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PRELIMINARY ANALYSIS ON ESSENTIAL OIL COMPOSITION OF *PERILLA* L. CULTIVATED IN LITHUANIA

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Abstract: This study represents the investigation of the essential oil of *Perilla frutescens* (L.) Britton, *Perilla frutescens* (L.) Britton var. *crispa* f. *viridis*, *Perilla ocymoides* L. var. *bicolorlaciniata* leaves collected at full flowering stage in August 2007, in Lithuania. As biogenesis and composition of essential oil depends on geographical location, environmental factors, plants with same chemotypes but growing in different conditions have different composition of major components. Plants have been cultivated in the collection of medicinal plants at Kaunas Botanical Garden of Vytautas Magnus University in Lithuania. Essential oil was analyzed using gas chromatography methods with flame ionization and mass spectrometry detectors. Thirteen constituents of essential oil, representing 96.42% of identified compounds, were indentified in the essential oil of *Perilla frutescens* (L.) Britton. Essential oil was ascertained to perillaketone chemotype. Principal compounds were found to be perillaketone and egomaketone. In *Perilla ocymoides* L. var. bicolorlaciniata aerial parts 97.70% of essential oil components were indentified with abundant amounts of perillaldehyde (72.07%) and limonene (13.15%). Essential oil contained two phenylpropanoids elemicin (1.91%) and myristicin (1.41%). In the essential oil of *Perilla frutescens* (L.) Britton var. *crisp*a f. *viridis* 83.18% compounds were indentified. Principal compounds were found to be perillaldehyde (49.47%), limonene (11.76%), limonene oxides (9.85%) and caryophyllene oxide (7.21%). β-Caryophyllene and perillaldehyde were the only compounds identified in all investigated perilla plants. Considering a broad diversity of *Perilla* L. species and chemotypes, it is important to discriminate cultivated *Perilla* L. species with identified chemotypes.

Keywords: essential oil, *Perilla frutescens* L., perillaketone, perillaldehyde, chemotype

Perilla L. is the genus of annual herbaceous plants of Lamiaceae Lindl. family, originated in Eastern Asia (1, 2). According to the plant genetic analysis, Perilla L. contains four species: most commonly cultivated *Perilla frutescens* L. Britton, and three wild species: *Perilla hirtella* Nakai, *Perilla seytoensis* G. Honda and *Perilla citriodora* Nakai (3, 4). The other species e.g. *Perilla aguta* Benth., *Perilla albiflora* Odash., *Perilla ocimoides* L. are ascribed to Perilla frutescens species, which are medicinal and spice plants with a long application history in Asia (1, 5).

Perilla frutescens L. Britton has been cultivated in the collection of medicinal plants at Kaunas Botanical Garden of Vytautas Magnus University in Lithuania since 1990. *P. frutescens* is a new perspective medicinal, spice and decorative plant which has already enriched national genetic resources and diversity of species. The investigations of plant growth, vegetation rhytmics and its dependence upon ecological factors, has been carried out since 1998 (6, 7).

Perilla is an essential oil accumulating plant. Essential oil is accumulated in the glandular trichomes on the surface of stems and leaves (1, 8). Herbal raw material is composed of fresh and dried terraneous parts: leaves (*Perillae folium*), fruit (*Perillae fructus*) (9).

According to the main volatile component of essential oil, plants can be classified into the chemotypes (1, 10, 11). Perillaldehyde type (PA) is used in culinary and traditional Chinese medicine. Major constituent is perillaldehyde, other constituents are limonene, linalool, β-caryophyllene, 1-menthol, α-

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pinene, perillene (12). Perillaldehyde can cause contact dermatitis. Main component of perillaketone (PK) chemotype essential oil is perillaketone; other constituents are isoegomaketone, perillene, and egomaketone. PK chemotype is common among wild species. Elsholtzoaketone type (EK) has considerable amounts of elsholtziaketone and naginataketone. In perillene type (PL) essential oil perillene is the predominating compound with lesser amounts of citral, perillaketone and isoegomaketone. Phenylpropanoid type (PP) contains no monoterpenoids, but synthesizes phenylpropanoids myristicin, elemicin, and dillapiol (10, 13). In citral type (C) essential oil citral is a predominant constituent with lesser amounts of perillene, perillaketone and isoegomaketone. Rosefuran containing type (R) can be used as a cheaper substitute in the rose oil manufacturing process (14, 15).

After genetic analysis of *Perilla* L. plants was performed, it was ascertained that chemical composition of essential oil is specific to chemotype (1). Chemotypes are independent of morphological parameters of the plant (16). The biogenesis and composition of main essential oil component that determines the chemotype, depends on the geographical location of a plant, environmental conditions and medicinal raw material preparation time (17). Chemical composition of essential oil and chemotype determines the scent and the therapeutic properties of the plant (18). During the last decades various experiments confirming the variety of pharmacological effects of essential oil were carried out. Antibacterial, antiallergic, chemotherapeutic activities and effects on central nervous system were confirmed. Perillaldehyde, phenylpropanoids, and β-caryophyllene were the most active compounds.

Considering the different pharmacological effects of essential oil components it is very important to determine the chemotype and composition of essential oil of *Perilla* L. plants that are introduced in Lithuania. Essential oil analysis would provide information about the quality of essential oil and perspectives for medicinal usage. The aim of our study was to determine the composition of essential oil of *Perilla frutescens* (L.) Britton, *Perilla frutescens* (L.) Britton var. *crispa* f. *viridis*, and *Perilla ocymoides* L. var. bicolorlaciniata growing in Lithuania.

EXPERIMENTAL

Plant material

Perilla plants were grown in the collection of medicinal plants and in the trial area at Kaunas Botanical Garden of Vytautas Magnus University. The seeds of *Perilla frutescens* (L.) Britton, *Perilla frutescens* (L.) Britton var. *crispa* f. *viridis*, and *Perilla ocymoides* L. var. bicolorlaciniata were purchased by seed catalogues of Kaunas Botanical Garden of Vytautas Magnus University. Plant material - leaves of *Perilla frutescens* (L.) Britton, *Perilla frutescens* (L.) Britton var. *crispa* f. *viridis*, and *Perilla ocymoides* L. var. bicolorlaciniata for essential oil analysis were collected in August, 2007 during full flowering stage. Essential oil was obtained by hydrodistillation.

Gas chromatography, gas chromatography-mass spectrometry

Gas chromatography methods with flame ionization and mass spectrometry detectors were used for essential oil analysis. Results are presented according to GS/MS obtained data. For restatement of results GC/FI was performed. Essential oil solutions in pentane were analyzed using Fisons GC 8000 series chromatograph with flame ionization detector equipped with nonpolar ZB-5 column (30 m × 0.25 mm × 0.25 μm). Nitrogen was used as a gas carrier at a flow rate of 2 mL/min. Temperature was programmed from 60°C to 250°C at 3°C/min rate. The analysis of gas chromatography-mass spectrometry was carried out using a Perkin Elmer Clarus 500 GC/MS system. Injections of 1 μL were made into a capillary column. A methylpolysiloxane (5% phenyl) GC phase capillary column Elite-5ms (30 m × 0.25 mm × 0.25 μm) was held at 60°C for 2 min and programmed from 60°C to 250°C at 3°C/min, and kept at 250°C for 30 min, using helium as gas carrier at a flow rate of 2 mL/min. The separated analytes were ionized in the positive EI mode at 70 eV ionizing energy and analyzed in a quadrupole mass spectrometer.

Qualitative and quantitative analyses

Compounds were identified by comparing retention indices (Kovats) and mass spectra. The retention indices were calculated in a reference of retention index of standard mixture of alkanes (Sigma-Aldrich, Inc. Milwaukee, USA). Mass spectra were compared with literature data and NIST/EPA/NIH Mass Spectral Database NBS75K) (19).

RESULTS

The constituents of essential oil of *Perilla frutescens* (L.) Britton, *Perilla frutescens* (L.) Britton var. *crispa* f. *viridis* and *Perilla ocymoides* L.

Table 1.Essential oil composition of *Perilla frutescens* (L.) Britton, *Perilla frutescens* (L.) Britton var. *crispa* f. *viridis* and *Perilla ocymoides* L. var. *bicolorlaciniata*

Compound	*Perilla frutescens* L. Britton				*Perilla ocymoides* L. var. *bicolorlaciniata*				*Perilla frutescens* L. Britton var. *crispa* f. *viridis*			
	GC/MS	%	GC/FI	%	GC/MS	%	GC/FI	%	GC/MS	%	C/FI	%
3- octanol	991	0.23	985	017								
myrcene									1003	0.63	992	0.45
p-cymene									1022	0.15	1022	0.07
limonene					1029	13.15	1027	13.77	1027	11.76	1025	15.36
eucalyptol									1030	0.17		
linalool oxide												
***trans* furanoid**									1069	0.96		
linalool oxide												
***cis* pyranoid**									1086	0.92		
terpinolene									1099	0.50	1093	1.89
linalool	1099	1.15	1095	1.17	1101	1.42	1097	1.41				
limonene oxi de *cis*									1133	6.81	-	-
limonene oxi de *trans*									1137	3.04		
linalyl oxide *cis*									1170	0.38	1184	0.18
linalool oxide *trans*									1175	0.16	1196	0.21
α-terpineol					1196	0.22	1190	0.29				
dihydrocarveol					1222	0.33	1222	0.35				
β-cyclocitral									1227	0.23	1224	0.10
carvone									1246	0.18	-	-
perillaketone	1257	55.60	1258	53.90								
perillaldehyde	1278	0.17	1274	0.18	1284	72.07	1290	70.41	1280	49.47	1280	66.73
bornyl acetate									1287	0.17	1296	0.12
isoegomaketone	1297	2.63	1295	0.99								
perilla alcohol					1302	0.22	1305	0.18				
egomaketone	1308	28.12	1310	29.80								
methyl geranate	1324	0.12	1324	0.12								
isodihydrocarveol acetate									1334	0.17	1335	0.07
β-caryophyllene	1417	3.21	1425	3.51	1418	3.35	1431	3.58	1415	0.14	1429	0.10
α-caryophyllene	1452	0.26	1458	0.32	1453	1.08	1463	1.14				
β-murolene	1479	0.14	1498	1.41								
germacrene D					1479	1.50	1473	0.05				
E,E-α-farnesene					1492	3.36	1490	1.84				
Z,E-farnesene	1493	3.92	1486	0.14								
α-farnesene	1506	0.18	1512	0.31								
myristicin					1524	1.41	1522	1.04				
elemicin					1554	1.91	1562	1.52				
caryophyllene oxide	1582	0.45	1591	0.61	1583	7.21	1601	3.73				
humulene epoxide II									1609	0.13	-	-

GC/MS = Retention index for GC/MS, GC/FI = Retention index fot GC/FI, % = amount in percent.

var. bicolorlaciniata are presented in Table 1. Retention indices and amounts in percent, obtained by gas chromatography with flame ionization and gas chromatography with mass spectrometry detectors, are indicated. The essential oil of *Perilla frutescens* (L.) Britton, *Perilla frutescens* (L.) Britton var. *crispa* f. *viridis* and *Perilla ocymoides* L. var. bicolorlaciniata was obtained in a yield of 0.3%, 0.5% and 0.4%, respectively.

Thirteen constituents of essential oil, representing 96.42% of identified compounds, were indentified in the essential oil of *Perilla frutescens* L. Britton. Principal compounds were found to be perillaketone (Fig. 1 A) and egomaketone. Isoegomaketone and egomaketone were identified in reference to outlet nature and mass spectra characteristics (11). Z,E-farnesene, β-caryophyllene and isoegomaketone collectively yielded 9.76%. Other identified compounds (linalool, caryophyllene oxide, α-caryophyllene, 3-octanol, α-farnesene, perillaldehyde, γ-murolene, methyl geranate) in total were 2.94% of essential oil. Perillaketone and egomaketone yielded 83.72% of essential oil with considerable amount (55.6%) of perillaketone. It has been determined that extracts containing perillaketone promotes intestinal propulsion, by stimulation of intestinal circular muscles. The effect is determined by perillaketone, which acts as an antidote in food poisoning and digestive disorders (1). The toxicity of this compound was proved during experiment with mice. It was stated that using regular doses of perilla, the amount of perillaketone is minor and insignificant. However, toxic doses for humans have not been assessed yet (1). During essential oil analysis in Turkey perillaketone (35%) and isoegomaketone (35%) prevailed over other components in 70.7% of total constituents of essential oil. Isoegomaketone and perillaketone are synthesized *via* egomaketone biosynthetic pathway (1, 20). As biogenesis and composition of essential oil depends on geographical location, environmental factors, plants with same chemotypes but growing in different conditions have different composition of major components (17). Further studies are needed to ascertain composition of essential oil throughout the vegetation period.

In *Perilla ocymoides* L. var. *bicolorlaciniata* 97.70% of essential oil components were indentified with abundant amounts of perillaldehyde (72.07%) (Fig. 1B) and limonene (13.15%). The presence of limonene in essential oil indicates that limonene biosynthetic pathway dominates in biosynthesis of essential oil (13). Ketones (perillaketone, egomaketone, isoegomaketone) were not detected in the

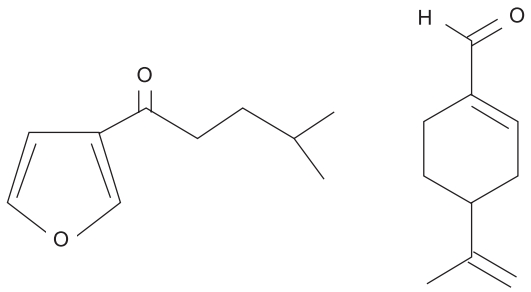

Figure 1. Perillaketone (A) and perillaldehyde (B)

essential oil of *Perilla ocymoides* L. var. bicolorlaciniata, and in *Perilla frutescens* L. Britton with perillaketone as principal compound, limonene was not detected. Although synthesis of perilla essential oil compounds commonly is limited to phenylpropanoids or monoterpenoids, in *Perilla ocymoides* L. *bicolorlaciniata* essential oil two phenylpropanoids elemicin (1.91%) and myristicin (1.41%) were detected. Phenylpropanoids were not detected in *Perilla frutescens* L. Britton and *Perilla frutescens* L. Britton var. *crispa* f. viridis. Traditional Chinese medicine proposes perilla leaves in treating neurosis. Medicinal studies have proved that phenylpropanoid chemotype containing myristicin and dillapiol is the most potent (in comparison with perillaldehyde and perillaketone chemotypes) in hexobarbital induced sleeping time prolongation observed in mice (1). Other identified compounds (E,E-α-farnesene, β-caryophyllene, germacrene D, linalool, α-caryophyllene, dihydrocarveol, α-terpineol, perilla alcohol) yielded 11.46% in total, 0.22% whereof was perilla alcohol detected only in this plant. Scientific research data revealed that perilla alcohol inhibits tumor cell growth in various tissues. Clinical trials regarding toxicity and chemotherapeutical effects of perilla alcohol has been performed (21, 22).

In *Perilla frutescens* (L.) Britton var. *crispa* f. *viridis* essential oil 83.18% compounds were indentified. Principal compounds were found to be perillaldehyde (49.47%), limonene (11.76%), limonene oxides (9.85%) and caryophyllene oxide (7.21%). Linalool oxides, myrcene, terpinolene, β-cyclocitral, carvone, bornyl acetate, eucalyptol, isodihydrocarveol acetate, p-cymene, β-caryophyllene, humulene epoxide II yielded 4.89% in total identified compounds. Since perillaldehyde was prevailing constituent in essential oil of *Perilla frutescens* (L.) Britton var. *crispa* f. *viridis*, perillaldehyde chemotype was determined. It was experimentally proved,

that Perilla extracts show antimicrobial activity against *Salmonella choleraesuis* (14). The most active compounds were found to be perillaldehyde, limonene, β-caryophyllene and linalool. Perillaldehyde strongly inhibits dermatophytic fungi. These effects confirm the uses of perilla leaves in traditional Chinese medicine for detoxifying the fish and crab poisons, and treating ringworm.

Essential oil of perilla plants is also beneficial in treating allergies. β-caryophyllene, l-menthol, α-pinene and elemicin show antiallergic effects on type I allergy. Perillaldehyde itself possesses strong sensitizing effect, therefore perillaldehyde containing plant material can not be used in treating allergies.

CONCLUSION

After the investigations of essential oil composition of perilla plants cultivated in Lithuania has been performed, it was determined that *Perilla frutescens* (L.) Britton essential oil is ascertained to perillaketone chemotype. *Perilla frutescens* (L.) Britton var. *crispa* f. *viridis* and *Perilla ocymoides* L. var. *bicolorlaciniata* are ascribed to perillaldehyde chemotype. β-Caryophyllene and perillaldehyde were the only compounds identified in all investigated perilla plants.

Considering a broad diversity of *Perilla* L. species and chemotypes, it is important to discriminate cultivated *Perilla* L. species with identified chemotypes, whose provided medicinal raw material could be standardized and optimal for further pharmacological investigations and manufacturing of medicinal preparations.

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