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Evaluation of the Physical and Chemical Properties of Vietnamese *Perilla frutescens* L. Essential Oil

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Perilla frutescens essential oil was studied in this work for its physico-chemical characteristics and composition. The essential oil was obtained yields by 0.1%. The physico-chemical properties of the oil were also discovered including the physico-chemical parameters averaged 0.944 g/cm^3 for specific gravity, acid index (9.185 mg KOH/g), ester index (28.66 mg KOH/g), refractive index (1.4976). Thought gas chromatography/mass spectrometry (GC/MS). The results revealed that the oil is extremely rich in myristicin (43.896%), elemicin (28.793%), α-caryophyllene (8.327%), perillaldehyde (7.973%), (*Z*,*E*)-farnesene (2.807%), D-limonene (1.043%). The results showed that the morphological characteristics and the polymorphism in the essential oil composition of perilla leaves were reliant on the harvesting time and geographical cultivate. Perilla essential oil is antioxidant, antibacterial and used in many studies, helping to find its effect on Alzheimer's disease, memory loss, concentration and anti-depressive concentration, because it contains unique compounds as above.

Keywords: Physical properties, Chemical properties, *Perilla frutescens* L., Essential oil.

INTRODUCTION

Nowadays, traditional medicines use of natural products (such as plants, animals, microorganisms and marine organisms), are of great importance [1-4]. Among all products, essential oils with many wonderful uses have been used in many fields of production and life such as antimicrobial compounds, medicine, food additives and so on. Essential oils are odorous oily liquids of complex mixtures extracted from several plant organs such as flowers, seeds, *etc*.

Perilla (*Perilla frutescens*) belongs to the Lamiaceae family, which consisted of about 200 species and cultivated mainly in America, Africa and Asia. The previous study reports that the perilla essential oils play an indispensable role in the treatment of different diseases such as diarrhea, headache, fever and insecticidal activities. Perilla is one of spice and medicinal herb, which used as vegetable on soup and also enhance the flavour and organoleptic properties. Perilla essential oil is powerful antibacterial, antipyretic, anticarcinogenic and other activities [5-7]. There are different highly valuable compounds in the essential oil of Perilla such as flavonoids, phenolic acid and glucosides.

The main constituents in the essential oil of *Perilla frutescens* (L.) are perillaldehyde and α-caryophyllene [8]. Previous study showed that there are eight chemotypes with the different chemical composition such as perillaldehyde and perilla ketone [9]. Many studies have exhibited that the variations in the chemical composition of the essential oils in different cultivars can be affected by multiple factors, including temperature, humidity, soil conditions and seasonality [10-12]. *Perilla frutescens* L. essential oil has antibacterial properties and is a natural source of ingredients widely used in cosmetics, pharmaceuticals and food [13]. Their chemical composition may be affected by environmental conditions and plant growth stages. Chromatography coupled with mass spectrometry (GC-MS) methods help separate and identify different constituent of the essential oil in case of adulterated oil. The hydrodistillation method became more and more popular for extract essential oils from plant materials due to the simplicity of installations, easy of method performing. Current assessments provide a comprehensive summary of possible changes in essential oils and factors affecting the stability of essential oil. In particular, available temperature, light and oxygen are considered to have an important influence on intact essential oils [14]. The chemical composition of essential oil is complex, unstable and always changes with the growth time of the tree or changes according to the climatic conditions of the weather. In recent years, cultivating *Perilla frutescens* L. have been receiving a great deal of public attention due to the essential oil extracted from its flowers and leaves [15].

The aim of this study is to determine a physico-chemical of Vietnamese *Perilla frutescens* L. and characterization of its essential oil. Chemical composition analysis were done using gas-chromatography coupled with mass spectrometry (GC-MS).

EXPERIMENTAL

Isolation of *Perilla frutescens* essential oils: *Perilla frutescens* leaves were bought from Thu Duc market in Ho Chi Minh city, located in the southeast of Vietnam. Essential oils were extracted by steam distillation methol. Leaves were washed with water, allowed to dry at room temperature and grounded in blender. After the perilla leaves were mashed, proceed to distill the essential oils in Clevenger′s equipment with technical conditions 200 g of plant material with 400 mL of water and the extraction time was about 3 h in average. Essential oil was steam distilled and collected after reflux condensation. The obtained essential oil was dehydrated with anhydrous Na_2SO_4 and kept in a sealed glass vials for further study.

Physico-chemical analyses of essential oils: Some basic physical and chemical parameters of the essential oil analyzed by TCVN: density of essential oils, acid index (TCVN 8450: 2010, ester index (TCVN 8451:2010), rotator power and refractive index. The experiments were repeated three times.

Density: The proportion of essential oils is the ratio of essential oils at 25 ºC with the mass of the oil. The same volume of distilled water was also at 25 ºC.

Acid index: The acid index is the number of milligrams of KOH needed to neutralize free acids in 1 g of fat.

Ester index: The soap index is the number of milligrams of KOH needed to neutralize all free acids and acid combined as esters in 1 g of fat.

Chemical identification: In order to determine the chemical composition in the essential oil sample, 25 µL of essential oil from the optimized process was added with 1 mL *n*-hexane and dehydrated with Na_2SO_4. The instrument was GC Agilent 6890 N, HP5-MS column, MS 5973 inert, head column pressure of 9.3 psi.

The gas chromatography-mass spectroscopy (GC-MS) was employed to analyze the chemical composition of the essential oil samples. GC-456 SQ with SCION performance RESTEK Rxi-5ms (30 m × 0.25 mm, 0.25 µm df, in the presence of helium gas at a constant flow rate: 1 mL/min. Injector temperature was 250 ºC at the rate of division: 30 ºC.

Data analysis: The results obtained were analyzed statistically using Statgraphics Software.The mean total phenol content and antioxidant activity of the samples were compared using oneway ANOVA followed by LSD post hoc multiple comparisons. Statistical significant difference was performed at $p = 0.05$.

RESULTS AND DISCUSSION

The essential oil from *Perilla frutescens* leaf was obtained yield 0.1% by hydrodistillation. Essential oil content was much lower than regional countries. The essential oil from Kaili (China) had high yields of 1.11% whereas those from Turkey had the yields of 0.5% and other samples from India had yields average of 0.15-0.36%. The essential oil was the most yield at the time when the perilla is about to flower. For organoleptic properties (Table-1), perilla leaf essential oil was obtained as a transparent liquid, light yellow, slightly spicy taste, typical perilla flavour. These properties were the same as the properties of marketed perilla oil products. At room temperature, the liquid had a density less than one. Perilla oil is insoluble in water or less soluble, but missible well in organic solvents. The physico-chemical parameters calculated from the experiment with the density (d = 0.944) above showed that perilla oil is lighter than water. The acid index ($I_A = 9.185$) was relatively high and much higher than the Guenther study, indicated that the composition of essential oils containing small molecular free fatty acids is likely to cause oil damage. The acid index depends on the extraction method and the raw freshness of the material. With long-preserved materials, the acid index will increase due to oxidation and esters in the degraded essential oil. High or low refractive index depending on the composition of the essential oils contained in the oil is saturated, unsaturated or aromatic. If there are many components in multiple essential oils, there is a high refractive index. Refractive index is affected by temperature factor. When measured the refractive index at different temperatures, the result was different, the greater the temperature, the refractive index changes in the downward direction.

TABLE-1
SENSORY AND PHYSICO-CHEMICAL INDICATORS OF *Perilla frutescens* ESSENTIAL OIL

Organoleptic characteristics	Essential oil Vietnamese	Essential oil of Guenther [16]
Aspect	Liquid	Liquid
Colour	Light yellow	Pale yellow
Odour	Specidic	Specidic
Density	0.944	0.923-0.938
Acid index	9.185	1-1.5
Ester index	28.66	38.5-39
Refractive index	1.4976	1-1.5

The chemical components of hydrodistilled essential oils from *Perilla frutescens* were characterized by GC-MS and the results are shown in Table-2 and Fig. 1. A total of 14 different compounds with more than 90% compatibility were identified in the essential oil. Principal compounds were found to be β-caryophyllene (8.327%), D-limonene (1.043%), myristicin (43.896%), elemicin (28.793%), perillaldehyde (7.973%),

TABLE-2
CHEMICAL CONSTITUENTS OF
Perilla frutescens ESSENTIAL OIL

Peak	Retention time (min)	Compound	Concentration (%)
1	11.925	D-Limonene	1.043
2	24.316	*trans*-Shisool	0.354
3	24.421	Perillaldehyde	7.973
4	28.342	β-Elemene	0.173
5	29.158	β-Caryophyllene	8.327
6	30.13	α-Caryophyllene	0.876
7	30.915	Germacrene D	1.093
8	31.302	(Z,E)-α-Farnesene	2.807
9	32.075	Myristicin	43.896
11	32.797	Elemicin	28.793
13	33.32	Caryophyllene oxide	0.519
14	34.627	α-Cadinol	0.173

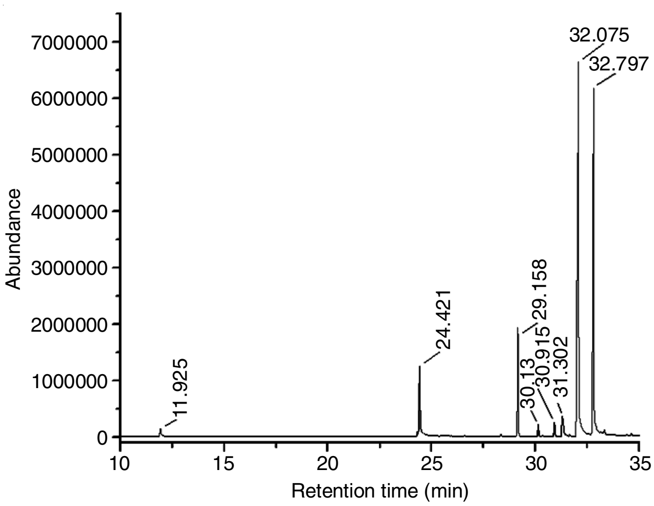

Fig. 1. GC-MS analysis results of volatile compounds present in the essential oils of the studied perilla leaves

(*Z,E*)-α-farnesene (2.807%) and some other components < 1%. Myristicin (43.896%) and elemicin (28.793%) were identified as the most abundant components in the essential oils. Earlier work done on *Perilla frutescens* in Lithuania reported 13 components in oil, where peril laketone was found around 55.60%, linalool was 1.15% and caryophyllene oxide was 0.45% [17]. A recent study in Korea reported 36 compounds in the essential oil in which the perillaketone was about 90.19% [18]. Similar study in China reported caryophyllene oxide to be 0.12% [15]. One of the studies in Turkey reported peri llaketone 35.6%, linalool 1.7% and caryophyllene oxide 0.9% [19]. A recent report from India on volatile oil composition of Indian *Perilla frutescens* revealed perillaketone (39.5%), caryophyllene (59.3%) and caryophyllene oxide (0.4%) [20]. Yet another study on compositional variability in volatiles from different plant organs of *Perilla frutescens* cultivated in Uttarakhand, India reported linalool (0.64-1.16%), 1-methyl-2-methyltransdecalin (22.81-32.98%), perillaketone (47.73-58.94%) and caryophyllene oxide (0.53-2.80%) in leaves [21]. Moreover, in the ingredients of Vietnamese perilla oil, there are no PK compounds (perillaketone, egomaketone, isoegomaketone), which are toxic. This shows that the essential oil obtained from Vietnamese perilla leaves is safe for the health of users, while Indian *Perilla frutescens* reported perillaketone (43.28%) [20], Lithuanian perillaketone and egomaketone [17], South Korean perillaketone (95%) [22], Turkish perilla ketone (35.6%) and isoegomaketone (35.1%) [19]. The other constitutents *viz*. D-limonene, perillaldehyde and β-caryophyllene in perilla oil have effective antimicrobial, antifungal, anti-inflammatory, anti-infection properties. The chemical composition of essential oils includes terpenes and oxygen-containing derivatives of terpenes.

The chemical composition of perilla oil is complex, unstable and always changes with the time of growth of the tree or also changes according to the climatic conditions of the weather. The amount of essential oil in the parts of the plant is different, so, it is essential to determine the time of collection to give the essential oil in the best quality (Table-3).

TABLE-3
MAIN CHEMICAL COMPOSITION OF *Perilla frutescens*
ESSENTIAL OIL IN SOME COUNTRIES

Main ingredients	Concentration % of essential oils in countries		
	Turkey [19]	China [23]	Vietnamese
Perillaketone	35.6	29.6	–
Isoegomaketone	35.1	15.6	–
β-Caryophyllene	4.3	13.8	8.327
(Z,E)-α-Farnesene	2.7	9.2	2.807
Myristicin	–	–	43.896
Elemicin	–	–	28.793
Perillaldehyde	–	–	7.973

Conclusion

In this research, fresh *Perilla frutescens* leaves were used for extraction of essential oil using hydrodistillation method and evaluation of chemical compositions and physico-chemical characteristics. The essential oil was obtained yields at 0.1%. The physico-chemical parameters averaged specific gravity (0.944 g/cm^3), acid index (9.185 mg KOH/g), ester index (28.66 mg KOH/g), refractive index (1.4976). Using GC/MS analysis, fourteen components were identified in *Perilla frutescens* extracted oils. The concentration of myristicin takes the lead (43.896%), followed by elemicin (28.793%) and β-caryophyllene (8.327%). It can be concluded that the chemical composition of *Perilla frutescens* essential oils correlates with the climatic conditions in which it is grown, as well as a genetic variation.

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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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