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Original Article

Compositional variability in volatiles from different plant organs of *Perilla frutescens* L. cultivated in Uttarakhand (India)

Nirpendra K. Chauhan [a], *Sher Singh* [a], *S. Zafar Haider* [a,*], *Hema Lohani* [a], *Babu Lal Kushwaha* [b]

[a] *Centre for Aromatic Plants (CAP), Industrial Estate, Selaqui 248197, Dehradun, Uttarakhand, India*
[b] *R.M.P. (P.G.) College, Gurukul Narsan, Haridwar, Uttarakhand, India*

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ABSTRACT

Background: This investigation aims to determine the variation in essential oil compositions in plant parts (whole plant, leaves, spikes and husk) of Perilla (*Perilla frutescens* L.) at 3 different sowing times and also to ensure the suitability of this crop in Doon valley climatic conditions for commercial cultivation.
Methods: During the course of study, seeds were sown at 25 days intervals in the month of May, June and July with 60 × 30 cm spacing. The essential oils from all the samples were hydro-distilled using a Clevenger-type apparatus and analyzed by GC/MS.
Results and discussion: In all the oils, perilla ketone (43.49–90.28%) and 1-methyl-2-methylene trans-decalin (4.49–36.11%) were found to be the most abundant compounds.
Conclusion: On the basis of comparative composition, D_1 stage (seeds sown on 20th May) showed better results as compared with D_2 (seeds sown on 15th June) and D_3 (seeds sown on 10th July).

1. Introduction

Perilla (*Perilla frutescens* L.), commonly known as "Bhanjira" in India, belonging to Lamiaceae family, is an underutilized crop of Indian Himalayas with potential utility in agriculture. It is cultivated as a traditional crop in Asia for its medicinal and nutritional value due to the bio-actives, fatty acid constituents and essential oil. In India, the plant is grown in Himalayas but there is no organized cultivation of the herb.[1] In Uttarakhand, villagers generally used seeds and leaves of the plants for the preparation of 'food chutney' and flavoring curry materials.[2] Literature survey has shown different chemotypes in the essential oil of *P. frutescens* and other Perilla species such as perilla ketone,[3,4] perilla ketone-isoegomaketone,[5] perilla ketone-egomaketone,[6] perilladehyde,[6,7] limonene-piperitone,[8] β-caryophyllene,[9,10] caryophyllene oxide[4] and rosefuran.[11] Perilla also showed high antioxidant and anti-inflammatory activity.[12–15]

Keeping in view, that *Perilla* crop can play an important role in national economy both as raw material, essential oil and fatty oil for pharmaceutical industry and also as a foreign exchange earner through export, we started to study on quality and crop improvement of this plant.[3,16] Therefore, this investigation aims to determine the compositional variability in the essential oils of plant organs (whole plant, leaves, spikes and husk) at 3 different sowing times and also to ensure

* *Corresponding author. Tel./fax:* +91 0135 2698305.
E-mail address: zafarhaider.1@rediffmail.com (S. Zafar Haider).
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the suitability of this crop in Doon valley climatic conditions of Uttarakhand for commercial cultivation.

2. Materials and methods

2.1. Experimental procedure

The study was performed in Doon valley (Uttarakhand) climatic conditions at the experimental field of Centre for Aromatic Plants (CAP), Selaqui, Dehradun located at 30° 9′ N latitude and 78° 2′ E longitude at 680 m above sea level. The seeds were sown at 25 days intervals on 20th May, 15th June and 10th July, 2010 in the experimental plots with 60 × 30 cm spacing. All agronomical management practices were performed as needed. The samples of leaves and whole plants were collected at pre flowering and full flowering stages.

2.2. Isolation of essential oils

Samples of whole plant, leaves, spikes and husk were subjected to hydro-distillation for 4 h using a Clevenger-type apparatus to produce oil. The oils were dried over anhydrous sodium sulphate and stored in sealed vial at low temperature before analysis.

2.3. Gas chromatography/mass spectrometry (GC/MS)

GC/MS analyzes were performed with a Perkin Elmer *Clarus 500* gas chromatograph equipped with a split/splitless injector (split ratio 50:1) data handling system. The column was Rtx®-5 capillary columns (60 m × 0.32 mm, 0.25 μm film thickness). Helium (He) was the carrier gas at a flow rate 1.0 ml/min. The GC was interfaced with (Perkin Elmer *Clarus 500*) mass detector operating in the EI^+ mode. The mass spectra were generally recorded over 40–500 amu that revealed the total ion current (TIC) chromatograms. Temperature program was used as follows: initial temperature of 60 °C (hold: 2 min) programmed at a rate of 3 °C/min to a final temperature of 220 °C (hold: 5 min). The temperatures of the injector, transfer line and ion source were maintained at 210 °C, 210 °C and 200 °C, respectively.

The components of the oils were identified by comparison of their mass spectra with those of commercial libraries (*NIST/Pfleger/Wiley*) or with authentic compounds and confirmed by comparison of their retention indices either with those of authentic compounds or with data published in literature.[17]

3. Results

The average oil content in different plant parts were obtained as 0.06–0.10% (whole plant), 0.10–0.14% (leaves), 0.13–0.23% (spike) and 0.10–0.13% (husk) during different sowing times. The highest oil content obtained in all the spike samples at different sowing times, which ranged from 0.16 to 0.23% (D_1), 0.15–0.20% (D_2) and 0.13–0.18% (D_3), whereas lowest oil yield obtained in whole plant, varied between 0.06 and 0.09% (D_1), 0.06–0.10% (D_2 and D_3). Table 1 shows the identified constituents and their relative content in the essential oils obtained from whole plant, leaves, spikes and husk of *Perilla frutescens* at 3 sowing times, D_1-seeds sown on 20th May, D_2-seeds sown on 15th June and D_3-seeds sown on 10th July.

D_1 stage: The major compound was found as perilla ketone (52.34–90.28%) followed by 1-methyl-2-methylene trans-decalin (4.49–32.98%). The percentage of perilla ketone, the first major compound in all the oils, was found maximum in spikes (90.28%) followed by husk (64.54%), leaves (54.56%) and whole plant (52.34%). 1-Methyl-2-methylene trans-decalin was higher in leaves oil (32.98%) and lower in spikes essential oil (4.49%). The amount of trans-caryophyllene was higher in the essential oil obtained from whole plant (8.54%) and also in husk (5.08%).

D_2 stage: In this stage of sowing the major compounds were same as in D_1, but the amount of perilla ketone decreased in all the plant parts. In whole plant and leaves oils it ranged from (43.49–47.73%), whereas in spikes and husk,

Table 1 – Identified compounds in the essential oils of *Perilla frutescens* during different sowing times.

Components	D_1 stage				D_2 stage				D_3 stage			
	WP	L	S	H	WP	L	S	H	WP	L	S	H
1-Octen-3-ol	1.10	0.40	0.22	–	0.33	0.35	0.50	–	0.43	0.16	0.78	–
Linalool	2.83	0.64	0.22	2.08	2.44	0.67	1.31	2.82	4.09	1.16	4.67	3.18
Valeric acid, pent-2-en-4-ynyl ester	1.27	1.13	0.03	0.84	0.81	0.96	0.64	0.75	0.95	0.48	0.84	0.76
1H-Indene, 1-ethylidene octa hydro-7a-methyl	3.58	5.58	–	4.58	2.59	5.58	1.73	3.60	3.19	6.84	1.54	3.64
Perilla ketone	52.34	54.56	90.28	64.54	43.49	47.73	60.06	56.80	51.17	58.94	49.31	61.12
Imidazole, 4-trifluoroacetyl	2.28	2.54	–	1.18	1.38	2.30	–	0.95	2.23	2.04	0.92	0.92
1-Methyl-2-methylene trans-decalin	21.16	32.98	4.49	16.39	16.69	36.11	9.08	12.20	14.85	22.81	6.47	13.32
α-Cubebene	–	–	–	–	–	–	0.51	0.13		–	0.24	0.09
β-Bourbobene	–	–	–	–	–	–	0.70	0.37		–	0.39	0.25
Trans-caryophyllene	8.54	1.58	0.02	5.08	15.85	2.55	6.32	9.70	9.74	1.89	16.44	7.08
Trans α-bergamotene	2.40	0.31	–	1.20	4.49	0.81	4.41	3.35	3.31	0.57	5.49	2.69
α-Humulene	0.80	–	–	0.45	1.77	0.13	1.37	0.99	1.09	0.22	1.75	0.71
Germacrene-D	1.33	–	–	0.56	1.90	0.29	1.10	1.70	1.09	–	3.17	0.10
Nerolidol	0.33	–	–	0.22	0.50	0.31	1.02	0.48	0.36	0.30	0.51	0.39
Caryophyllene oxide	0.53	0.11	–	1.72	2.14	1.22	1.45	2.45	3.36	2.80	2.21	2.34
Total identified (%)	98.49	99.83	95.26	98.84	94.38	99.01	90.2	96.29	95.86	98.21	94.73	96.59

Date of sowing – D_1 = 20th May; D_2 = 15th June; D_3 = 10th July; WP: Whole Plant; L: Leaves; S: Spikes; H: Husk.

the compound constituted 60.06% and 56.80%, respectively. The amount of 1-methyl-2-methylene trans-decalin was also decreased in whole plant (16.69%) and husk (12.20%), while increased in leaves (36.11%) and spikes (9.08%) as compared to D_1. In D_2 stage, the amount of trans-caryophyllene was increased which ranged from (2.55–15.85%).

D_3 stage: In D_3 stage of seed sowing the percentage of first major compound (perilla ketone) was found 51.17%, 58.94%, 49.31% and 61.12% in whole plant, leaves, spikes and husk, respectively. The average amount of 1-methyl-2-methylene trans-decalin was also found lesser as compared with D_1 and D_2. trans-Caryophyllene was detected in appreciable amounts in D_3 sowing stage (1.89–16.44%).

Earlier studies on the essential oils of *P. frutescens* and other species[3–11] revealed that perilla ketone and perillaldehyde are the two major chemotypes which were reported in different countries, though in some studies egomaketone, limonene, piperitone, β-caryophyllene and rosefuran were also reported as the major components in perilla species.

4. Discussion

On the basis of comparative composition of the essential oils of whole plant, leaves, spikes and husk at three sowing times, it was found that the amounts of first 2 major compounds, especially perilla ketone were higher in D_1 as compared with D_2 and D_3 sowing times. Although all the samples were found qualitatively similar yet quantitative variations were occurred in their compositions. The other components which were present in remarkable amounts were linalool; 1H-indene, 1-ethylidene octa hydro-7a-methyl; imidazole, 4-trifluoroacetyl; trans-α-bergamotene and caryophyllene oxide. Perilla ketone was also found in appreciable amounts in two previous studies on the essential oils of *P. frutescens*, which constituted 35.6%[5] and 55.6%[6] of the oil, but in present investigation, the samples from all the stages were found rich in the name of perilla ketone.

Conflicts of interest

All authors have none to declare.

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REFERENCES

1. Anonymous. *The Wealth of India-Raw Materials*. vol. 7. New Delhi: CSIR; 2001. 311–313.
2. Miyuki N, Lee Ju K, Ohmi O. Asian perilla crops and their weedy forms: their cultivation, utilization and genetic relationships. *Econ Bot*. 2003;57:245–253.
3. Chauhan NK, Singh S, Haider SZ, Lohani H, Kushwaha BL. Quality evaluation of *Perilla frutescens* L. cultivated in Doon Valley climatic conditions of Uttarakhand: a source of perillaketone. *Natl Acad Sci Lett*. 2012;35:91–93.
4. Ito M, Toyoda M, Honda G. Essential oil composition of hybrids and amphidiploids of Japanese wild. *Perilla Nat Med*. 1999;53:118–122.
5. Baser KHC, Demirci B, Donmez A. Composition of the essential oil of *P. frutescens* L. Britton from Turkey. *Flav Frag J*. 2003;18:122–123.
6. Bumblauskiene L, Jakstas V, Janulis V, Mazdzieriene R, Ragazinskiene O. Preliminary analysis on essential oil composition of *Perilla* L. cultivated in Lithuania. *Acta Polo Pharma*. 2009;66:409–413.
7. Omer EA, Khattab ME, Ibrahim ME. First cultivation trial of *P. frutescens* L. in Egypt. *Flav Fragr J*. 1998;13:221–225.
8. Dung NX, Moi LD, Cu LD, Leclercq PA. Essential oil constituent from the aerial parts of *Perilla frutescens* (L.) Britton. *J Essent Oil Res*. 1995;7:429–432.
9. Chen G, Zhang J, Guo Y. Analysis of volatile components of fresh *P. frutescens* L. Britt. var. *acuta* (Thunb.) Kudo by head space GC/MS. *J Essent Oil Res*. 2004;16:435–436.
10. Ito M, Toyoda M, Nakano Y, Kiuchi F, Honda G. Chemical composition of the essential oils from *Perilla setoyensis*, A new species of wild *Perilla* in Japan. *J Essent Oil Res*. 1999;11:669–672.
11. Mishra N, Husain A. The essential oil of *Perilla ocimoides*: a rich source of Rosefuran. *Planta Med*. 1987;53:379–380.
12. Chou H, Kuo J, Lin E. Comparative antioxidant properties of water extracts from different parts of beefsteak plant (*Perilla frutescens*). *J Food Drug Anal*. 2009;17:489–496.
13. Oh HA, Park CS, Ahn HJ, Park YS, Kim HM. Effect of *Perilla frutescens* var. acuta Kudo and rosmarinic acid on allergic inflammatory reactions. *Exp Biol Med*. 2011;236:99–106.
14. Park YD, Jin CH, Choi DS, Byun MW, Jeong IY. Biological evaluation of isoegomaketone isolated from *Perilla frutescens* and its synthetic derivatives as anti-inflammatory agents. *Arch Pharm Res*. 2011;34:1277–1282.
15. Saita E, Kishimoto Y, Tani M, et al. Antioxidant activities of *Perilla frutescens* against low-density lipoprotein oxidation in vitro and in human subjects. *J Oleo Sci*. 2012;61:113–120.
16. Chauhan N, Haider SZ, Singh S, Lohani H. Popularization of Perilla seed oil as a functional food source. *Curr Sci*. 2013;104(3):284–285.
17. Adams RP. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*. 4th ed. Carol Stream, IL: Allured Business Media; 2009.