26]. Tabanca et al. used a hydrodistillation method to extract essential oils from two different plant sources (green perilla and red perilla) collected from southern Poplarville, USA, resulting in similar yields, at 0.08% and 0.02% respectively [16]. In other publications, Başer et al. conducted extracting essential oils on perilla from Turkey, indicating the optimum yield of 0.5% [27]. These variability are possibly due to the influence of climatic conditions, soil, extraction methods and growing times between different regions of the world [26].

Composition wise, GC-MS method identified a total of twenty-four peaks, corresponding to 24 compounds accounting for 97.58% of the total essential oil content as shown in Figure 2 and Table 1. The obtained essential oil was rich in volatile oxygenated monoterpenes compounds. However, three unknown compounds were found, accounting for a relatively content (14.41%) of the oil. The content of Perilla aldehyde at 62.13% accounts for the most abundant component in essential oils and is considered the compound that determines the scent and serves as the quality indicator of perilla essential oil. Some other identified ingredients were Limonene (4.99%), Linalool (1.41%), Caryophyllene &E- (6.72%), Humulene &a- (1.11%), Caryophyllene oxide (2.45%), and Perilla alcohol (2.45%).
Some ingredients such as perillaketone, egomaketone, and isoegomaketone, which were previously found in perilla essential oil in Korea (Perilla Ketone of 90.19%) and India (Perillaketone of 39.5%), were absent in this study [28, 29]. Tabanca et al. analyzed volatile compounds in essential oil of perilla harvested from Poplarville (USA) and found that the compound class with the highest content was Oxygenated monoterpenes (63.3%), followed by Oxygenated sesquiterpenes (14.8%) and Monoterpene hydrocarbons (13.9%) [16], as compared to the present study in which Perilla aldehyde accounted for only 48.4%. Bumblauskiené et al. (2009) used hydodistillation method to extract perilla essential oil in Lithuania and identified 83.18% volatile compounds. The main compounds found were perillaldehyde (49.47%), limonene (11.76%), limonene oxide (9.85%) and caryophyllene oxide (7.21%) [30].

In Thailand, Ito et al. found that Perilla frutescens mostly contained piperitenone (36.3%) and limonene (23.7%), indicating the presence of a dominant gene involved in the transformation of essential oils between piperitenone and perillaldehyde [31]. The biotransformation and the composition of the essential oil depend on the geographical location, environmental factors, seasonal climate, or extraction process. In addition, plants with the same phenotype but different growing habitats may result in different volatile compositions.

Table 1. Chemical composition of the Perilla frutescens Eos.

| Retention Time (min) | Chemical name               | %   |
|----------------------|------------------------------|-----|
| 11.52                | Octen-3-ol <1->              | 0.11|
| 13.37                | Limonene                     | 4.99|
| 15.66                | Linalool                     | 1.41|
| 15.77                | Perillene                    | 0.11|
| 19.04                | Terpineol <a->               | 0.52|
| 20.21                | Nerol                        | 0.16|
| 21.89                | unknown (93, 154, RI 1280)   | 5.95|
| 22.16                | Perilla aldehyde             | 62.13|
| 22.7                 | Menth-1-en-9-ol              | 0.12|
| 22.76                | Perilla alcohol              | 0.80|
| 25.53                | unknown (75, 204, RI 1389)   | 3.66|
| 25.98                | Elemene <cis-h->             | 0.24|
| 27.04                | Caryophyllene <E-> (=Caryophyllene <h->) | 6.72|
| 28.11                | Humulene <a->                | 1.11|
| 28.94                | unknown (93, 204, RI 1497)   | 4.80|
| 29.36                | Farnesene <(E,E)-a->         | 0.22|
| 30.1                 | Cadinene <d->                | 0.20|
| 30.17                | Nerolidol <Z-> (=Peruviol)   | 0.13|
| 31.06                | Nerolidol <E->               | 0.65|
| 31.78                | Scapanol                     | 0.19|
| 32.11                | Caryophyllene oxide          | 2.45|
| 32.86                | Humulene Epoxide II          | 0.39|
| 34.02                | Cadinol <a->                 | 0.34|
| 45.19                | Phytol                       | 0.18|
| Total                |                              | 97.58|
Figure 2. Chromatogram of *Perilla frutescens* essential oil by GC-MS.

Figure 3. Perilla aldehyde in *Perilla frutescens* essential oil worldwide (Lithuania [30], Japan [15], USA [16], China [32] and Vietnamese).

4. Conclusion
This study successfully attempted the microwave-assisted extraction of perilla leaf essential oil. The materials was collected in the period where the plant started flowering. The obtained yield was 0.0941%. Obtained essential oil had a specific aroma that meets the quality criteria for use in food. By mass gas chromatography, some main constituents with high medicinal properties were identified including Perilla aldehyde (62.13%), Limonene (4.99%), Linalool (1.41%), Caryophyllene <E-> (6.72%), Humulene <a-> (1.11%), Caryophyllene oxide (2.45%), and Perilla alcohol (0.8%). The high content of Perillaldehyde suggests that obtained perilla leaf oil is of high quality and commercial value.
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