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Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage: http://ees.elsevier.com/apjtm

Original research http://dx.doi.org/10.1016/j.apjtm.2017.07.004

GC–MS analysis of volatile compounds of *Perilla frutescens* Britton var. *Japonica* accessions: Morphological and seasonal variability

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ARTICLE INFO

Article history:
Received 6 Feb 2017
Received in revised form 15 May 2017
Accepted 25 Jun 2017
Available online 28 Jul 2017

Keywords:
Perilla frutescens
Essential oil
GC–MS analysis
Morphological character
Harvesting time

ABSTRACT

Objective: To investigate the composition of volatile compounds in the different accessions of *Perilla frutescens* (*P. frutescens*) collected from various habitats of China and Japan.

Methods: In the present study, the essential oil from the leaves of *P. frutescens* cultivars from China and Japan was extracted by hydro-distillation and the chemical composition and concentration of the volatile components present in the oils were determined by gas chromatography–mass spectrometry (GC–MS) analysis.

Results: Among the volatile components, the major proportion was of perilla ketone, which was followed by elemicin and beta-caryophyllene in the Chinese *Perilla* cultivars. The main component in the oil extracted from the Japanese accessions was myristicin, which was followed by perilla ketone and beta-caryophyllene. We could distinguish seven chemotypes, namely the perilla ketone (PK) type, perilla ketone, myristicin (PM) type, perilla ketone, unknown (PU) type, perilla ketone, beta-caryophyllene, myristicine (PB) type, perilla ketone, myristicin, unknown (PMU) type, perilla ketone, elemicine, myristicin, beta-caryophyllene (PEMB) type, and the perilla ketone, limonene, beta-cryophyllene, myristicin (L) type. Most of the accessions possessed higher essential oil content before the flowering time than at the flowering stage. The average plant height, leaf length, leaf width of the Chinese accessions was higher than those of the Japanese accessions.

Conclusion: The results revealed that the harvest time and geographical origin caused polymorphisms in the essential oil composition and morphological traits in the *Perilla* accessions originating from China and Japan. Therefore, these chemotypes with desirable characters might be useful for industrial exploitation and for determining the harvest time.

1. Introduction

Perilla frutescens L. (Labiatae) (*P. frutescens*), a traditional crop of Korea, China, Japan, India, Nepal, and Thailand, is cultivated for its essential oils and medicinal uses [1]. In Korea, it is commonly known as "Tilkae" and is used as sushi, salad, and traditional oil [2]. It is an annual bush, growing to a height of 4 feet, and has a characteristic odor due to the presence of various oil components [3] in the glandular trichomes distributed on the abaxial surface of leaves [4]. The plant is used to treat depression, inflammation, bacterial and fungal infections, intoxication, some intestinal disorders and tumors [5–7]. It also has anti-inflammatory, anti-allergic [8], detoxicant, antitussive, antibiotic, and antipyretic properties [9]. The oil of this plant is a good source of natural phenolic antioxidants, including sesamol and sesamin [10], and omega-3 alphalinolenic acid [11].

There is a growing interest in ethnic and traditional use of aromatic medicinal plants as they contain essential oils and volatile compounds. The essential oils are rich in complex

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Peer review under responsibility of Hainan Medical University.

mixtures of secondary metabolites [12]. The volatiles compounds are mainly produced by the oxidation of fatty acids through intracellular biogenic pathway [13]. The volatiles compounds are helpful in quality control of oils because they are used for detection of adulterants [14] and rancidity [15]. Moreover, the volatile compounds exert toxic effects on herbivores [16], attract pollinators [17] and seed dispersers [18,19], provide protection against pathogens and insect [20], are involved in plant–plant signaling [21], effective in larvicidal activity [22], repellent activity [23], anti-inflammatory activity [24], inhibit apoptosis [25], and protect the plants against abiotic and oxidative stress [26].

A number of studies have reported on the volatile and non-volatile constituents of *P. frutescens* [27,28]. *Perilla* seeds are especially good sources of linolenic, linoleic, oleic, stearic, palmitic acids [29], perilla aldehyde, benzaldehyde, limonene [30], caffeic acid, rosmarinic acid, rosmarinic acid methyl ester [31,32], luteolin, apigenin, chrysoeriol, and rosemarinic acid [33]. The different cultivars show great variation in terms of the chemical composition and concentration of the bioactive compounds present in them. A number of studies have revealed that the variations in the chemical composition of the essential oils in different cultivars can be affected by different factors, including humidity, soil conditions, temperature, and seasonality [34–37], harvest time [38], sowing time [39], geographic region [40], and phenological stages [41]. However, as of date, no comparative study on the seasonal variability of the volatile compounds and the morphological analysis of leaves from different *Perilla* accessions has been attempted.

The present study was undertaken to investigate the composition of volatile compounds in the different accessions of *P. frutescens* collected from various habitats of China and Japan. Furthermore, to study their chemotypes, the influence of harvesting time on the essential oil content and morphological traits of 18 accessions of *Perilla* were assessed. The data obtained in the present study should prove to be useful for screening of new and potential sources of natural antioxidants that can be used for treatment of various diseases.

2. Material and methods

2.1. Experimental plant material

Eighteen accessions of *P. frutescens* used in the present study, originated from China and Japan, and were kindly provided by the Rural Development Administration (RDA), Suwon, South Korea. All the accessions of *Perilla* were grown under similar field management.

2.2. Morphological variations in the Perilla accessions

Morphological variations in the 18 accessions of *Perilla* were scored by using 10 individuals of each accession grown in a field at Kangwon National University Experimental Farm, with latitudinal and longitudinal coordinates of 37°56′24.63 (N) and 127°46′ (E), respectively, and an altitude of 117.22 m above the mean sea level. Twenty seeds of each accession were sown in a nursery bed in the last week of April, 2014 and grown in a glass house for a month. Ten seedlings of each accession were subsequently transplanted into the field in early June, 2014. The field trial was performed in a randomized complete block design, with three replications of a plot size of 10.0 m × 8.0 m and a line-to-line distance of 30 cm and a plant-to-plant distance of 20 cm in rows. The temperature in the experimental field ranged from 25 °C to 34 °C in the summer and −10 °C to 8 °C in the winter and the total annual rainfall was 505.5 mm. The quantitative traits evaluated were the plant height (cm), leaf length (cm), leaf width (cm), ratio of length/width, total number of leaves picked, total picking days, number of picking, picking interval, yield per plant, number of branches, 1 000 seed weight (g), number of nodes, number of panicle, number of pods per panicle, panicle length (cm), days from sowing to flowering, and days from sowing to maturity. The qualitative traits assessed were the distribution of pubescence, leaf color of the adaxial surface, leaf color of the abaxial surface, leaf shape, seed coat color, seed shape, and seed size. All the leaf related traits were recorded from the fully expanded leaves of the three major branches.

2.3. Isolation of volatile components

The fresh leaves of *P. frutescens* (500 g) were subjected to hydro-distillation using 500 mL H_2O in Lickens and Nickerson apparatus for a minimum of 2 h each. Diethyl ether (100 mL) used as a solvent was heated to boiling temperature in a 200 mL round-bottom flask and the volatile compounds were extracted by simultaneous steam distillation process. The resulting extract was obtained at a yield of 0.01% w/w. The extract was dried over anhydrous sodium sulfate and then stored at 4 °C until gas chromatography–mass spectroscopy (GC–MS) analysis.

2.4. Gas chromatography-mass spectrometry analysis of volatile components

The samples extracts were first diluted in hexane (spectroscopy grade) and analyzed in a Finnigan Focus GC/Finnigan Focus DSQ MS system (Thermo Co., Braunschweig, Germany) equipped with VB-WAX bonded PEG capillary column (30 m × 0.25 mm internal diameter, 0.25 μm film thickness). The flow rate of the carrier gas (He) was 1 mL/min. A sample volume of 10 μL was injected in the split mode (split less). The temperature program used for the analysis was as follows: the injector and detector were set to 150 °C and 250 °C, respectively; the column oven temperature was maintained at 50 °C for 10 min and then programmed to 200 °C and subsequently held for 0.5 h at 200 °C. The MS was operated in EI mode at 70 eV and the spectra were scanned from 25 to 350 *m/z*. The individual components were identified by matching their recorded mass spectra of the GC–MS data system. The relative percentages of the individual components were obtained from electronic integration peak areas and by comparing their retention time and mass spectra library with those found in a library of mass spectra (The Wiley Registry of Mass Spectral Data, 7th ed.). The analyses were performed in triplicate.

2.5. Statistical analysis

Statistical analyses of the data were carried out using the SAS statistical package (version 9.3–2012, SAS Institute, Cary, North Carolina, USA). Duncan's multiple range test (DMRT) was

Table 1

The concentrations of volatile components in the 18 accessions of *P. frutescens* (%).

Accession no.	Mean of GC–MS area (%)										
	Limonene	*trans*-2-hexenal	6-methyl-5-hepten-2-one	1-octene-3-ol	linalool	Beta-caryophyllene	Alpha-humulene	Beta-farnesene	Germacrene D	ç-elemene	*trans*-alpha-bergamotene
K100	0.00 ± 0.00^{b}	0.14 ± 0.01^{gh}	0.21 ± 0.01^{fgh}	0.15 ± 0.02^{f}	0.73 ± 0.01^{b}	24.28 ± 0.99^{a}	0.51 ± 0.16^{ef}	0.57 ± 0.18^{bcd}	0.91 ± 0.28^{a}	0.00 ± 0.00^{g}	1.28 ± 0.17^{de}
K102	0.00 ± 0.00^{b}	0.18 ± 0.01^{gh}	0.16 ± 0.01^{j}	0.21 ± 0.02^{de}	0.31 ± 0.10^{gh}	23.39 ± 4.90^{a}	1.08 ± 0.21^{bc}	0.56 ± 0.11^{bcd}	0.56 ± 0.22^{bc}	0.35 ± 0.07^{bc}	2.34 ± 0.51^{bc}
K104	0.00 ± 0.00^{b}	0.21 ± 0.05^{defg}	0.16 ± 0.02^{j}	0.29 ± 0.03^{b}	0.63 ± 0.03^{abc}	21.89 ± 0.86^{a}	0.68 ± 0.17^{def}	0.43 ± 0.09^{cd}	0.23 ± 0.06^{d}	0.22 ± 0.05^{cdef}	1.15 ± 0.23^{e}
K107	0.00 ± 0.00^{b}	0.00 ± 0.00^{i}	0.23 ± 0.01^{ef}	0.09 ± 0.02^{g}	0.48 ± 0.10^{cdef}	20.81 ± 3.41^{ab}	0.99 ± 0.21^{bcd}	0.88 ± 0.16^{b}	0.33 ± 0.08^{d}	0.14 ± 0.04^{f}	1.76 ± 0.16^{cde}
K108	0.00 ± 0.00^{b}	0.57 ± 0.10^{b}	0.06 ± 0.01^{k}	0.48 ± 0.030^{a}	0.57 ± 0.05^{bcd}	16.63 ± 2.28^{bc}	0.89 ± 0.31^{cde}	0.43 ± 0.15^{cd}	0.26 ± 0.05^{d}	0.14 ± 0.05^{fg}	1.06 ± 0.14^{e}
K109	0.00 ± 0.00^{b}	0.51 ± 0.06^{b}	0.00 ± 0.00^{l}	0.28 ± 0.03^{b}	0.66 ± 0.08^{ab}	15.68 ± 2.03^{cd}	0.53 ± 0.19^{ef}	0.38 ± 0.23^{cd}	0.32 ± 0.07^{d}	0.25 ± 0.06^{cdef}	1.12 ± 0.23^{e}
K113	0.00 ± 0.00^{b}	0.30 ± 0.02^{de}	0.18 ± 0.0^{j}	0.49 ± 0.03^{a}	0.45 ± 0.10^{defg}	13.25 ± 1.50^{cde}	0.44 ± 0.17^{f}	0.27 ± 0.08^{d}	0.17 ± 0.04^{d}	0.00 ± 0.00^{g}	1.05 ± 0.08^{e}
K114	0.00 ± 0.00^{b}	0.30 ± 0.01^{de}	0.27 ± 0.01^{ed}	0.23 ± 0.03^{cd}	0.57 ± 0.04^{bcd}	12.67 ± 3.33^{cdef}	0.88 ± 0.23^{cde}	0.43 ± 0.15^{cd}	0.15 ± 0.05^{d}	0.11 ± 0.03^{g}	1.24 ± 0.15^{e}
K118	0.00 ± 0.00^{b}	0.27 ± 0.03^{de}	0.21 ± 0.01^{hij}	0.00 ± 0.00^{h}	0.70 ± 0.11^{ab}	11.83 ± 1.02^{efdg}	0.46 ± 0.21^{f}	0.28 ± 0.13^{d}	0.16 ± 0.06^{d}	0.20 ± 0.07^{def}	1.41 ± 0.31^{cde}
K119	0.00 ± 0.00^{b}	0.27 ± 0.01^{de}	0.17 ± 0.02^{ij}	0.16 ± 0.02^{f}	0.31 ± 0.12^{gh}	10.64 ± 2.28^{efg}	0.99 ± 0.29^{bcd}	0.58 ± 0.15^{cd}	0.32 ± 0.07^{d}	0.21 ± 0.06^{ef}	2.97 ± 0.35^{b}
K133	0.00 ± 0.00^{b}	0.25 ± 0.01^{def}	0.18 ± 0.03^{hij}	0.03 ± 0.01^{g}	0.35 ± 0.05^{efgh}	10.49 ± 1.11^{efg}	1.33 ± 0.13^{ab}	0.29 ± 0.09^{d}	0.23 ± 0.08^{d}	0.79 ± 0.11^{a}	2.30 ± 1.01^{bcd}
K134	0.00 ± 0.00^{b}	0.31 ± 0.04^{d}	0.23 ± 0.01^{fg}	0.00 ± 0.00^{h}	0.32 ± 0.04^{fgh}	9.57 ± 0.82^{efg}	1.58 ± 0.18^{a}	0.21 ± 0.08^{d}	0.27 ± 0.07^{d}	0.25 ± 0.05^{cdef}	3.03 ± 0.94^{b}
K136	0.00 ± 0.00^{b}	0.29 ± 0.05^{de}	0.33 ± 0.03^{b}	0.18 ± 0.01^{ef}	0.41 ± 0.05^{defg}	8.85 ± 2.39^{efg}	0.75 ± 0.16^{cdef}	0.27 ± 0.11^{d}	0.23 ± 0.07^{d}	0.34 ± 0.06^{bcd}	1.62 ± 0.12^{cde}
K137	0.00 ± 0.00^{b}	0.68 ± 0.07^{a}	0.31 ± 0.02^{bc}	0.26 ± 0.01^{bc}	0.50 ± 0.06^{cde}	8.58 ± 1.34^{efg}	0.54 ± 0.22^{ef}	0.26 ± 0.08^{d}	0.19 ± 0.05^{d}	0.17 ± 0.08^{ef}	1.55 ± 0.45^{cde}
K138	0.00 ± 0.00^{b}	0.41 ± 0.07^{c}	0.58 ± 0.03^{a}	0.14 ± 0.01^{f}	0.76 ± 0.15^{a}	8.53 ± 2.37^{efg}	0.75 ± 0.18^{cdef}	0.75 ± 0.17^{bc}	0.74 ± 0.11^{ab}	0.29 ± 0.07^{bcde}	1.67 ± 0.70^{cde}
K139	0.00 ± 0.00^{b}	0.21 ± 0.01^{efgh}	0.28 ± 0.03^{cd}	0.14 ± 0.02^{f}	0.26 ± 0.07^{h}	8.31 ± 4.07^{efg}	1.58 ± 0.26^{a}	0.75 ± 0.15^{bc}	0.65 ± 0.15^{bc}	0.31 ± 0.08^{bcde}	2.88 ± 0.91^{b}
K140	0.00 ± 0.00^{b}	0.51 ± 0.10^{b}	0.00 ± 0.00^{l}	0.25 ± 0.04^{bc}	0.00 ± 0.00^{i}	7.76 ± 1.25^{fg}	0.56 ± 0.16^{ef}	10.57 ± 0.39^{a}	0.53 ± 0.06^{c}	0.40 ± 0.09^{b}	2.35 ± 0.38^{bc}
K141	0.88 ± 0.04^{a}	0.13^{h} ± 0.03^{h}	0.18 ± 0.03^{hij}	0.00 ± 0.00^{h}	0.25 ± 0.02^{h}	7.25 ± 3.83^{g}	1.64 ± 0.18^{a}	019 ± 0.08^{d}	0.29 ± 0.09^{d}	0.70 ± 0.16^{a}	6.58 ± 0.91^{a}

Within column means with the same letter are not significantly different at $P \le 0.05$ using SAS 9.3 (2012).

Table 2

The concentrations of volatile components in the 18 accessions of *P. frutescens* (%).

Accession no.	Mean of GC/MS area (%)										
	Perillaldehyde	Perilla ketone	Unknown	Phytol	Nerolidol	Spathulenol	Eugenol	Elemicin	Myristicin	Apiol	Others
K100	0.35 ± 0.14^{c}	54.18 ± 8.19^{ab}	9.35 ± 1.86^{ef}	1.34 ± 0.14^{ab}	0.22 ± 0.03^{bcd}	0.32 ± 0.08^{abc}	0.00 ± 0.00^{h}	0.44 ± 0.08^{g}	19.31 ± 4.04^{b}	0.00 ± 0.00^{h}	0.91 ± 0.10^{h}
K102	0.23 ± 0.08^{c}	29.30 ± 2.11^{fg}	6.41 ± 0.48^{fgh}	0.64 ± 0.15^{def}	0.15 ± 0.05^{cde}	0.16 ± 0.03^{g}	0.15 ± 0.03^{ef}	1.06 ± 0.16^{fg}	34.17 ± 3.96^{a}	0.22 ± 0.02^{gh}	1.83 ± 0.24^{f}
K104	0.29 ± 0.08^{c}	46.10 ± 4.50^{bcde}	13.60 ± 3.39^{cd}	0.64 ± 0.13^{def}	0.06 ± 0.02^{ef}	0.22 ± 0.04^{defg}	0.00 ± 0.00^{h}	1.79 ± 0.12^{efg}	20.19 ± 2.73^{b}	0.36 ± 0.05^{fgh}	1.56 ± 0.07^{fg}
K107	0.18 ± 0.08^{c}	46.08 ± 1.03^{bcde}	9.27 ± 1.75^{ef}	0.75 ± 0.14^{de}	0.21 ± 0.04^{bcd}	0.29 ± 0.04^{abcd}	0.11 ± 0.02^{fg}	1.85 ± 0.05^{efg}	10.63 ± 2.65^{cd}	7.71 ± 0.57^{b}	1.74 ± 0.14^{f}
K108	0.31 ± 0.10^{c}	42.79 ± 3.49^{cde}	9.78 ± 1.18^{ef}	0.58 ± 0.14^{def}	0.21 ± 0.06^{bcd}	0.17 ± 0.02^{fg}	0.35 ± 0.02^{c}	9.74 ± 0.97^{c}	14.06 ± 2.91^{bc}	2.73 ± 0.45^{c}	4.78 ± 0.98^{ab}
K109	0.26 ± 0.08^{c}	47.03 ± 6.55^{abcd}	21.76 ± 1.07^{b}	1.18 ± 0.09^{bc}	0.17 ± 0.07^{cd}	0.26 ± 0.02^{cde}	0.14 ± 0.04^{fg}	3.38 ± 0.38^{e}	10.52 ± 2.41^{cd}	0.74 ± 0.17^{ef}	2.12 ± 0.19^{ef}
K113	0.27 ± 0.07^{c}	53.66 ± 5.50^{ab}	15.13 ± 1.16$_{c}$	0.87 ± 0.12^{cd}	0.36 ± 0.08^{a}	0.35 ± 0.04^{a}	0.15 ± 0.02^{ef}	1.91 ± 0.80^{efg}	14.06 ± 1.40^{bc}	1.68 ± 0.18^{d}	1.18 ± 0.20^{gh}
K114	0.33 ± 0.09^{c}	40.65 ± 5.50^{de}	9.97 ± 1.24^{def}	1.94 ± 0.14^{a}	0.24 ± 0.06^{bcd}	0.28 ± 0.05^{abcde}	0.13 ± 0.01^{fg}	11.83 ± 0.91^{b}	14.26 ± 3.02^{bc}	1.01 ± 0.21^{e}	3.21 ± 0.31^{d}
K118	0.42 ± 0.11^{bc}	46.54 ± 5.76^{abcd}	18.77 ± 2.14^{b}	0.89 ± 0.33^{cd}	0.19 ± 0.05^{bcd}	0.34 ± 0.03^{ab}	0.62 ± 0.08^{a}	5.76 ± 0.29^{d}	13.57 ± 2.61^{bc}	0.67 ± 0.07^{efg}	1.10 ± 0.17^{gh}
K119	0.47 ± 0.19^{bc}	26.86 ± 4.10^{gh}	8.84 ± 1.95^{efg}	0.89 ± 0.30^{cd}	0.21 ± 0.05^{bcd}	0.24 ± 0.03^{def}	0.52 ± 0.06^{b}	21.32 ± 3.23^{a}	13.29 ± 1.23^{bc}	2.43 ± 0.43^{c}	3.78 ± 0.16^{c}
K133	0.36 ± 0.05^{c}	24.80 ± 5.33^{gh}	5.33 ± 0.59^{gh}	0.59 ± 0.18^{def}	0.22 ± 0.05^{bcd}	0.28 ± 0.03^{abcde}	0.21 ± 0.02^{de}	3.03 ± 0.47^{ef}	33.63 ± 3.12^{a}	0.36 ± 0.09^{fgh}	5.21 ± 0.28^{a}
K134	0.34 ± 0.06^{c}	36.99 ± 5.19^{ef}	4.53 ± 0.35^{h}	0.64 ± 0.12^{def}	0.15 ± 0.02^{cde}	0.19 ± 0.02^{fg}	0.09 ± 0.02^{g}	1.04 ± 0.18^{fg}	37.76 ± 2.60^{a}	0.21 ± 0.02^{gh}	4.43 ± 0.46^{b}
K136	0.57 ± 0.19^{bc}	50.69 ± 6.40^{abc}	49.67 ± 0.94^{h}	0.47 ± 0.03^{ef}	0.29 ± 0.05^{ab}	0.26 ± 0.04^{bcde}	0.00 ± 0.00^{h}	8.70 ± 1.51^{c}	15.30 ± 2.13^{bc}	0.16 ± 0.03^{h}	2.12 ± 0.10^{ef}
K137	0.54 ± 0.14^{bc}	53.76 ± 5.42^{ab}	11.83 ± 2.16^{cde}	0.79 ± 0.29^{de}	0.19 ± 0.05^{bcd}	0.21 ± 0.02^{efg}	0.00 ± 0.00^{h}	0.54 ± 0.09^{g}	14.97 ± 1.69^{bc}	0.22 ± 0.03^{gh}	3.68 ± 0.91^{cd}
K138	0.55 ± 0.18^{bc}	56.10 ± 5.42^{a}	4.17 ± 1.08^{h}	0.39 ± 0.09^{f}	0.26 ± 0.08^{bc}	0.22 ± 0.06^{defg}	0.00 ± 0.00^{h}	0.40 ± 0.97^{g}	16.76 ± 3.56^{bc}	0.15 ± 0.05^{h}	0.87 ± 0.16^{h}
K139	0.87 ± 0.25^{b}	25.50 ± 5.18^{gh}	4.04 ± 0.83^{h}	0.60 ± 0.08^{def}	0.24 ± 0.08^{bcd}	0.16 ± 0.02^{g}	0.00 ± 0.00^{h}	0.36 ± 0.10^{g}	35.41 ± 6.07^{a}	0.14 ± 0.02^{h}	1.16 ± 0.10^{gh}
K140	0.11 ± 0.04^{c}	17.63 ± 1.63^{h}	27.59 ± 1.79^{a}	0.00 ± 0.00^{g}	0.00 ± 0.00^{f}	0.00 ± 0.00^{h}	0.22 ± 0.04^{d}	0.27 ± 0.03^{g}	5.69 ± 1.44^{d}	14.72 ± 0.46^{a}	2.56 ± 0.12^{e}
K141	3.02 ± 0.91^{a}	21.04 ± 5.88^{gh}	3.40 ± 0.50^{h}	0.48 ± 0.11^{ef}	0.15 ± 0.02^{de}	0.27 ± 0.03^{abcde}	0.00 ± 0.00^{h}	0.36 ± 0.04^{g}	34.28 ± 7.08^{a}	0.38 ± 0.07^{fgh}	0.92 ± 0.10^{h}

Within column means with the same letter are not significantly different at $P \le 0.05$ using SAS 9.3 (2012).

performed for each parameter to compare the morphological traits and the volatile compound composition of 18 accessions of *Perilla* collected from China and Japan. P values ≤ 0.05 were considered statistically significant. Principle components analysis (PCA) was implemented to analyze the patterns in the morphological data distribution using the SAS statistical package (9.3–2012, SAS Institute, Cary, North Carolina, USA). The number of principal components to retain in the analysis was determined using the minimum eigenvalue criterion. The genetic similarity/distances carried out on the matrix of Euclidean distances were assessed using cluster analysis (Ward) method [42]. Dendrogram of relationships among the 18 accessions of *P. frutescens* obtained by UPGMA cluster analysis based on the morphological traits [43].

3. Results

3.1. Composition of volatile compounds in P. frutescens

The volatile components of the *Perilla* leaves were analyzed by GC–MS. Twenty-two volatile compounds were identified in the 18 accessions of *P. frutescens* collected from different places of China and Japan; their types, concentrations, and relative percentages are described in Tables 1 and 2. The total ion chromatograms of the identified volatile compounds are shown in Figure 1. Significant differences ($P < 0.05$) were observed in the concentration and composition of the volatile compounds among the accessions collected from different geographic bioregions of China. The dominant volatile component was perilla ketone, with an average content of 43.34%, followed by myristicin (average content = 16.3%) and beta-caryophyllene (average content = 11.88%). Other compounds with relative contents more than 1% were elemicin, apiol, *trans*-alpha-bergamotene, alpha-humulene, and phytol. The concentration of other identified components was found to be less than 1%. Beta-bourbonene, egomaketene, and limonene were not detected in the Chinese accessions. The highest perilla ketone concentration (54.62%) was observed in the accession 100.

The most dominating volatile component in the *Perilla* accessions from Japan was the perilla ketone (average = 38.50%), followed by myristicin (26.86%) and beta-caryophyllene (16.00%). The other components with concentrations higher than 1% included elemicin, perillaldehyde, *trans*-alpha-bergamotene, and alpha-humulene. The concentrations of other identified components were found to be less than 1%. Eugenol was found only in the Japanese accessions 133 & 141, whereas, limonene was detected only in the accession 141, suggesting the existence of cultivar specific volatile compounds. The average perillaldehyde (0.89%), myristicin (26.85%), bergamotene (2.78%), and apiol (2.62%) concentrations were detected to be higher in Japanese accession compared to the Chinese accessions, whereas, the average perilla ketone (43.34%) and elemicin (5.92%) contents were higher in the Chinese accessions compared to the Japanese accession. Beta-caryophyllene and beta-farnesene were the most abundant sesquiterpenes found in the Japanese and Chinese *Perilla* accessions. All the six alcohols (1-octene-3-ol, linalool, phytol, nerolidol, spathulenol, and eugenol) were detected at lower concentrations in the Chinese and Japanese accessions. Perilla ketone was the most dominant ketone in both the Chinese and Japanese accession, whereas egomaketone was not detected in both the populations. Among the aldehydes, perillaldehyde was higher than *trans*-2-hexenal in both the Chinese and Japanese accessions.

3.2. Chemotypes

Due to its wide geographical distribution, a huge variation occurs in the composition and concentration of volatile components in *P. frutescens*. We could classify the *Perilla* accessions into seven chemotypes based on the combination of major chemical components *viz*., the perilla ketone (PK) type, perilla ketone, myristicin (PM) type, perilla ketone, unknown (PU) type, perilla ketone, beta-caryophyllene, myristicine (PB) type, perilla ketone, myristicin, unknown (PMU) type, perilla ketone, elemicine, myristicin, beta-caryophyllene (PEMB) type, and perilla ketone, limonene, beta-cryophyllene, myristicin (L) type.

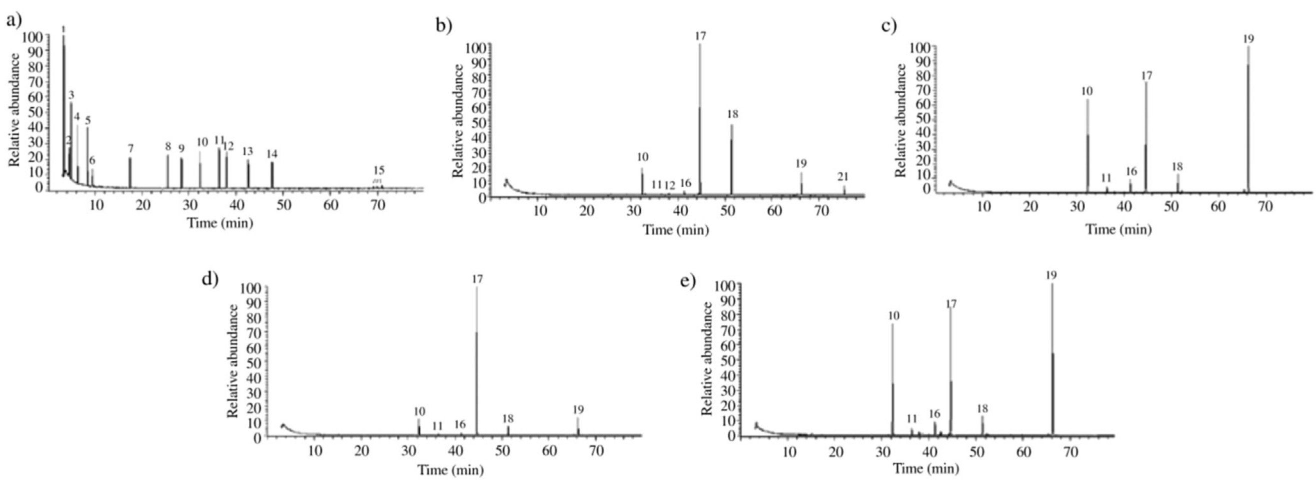

Figure 1. GC–MS chromatogram of essential oil components from the leaves of the 18 accessions of *P. frutescens* from China and Japan.
(a) GC–MS chromatogram of the 15 standards. (b) GC–MS chromatogram of the PU-type (K107). (c) GC–MS chromatogram of the PM-type (K134). (d) GC–MS chromatogram of the PK-type (K136). (e) GC–MS chromatogram of the PMB-type (K139). 1. alpha-pinene, 2. hexanal, 3. beta-pinene, 4. delta-3-careen, 5. limonene, 6. *trans*-2-hexanal, 7. 6-methyl-5-hepten-2-one, 8. 1-octene-3-ol, 9. benzaldehyde, 10. beta-caryophyllene, 11. alpha-humulene, 12. beta-farnesene, 13. perillaldehyde, 14. geraniol, 15. farnesol, 16. *trans*-alpha-bergamotene, 17. perilla ketone, 18. unknown, 19. myristicin 20. elemicin 21. apiol.

3.3. Harvesting time and the content of volatile oils

In this study, the time of leaves collection influenced the volatile oils contents in the accessions. The essential oil content in the Chinese *Perilla* accessions was higher before flowering stage. The average percentage of the essential oil content in the Chinese *Perilla* accessions ranged from (8.10 ± 1.24) mg/g to (19.5 ± 0.48) mg/g dry leaf in K108 and K118, respectively. The differences among the volatile compounds investigated were statistically significant at $P < 0.05$. The essential oil content in the Japanese *Perilla* accessions was higher before flowering stage. The average essential oil content in the Japanese *Perilla* accessions before the flowering stage ranged from (10.90 ± 0.50) to (21.20 ± 0.36) mg/g dry leaf in K139 and K136, respectively. Highest reduction in the volatile oil content was observed in the K139 accession (Table 3). The mean value of the essential oil content in the *Perilla* accessions was higher before flowering than at the flowering time.

Table 3

The average amount of essential oil content in the 18 accessions of *P. frutescens* from China and Japan (mg/fry leaf g).

Accession no.	Average amount of essential oil content	
	Before flowering	Flowering time
K100	19.0	18.6
K102	11.3	10.6
K104	10.3	10.5
K107	17.3	16.8
K108	8.1	8.0
K109	13.8	13.5
K113	18.0	17.5
K114	15.0	14.9
K118	19.5	19.3
K119	12.8	12.2
K133	18.0	16.6
K134	14.9	14.4
K136	21.3	21.2
K137	21.2	20.3
K138	13.2	13.1
K139	18.2	10.9
K140	12.8	12.5
K141	13.3	12.9

3.4. Morphological variation

The variations in the morphological characters among the 18 *P. frutescens* accessions are presented in Tables 4 and 5. The average number of lateral branches and the average number of nodes in the Chinese accessions of *Perilla* were more than in the Japan accessions. The highest average number of branches was observed in the accession numbers K107 and K139 from China and Japan, respectively.

Similarly, the number of panicles, number of pods per panicle, and the panicle length varied among the various accessions. The Chinese accessions had the lower average number of pods per panicle, whereas the *Perilla* accessions from Japan had the highest number of pods per panicle. The *Perilla* accessions from China were characterized by a higher average number of panicles, whereas the accessions from Japan had a lower average number of panicles. In particular, the accessions K119 and K140 showed higher number of panicles in their respective population. The accession K138, which revealed higher number of pods per panicle showed lower number of branches, nodes, 1 000 seed weight, days from sowing to, and days from sowing to maturity among the investigated accessions. Most of the Chinese accessions required longer time to flower and mature compared to the accessions from Japan. The accession 138 from Japan that required less number of days for flowering and maturation, produced the lowest total number of leaves that could be picked, with a lower yield per plant. In addition, the lowest total number of leaves picked was noted for the *Perilla* accession 100 from China and the highest for the accession 134 from Japan.

Table 4

Growth characteristics of the 18 accessions of *P. frutescens*.

Accession no.	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Ratio of length/width	Total no. of leaves picked	Total picking days	Picking interval (d)	Yield per plant (g/plant)
K100	184.1 ± 10.1^{bc}	15.4 ± 1.4^{bc}	11.4 ± 0.2^{bcd}	1.3 ± 0.1^{de}	175.0 ± 35.1^{m}	46.8 ± 2.1^{d}	8.3 ± 0.8^{bcde}	18.0 ± 2.5^{d}
K102	165.7 ± 9.2^{cde}	13.9 ± 0.8^{bc}	10.5 ± 0.1^{bcdef}	1.3 ± 0.1^{de}	289.0 ± 25.5^{l}	44.6 ± 1.8^{d}	7.0 ± 0.3^{e}	20.5 ± 2.7^{cd}
K104	172.0 ± 8.7^{cd}	15.7 ± 1.0^{b}	11.2 ± 0.2^{bcd}	1.4 ± 0.1^{cde}	780.0 ± 39.2^{b}	38.8 ± 2.6^{de}	7.3 ± 0.4^{de}	18.6 ± 2.3^{d}
K107	177.3 ± 12.5^{cd}	14.3 ± 0.9^{bc}	10.2 ± 0.2^{bcdef}	1.4 ± 0.1^{cde}	733.6 ± 40.2^{c}	56.1 ± 1.9^{cd}	8.1 ± 0.6^{bcde}	24.1 ± 2.9^{b}
K108	233.3 ± 11.6^{a}	18.0 ± 1.1^{a}	13.6 ± 0.5^{a}	1.3 ± 0.1^{de}	518.0 ± 24.7^{h}	74.0 ± 1.2^{b}	7.1 ± 0.3^{e}	22.5 ± 2.4^{bc}
K109	215.2 ± 13.2^{a}	15.1 ± 0.8^{bc}	11.6 ± 0.3^{bc}	1.4 ± 0.1^{cde}	495.0 ± 29.8^{i}	70.6 ± 2.6^{bc}	7.5 ± 0.3^{ed}	20.6 ± 2.6^{cd}
K113	167.8 ± 10.9^{cde}	17.9 ± 0.9^{a}	11.6 ± 0.3^{bc}	1.5 ± 0.1^{abc}	325.0 ± 31.3^{k}	86.2 ± 3.0^{ab}	8.0 ± 0.8^{cde}	22.0 ± 2.5^{bc}
K114	160.3 ± 7.2^{def}	15.5 ± 0.9^{b}	11.1 ± 0.1^{bcd}	1.4 ± 0.1^{cde}	713.0 ± 23.5^{d}	94.0 ± 3.1^{a}	7.2 ± 0.4^{e}	28.3 ± 2.1^{a}
K118	199.3 ± 13.1^{b}	14.0 ± 1.1^{bc}	8.7 ± 0.1^{f}	1.6 ± 0.1^{a}	286.0 ± 24.6^{l}	37.4 ± 1.4^{de}	7.7 ± 0.9^{cde}	9.0 ± 1.9^{fg}
K119	146.7 ± 10.3^{efg}	15.6 ± 1.0^{b}	9.8 ± 0.4^{cdef}	1.6 ± 0.1^{a}	664.0 ± 37.1^{f}	50.0 ± 2.6^{d}	7.6 ± 0.9^{de}	8.0 ± 1.9^{g}
K133	137.3 ± 10.5^{g}	13.0 ± 0.9^{d}	9.6 ± 0.2^{efd}	1.3 ± 0.1^{de}	458.0 ± 36.2^{j}	46.0 ± 1.9^{d}	9.2 ± 0.7^{abc}	10.2 ± 1.9^{fg}
K134	136.7 ± 11.0^{g}	13.0 ± 0.8^{d}	10.5 ± 0.2^{bcdef}	1.2 ± 0.1^{e}	982.0 ± 22.5^{a}	56.0 ± 2.3^{cd}	9.8 ± 0.9^{ab}	12.1 ± 2.3^{ef}
K136	176.7 ± 10.2^{cd}	14.0 ± 1.1^{bc}	9.9 ± 0.2^{bcdef}	1.4 ± 0.1^{cde}	670.0 ± 36.5^{f}	39.0 ± 2.8^{de}	8.3 ± 0.7^{bcde}	8.5 ± 2.1^{g}
K137	172.8 ± 9.5 ^{cd}	13.5 ± 0.9^{cd}	9.1 ± 0.2^{ef}	1.5 ± 0.1^{abc}	559.0 ± 21.5^{g}	54.2 ± 1.5^{cd}	10.5 ± 0.3^{a}	10.4 ± 2.2^{fg}
K138	146.7 ± 10.5^{efg}	15.6 ± 1.4^{b}	10.9 ± 0.2^{bcde}	1.4 ± 0.1^{cde}	229.0 ± 37.2^{m}	22.5 ± 1.4^{e}	9.6 ± 0.9^{abc}	14.8 ± 1.9^{e}
K139	141.7 ± 9.7^{fg}	13.5 ± 0.5^{cd}	10.2 ± 0.2^{bcdef}	1.3 ± 0.1^{de}	319.0 ± 32.6^{k}	46.2 ± 2.1^{d}	8.9 ± 0.4^{abcde}	12.3 ± 2.1^{ef}
K140	156.6 ± 9.4^{defg}	12.5 ± 0.8^{d}	9.0 ± 0.4^{ef}	1.3 ± 0.1^{de}	695.0 ± 28.5^{e}	86.0 ± 1.8^{ab}	7.2 ± 0.3^{e}	28.4 ± 2.5^{a}
K141	107.3 ± 10.6^{g}	15.2 ± 0.6^{bc}	11.7 ± 0.5^{b}	1.3 ± 0.1^{de}	710.0 ± 29.5^{d}	84.5 ± 1.7^{ab}	7.8 ± 1.1^{cde}	30.0 ± 2.0^{a}

Within column means with the same letter are not significantly different at $P \leq 0.05$ using SAS 9.3 (2012).

Table 5

Growth characteristics of the 18 accessions of *P. frutescens*.

Accession no.	No. of branches	No. of nodes	No. of panicle	No. of pods per panicle	Panicle length (cm)	1 000 seedweight (g)	Days from sowing to flowering	Days from sowing to maturity
K100	19.7 ± 1.9^{ab}	17.7 ± 1.8^{bcd}	173.4 ± 13.5^{d}	28.7 ± 2.1^{hi}	6.9 ± 1.6^{cde}	5.0 ± 0.1^{a}	213.3 ± 16.4^{a}	223.7 ± 20.4^{ab}
K102	15.4 ± 1.6^{defg}	17.6 ± 1.8^{bcd}	219.3 ± 19.5^{b}	31.6 ± 2.5^{gh}	22.8 ± 1.6^{a}	4.4 ± 0.3^{b}	172.1 ± 15.2^{de}	209.7 ± 16.5^{bcd}
K104	14.0 ± 2.1^{fg}	18.1 ± 1.9^{bcd}	159.3 ± 10.5^{de}	31.8 ± 2.2^{gh}	7.7 ± 0.9^{cde}	4.0 ± 0.2^{b}	192.7 ± 16.9^{b}	234.3 ± 23.0^{a}
K107	21.4 ± 2.0^{a}	18.3 ± 1.8^{abcd}	173.0 ± 13.5^{d}	32.0 ± 3.1^{gh}	6.6 ± 0.4^{de}	3.2 ± 0.1^{c}	192.0 ± 18.7^{b}	218.3 ± 17.0^{abc}
K108	15.5 ± 1.2^{defg}	17.4 ± 1.6^{bcd}	106.3 ± 10.6^{g}	50.8 ± 2.8^{c}	6.8 ± 0.9^{cde}	3.1 ± 0.1^{cd}	191.3 ± 15.5^{b}	223.5 ± 20.2^{ab}
K109	20.5 ± 2.3^{ab}	18.4 ± 2.1^{bcd}	152.3 ± 13.1^{e}	24.0 ± 2.4^{i}	6.9 ± 0.8^{cde}	3.1 ± 0.2^{cd}	190.7 ± 14.8^{bc}	221.0 ± 19.0^{ab}
K113	15.7 ± 2.1^{cdefg}	20.3 ± 1.9^{ab}	131.3 ± 16.5^{f}	42.0 ± 3.1^{de}	9.5 ± 0.9^{bcd}	3.1 ± 0.1^{cd}	183.0 ± 16.9^{bcd}	215.7 ± 16.0^{abcd}
K114	19.3 ± 1.5^{abc}	17.1 ± 1.8^{bcd}	105.0 ± 13.0^{gh}	41.2 ± 2.1^{de}	4.8 ± 1.6^{e}	2.9 ± 0.1^{cde}	190.3 ± 13.2^{bcd}	222.7 ± 20.5^{ab}
K118	12.5 ± 1.9^{g}	16.0 ± 2.1^{cd}	87.3 ± 12.5^{h}	45.3 ± 3.0^{d}	8.9 ± 1.9^{bcde}	2.9 ± 0.2^{cde}	181.7 ± 12.6^{bcde}	214.7 ± 17.3^{bcd}
K119	18.0 ± 1.9^{abcde}	21.7 ± 1.7^{a}	351.3 ± 15.1^{a}	42.2 ± 3.0^{de}	10.9 ± 1.4^{bc}	2.8 ± 0.2^{defg}	189.0 ± 13.6^{bcd}	223.0 ± 24.3^{ab}
K133	16.3 ± 2.1^{bcdefg}	16.7 ± 2.1^{cd}	166.7 ± 15.2^{de}	38.3 ± 2.5^{ef}	8.5 ± 1.6^{bcde}	2.6 ± 0.1^{defg}	172.0 ± 13.3^{de}	202.0 ± 17.2^{cd}
K134	18.7 ± 1.9^{abcd}	17.7 ± 1.9^{bcd}	124.5 ± 17.4^{f}	25.7 ± 2.1^{i}	5.3 ± 0.9^{e}	2.4 ± 0.1^{efgh}	181.0 ± 12.5^{bcde}	214.5 ± 21.0^{bcd}
K136	14.8 ± 2.1^{efg}	14.7 ± 1.9^{d}	195.3 ± 10.1^{bc}	58.3 ± 2.5^{b}	10.5 ± 1.9^{bcd}	2.3 ± 0.2^{fgh}	176.3 ± 19.0^{bcde}	202.1 ± 20.4^{cd}
K137	14.2 ± 2.0^{fg}	16.5 ± 1.9^{cd}	91.3 ± 11.3^{gh}	35.0 ± 2.5^{fg}	6.4 ± 1.5^{de}	2.3 ± 0.1^{fgh}	172.3 ± 18.2^{cde}	199.3 ± 12.5^{d}
K138	14.7 ± 2.0^{efg}	11.1 ± 1.8^{e}	124.3 ± 18.1^{f}	77.0 ± 1.7^{a}	6.7 ± 0.9^{de}	2.1 ± 0.1^{gh}	105.9 ± 13.9^{f}	141.6 ± 13.3^{f}
K139	20.2 ± 1.5^{ab}	19.0 ± 2.0^{abc}	208.3 ± 16.2^{bc}	32.3 ± 2.3^{gh}	11.9 ± 0.7^{b}	2.1 ± 0.1^{gh}	183.7 ± 16.7^{bcd}	217.3 ± 23.7^{abcd}
K140	16.7 ± 2.3^{bcdef}	17.4 ± 1.9^{bcd}	223.7 ± 15.1^{b}	41.0 ± 3.0^{de}	7.5 ± 0.4^{cde}	1.9 ± 0.1^{h}	164.7 ± 15.3^{e}	179.9 ± 14.1^{e}
K141	13.7 ± 2.1^{fg}	15.3 ± 1.8^{cd}	102.2 ± 16.1^{gh}	35.3 ± 3.5^{fg}	6.5 ± 0.3^{de}	1.9 ± 0.1^{h}	190.0 ± 13.5^{bcd}	226.6 ± 21.1^{ab}

Within column means with the same letter are not significantly different at $P \leq 0.05$ using SAS 9.3 (2012).

4. Discussion

4.1. Composition of volatile compounds in P. frutescens

The variation in essential oil of 18 *P. frutescens* accessions collected from different geographic bioregions of China was observed. The dominant volatile component was perilla ketone. This result is in accordance with those reported by Huang *et al.* [44], who also reported the highest relative perilla ketone content (54.5%–83.5%) in the Chinese *Perilla* cultivars (Baisu). In this study, perillaldehyde was found at low abundance (average content of 0.46%). In contrast, perillaldehyde was detected as a major volatile component in the Chinese *Perilla* cultivars [45]. In another study, carvone, perilla aldehyde, caryophyllene, and 2-furyl methyl ketone were the main volatile components in the Chinese accessions of *P. frutescens* [46]. Alpha-hexanoylfuran and asarone, which were detected in the present study, represent the major volatile components of the Chinese *Perilla* cultivars [27]. In contrast, in a previous report, beta-caryophyllene (24.2%), thujopsene (13.0%–20.8%), perillaldehyde (14.2%–15.1%) were reported to be the major volatile components present in the Chinese *Perilla* cultivars [47].

In the present study, the major volatile components of the Japanese *Perilla* accessions were perilla ketone. The data presented here are not in agreement with those described in earlier studies. For example, Tanaka *et al.* [48] and Martinetti *et al.* [49] reported that perillaldehyde was the major component in the Japanese accessions. However, Ito *et al.* [3] observed that shisofuran (73.8%–81.5%) was the dominating component, which was followed by 1-octen-3-ol (16.2%–21.8%) and beta-caryophyllene (18.3%–19.6%). In contrast, higher content of perilla ketone (92% in the SDE method) was followed by methyl sulphide (6.01%) and beta-caryophyllene (2.07%) [50]. In another study, the higher content of perilla ketone was followed by those of (Z)-3-hexanol and 1-octen-3-ol [51]. Similarly, Ha *et al.* [2] found that perilla ketone was the most intense volatile component (54%) and was followed by isoegomaketone (21%) and alpha-bergamotene (8.7%). Ayanoglu *et al.* [52] reported that the harvesting stages, harvesting hours, and drying methods could influence the content of the volatile compounds in plants. Other factors, such as environmental stress [53], agronomic conditions [54], and genotypes [55] can be attributed to the variation in the volatile components of the different accessions. However, in this study, all the accessions were planted in the same experimental field under the same growing conditions and field management. Moreover, all the samples were collected at the same time for chemical analysis. Therefore, these quantitative variations in the volatile components could be attributed to the genetic factors.

4.2. Chemotypes

In this study, different accessions of *P. frutescens* collected from China and Japan were classified according to the chemotype classification method proposed by Grayer *et al.* [56], which is based on the combination of major chemical components: perilla ketone, myristicin, beta-caryophyllene, myristicine, elemicine, and limonene. Several chemical investigations have been performed to determine the volatile components and chemotypes of the *Perilla* cultivars. For example, on the basis of the major chemical constituents, the essential oils of Japan were classified into five chemotypes, *viz*., perillaldehyde (PA), perilla ketone (PK), elsholtziaketone (EK), citral (C), and phenylpropanoid (PP) types [57]. Furthermore, Ito *et al.* [3] investigated the *Perilla* populations and classified *Perilla* plants into the following seven chemotypes based on the synthesis pathways: PA-type containing perillaldehyde, EK-type containing elsholtziaketone, naginataketone, and shisofuran, PK-type containing perilla ketone and isoegomaketone, PL-type containing perillene, C-type containing citral, PP-type containing phenylpropanoids, such as myristicin, elemicin, dillapiole, and PT-type containing piperitenone. A new chemotype (C type) of *P. frutescens* (Labiatae) that accumulates *trans*-citral as a main component of the essential oil in the leaf was reported by Yuba *et al.* [58]. The chemical diversity of essential oils in *Perilla* from China has also been reported earlier [59,60]. They classified the essential oils of *Perilla* into the following three chemotypes: PA-type containing perillaldehyde, PP-type containing myristicin, dillapiole, and/or elemicin, and PT-type containing piperitenone. More recently, Zhang *et al.* [61] identified and classified the *Perilla* essential oil compounds into eight chemotypes, viz., PK (perillaldehyde), DLP (D-limonene and piperitone), PT (piperitenone), MS (myristicin), AL (Apiol), EM (Elimicin), DEK (dehydroelsholtzia ketone), and PA (perilla aldehyde). Ohk and Chae [62] confirmed the occurrence of four distinct chemotypes in the *P. frutescens* populations from Korea (PA-type containing limonene and perillaldehyde, PK-type containing perilla ketone, ST-type containing sesquiterpene, beta-caryophyllene, and beta-farnesense, and PP-type containing phenylpropenes and sesquiterpenes).

4.3. Harvesting time and the content of volatile oils

We performed a comparative analysis of the volatile oil content of the 18 *P. frutescens* accessions from China and Japan. The results showed that the contents of volatile oil in the Chinese and Japanese accessions were influenced by the harvesting time. Most of the accession possessed higher essential oil content before the flowering stage. These results indicate that the developmental stage of the *Perilla* plant markedly influences the essential oil contents. However, harvesting time did not have any effect in some accessions (like K132), with similar content of essential oil present before and at the time of flowering. A number of studies have indicated that the harvest time influenced the essential oil content of plants [63,64]. In contrast to our results, Inan *et al.* [38] reported lowest essential oil content before the harvest of flowers in *Thymbra spicata* L. (*T. spicata*). In other studies, the highest increase in the total essential oil content was observed at the beginning of the blooming period in *Thymus vulgaris* (L.) [65], and *Thymus capitatus* (L.) [66]. The highest increase in the total essential oil was observed at the full blooming stage in *Thymus daenensis* subsp. daenensis Celak. [67], in *T. spicata* [54], and in *Origanum vulgare* L. [68]. The maximum yields of total essential oil after flowering were reported in *Coriandrum sativum* [69], and in *Myrtus communis* var. *italica* [70]. In contrast, in *Thymus citriodorus*, Omidbaigi *et al.* [71] found the maximum content of essential oils at the fruit-set stage. It has been argued that the stage, season of harvest, weather and soil conditions influence the content and composition of the active plant principles in the essential oil [72].

4.4. Morphological variation

We observed the variations in the morphological characters among the 18 *P. frutescens* accessions. Chinese accessions and Japanese accessions of *Perilla* distinguished by several morphological traits including number of lateral branches, number of nodes, number of panicles, number of pods per panicle, and the panicle length etc. Significant variations in the morphological differences in various accessions of *P. frutescens* were also reported by Hussain *et al.* [73], and Sa *et al.* [74]. These results indicate that the high morphological diversity among the different accessions of *P. frutescens* could perhaps be attributed to the inherited characters due to continued inbreeding. However, other studies indicated that domesticated plants become morphologically and physiologically divergent from their wild ancestors. These differences could also be due to the micro evolutionary changes in different environments by which the organisms respond adaptively to the local conditions [75].

The present study reports a comparative analysis of the composition and concentrations of volatile components in 18 accessions of *P. frutescens* collected from China and Japan using GC–MS method. We observed significant variations in the volatile oil composition between the Chinese and Japanese accessions. Perilla ketone was the most abundant component in all the accessions. The volatile oil contents in the Chinese and Japanese accessions were influenced by harvesting time. Most of the accessions possessed higher essential oil content before the flowering time. The results showed the existence of different chemotypes that distinguish the *P. frutescens* oil from the accessions of different origin. The 18 *P. frutescens* accessions were classified into seven chemotypes based on the main components of the volatile oils in their leaves; the chemotypes were named perilla ketone (PK) type (%), perilla ketone, myristicin (PM) type (5%), perilla ketone, unknown (PU) type (%), perilla ketone, beta-caryophyllene, myristicine (PB) type (%), perilla ketone, myristicin, unknown (PMU) type (%), perilla ketone, elemicine, myristicin, beta-caryophyllene (PEMB) type (%), and perilla ketone, limonene, beta-cryophyllene, myristicin (L) type (%). The intra- and inter-population variability in terms of morphological traits and the content of the volatile compounds were also observed, which revealed polymorphism in the *Perilla* accessions from China and Japan. The present study on the volatile components of *Perilla* accessions may be utilized as a significant tool in finding new promising sources for natural antioxidants, and for the identification and evaluation of the oil-quality in flavor and pharmaceutical industries.

Conflicts of interest statement

The authors declare that they have no conflicts of interest.

Acknowledgments

This work was supported by funding from the Konkuk University BrainPool.

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