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<SHORT COMMUNICATION>

Variations in Volatile Compounds from *Elshoizia cilliata*

Jong-Hee Kim* and Dae-Ho Jung
Department of Biological Sciences, Kyungnam University, Kyungnam, 631-701, Korea

We used gas chromatography-mass spectrometry (GC-MS) to analyze total yields and relative composition of the volatile compounds from leaf extracts of *Elshoizia cilliata*. This species contains 22 compounds. The major constituents of its essential oils are dihydrotagetone (62.7%), β-caryophellene (4.96%), germacrene-d (4.03%), and α-humulene (1.34%). Compounds in these leaf extracts are remarkably high in dihydrotagetones (40.5 to 81.6%). The total amount of monoterpenoids is 3.17 to 7.03 times greater than that of sesquiterpenoids, and is highly correlated with the level of dihydrotagetone ($r = 0.97$). Seasonal variations are significantly different only for dihydrotagetone ($p < 0.0005$), but not for the yields of other volatile compounds.

Key words: *Elshoizia cilliata*, GC-MS, monoterpenoids, sesquiterpenoids

The genus *Elshoizia*, a member of the Labiatae family, normally bears purple flowers (Fig. 1), although white, pink, and red-purple forms also exist. Of its 20 species of herbaceous perennials and sub-shrubs, 5 occur in Korea (Lee, 1996). Most have been collected from the wild and possess volatile compounds (Hilton et al., 1995).

The subject of numerous biological and chemical studies (Lange and Croteau, 1999; Skoula et al., 1999) have been studied, *E. cilliata* is known in Korea as HyangYoo, or flavor oil in Chinese characters. However, although each species contains essential oils, they have not been used for commercial production because their strong aroma does not meet marketing requirements. Such mixtures of volatile essential oils lend this characteristic odor to the plant foliage. Their terpenes have been widely used in studies of taxonomy (Skoula et al., 1999; Gross et al., 2002), phylogeny (Harborne and Tomas-Barberan (1991; Thompson et al., 2003), microbial activity (Cosentino et al., 1999; Vokou et al., 2002), and ecology (Zygadlo et al., 1996; Dudai et al., 1999; Palá-Paúl et al., 2001). Large fluctuations have been found in the concentrations of constituent compounds as a function of plant organ type as well as variations in season, location, and individual plant differences (Perry et al., 1999; Ahn et al., 2003). Moreover, the yield and composition of essential oils is affected by crop maturity at harvest, environmental conditions, and distillation practices. Therefore, the primarily objective in this study was to determine which particular volatile compound is most responsible for the distinctive odor produced by *E. cilliata*.

*Corresponding author; fax +82-55-244-6504
e-mail biokim@kyungnam.ac.kr

MATERIALS AND METHODS

Leaves of *Elshoizia cilliata* were gathered in 2002 from three environmentally similar sites at Mt. Muhak in Korea. The collections occurred at approximately two-week intervals during the maturing period of this species, with five plants at a similar development stage being sampled at each site. The leaves were sealed in plastic bags for transport. In the laboratory, 3-gram samples were ground with pure sand, then treated with n-pentane and 1 ml of 1% tetradecane as an internal standard. The extracts were filtered with sodium sulfate and concentrated by evaporation with a gentle stream of nitrogen gas (Kim and Langenheim, 1994).

Samples were assessed by gas chromatography-mass spectrometry (GC-MS; Hewlett Packard 5890, USA), using a 30-m-long HP5 capillary column (0.25 mm i.d.) with a flame ionization detector. Helium served as the carrier gas. For the terpene analysis, the temperature program included an initial 37°C for 5 min, followed by an increase to 180°C (rate of 5°C min^{-1}), then to a final temperature of 320°C (20°C min^{-1}). One μL of the final extract was used for the analysis. Individual terpenes were identified by comparing the GC-MS results with retention times (based on references) as well as with spectral data from the internal Wiley instrument library. The concentrations of peaks at selected retention times were estimated from the peak area, using the internal standard curve for tetradecane. Seasonal

Figure 1. *Elshoizia cilliata* in July at Mt. Muhak.

variations in monoterpenoid yields were examined with ANOVA, using the MS 2000 Excel program.

RESULTS AND DISCUSSION

Although we determined and identified many volatile compounds from *Elshoizia cilliata* by GC-MS, some were present only in small or trace amounts. Therefore, the

Table 1. Percentage composition of major essential oils in leaf extracts of *Elshoizia cilliata*. Only compounds found at levels >0.05% are included; values in bold type represent constituents of >1.00%.

Compound	Retention time	Percentage (%)
Monoterpenoids		
Trans-2-hexenal	7.12	0.979
α-Pinene	9.61	0.223
β-Pinene	11.08	0.626
β-Myrcene	11.66	0.228
β-Ocimene	13.26	0.864
Dihydrotagetone	13.88	62.732
1-Undecanol	14.55	0.791
Linanool	15.39	0.171
Alloocimeme	16.23	0.508
1,4-Pentadiene	16.81	0.125
Artemisia triene	22.45	0.344
Sesquiterpenoids		
β-Caryophyllene	24.77	4.955
β-Cubebene	25.01	0.155
GERMACRENE-D	25.42	0.356
α-Humulene	25.65	1.339
β-Farnesene	25.91	0.062
germacrene-d	26.39	4.043
α-Farnesene	26.63	0.438
E,E-α-Farnesene	26.97	0.997
γ-Cadinene	27.22	0.071
δ-Cadinene	27.44	0.179
1,6-Germacradien-5-ol	28.81	0.379

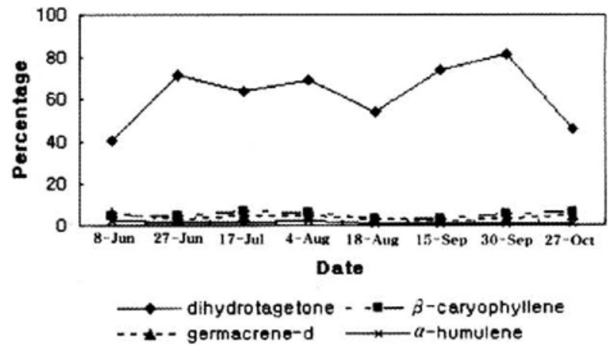

Figure 2. Time-course variations in relative contents of essential oils in leaf extracts of *E. cilliata*. Seasonal fluctuations were significantly different for dihydrotagetone ($p < 0.0005$), but not for any other compounds.

constituent yields, based on sample fresh weights, of only 22 components (each comprising >0.5% of the total) are included in Table 1. Of these, 11 were identified as monoterpenoids; 11, as sesquiterpenoids. Dihydrotagetone (62.73%), β-caryophellene (4.96%), germacrene-d (4.04%) and α-humulene (1.34%) were present in the greatest amounts, with the relative yields of monoterpenoids being very high.

The leaves of *E. cilliata* have a very strong odor throughout the growing season. We suggest that this is the result of the high level of dihydrotagetone, an observation also supported by the varying concentrations of this compound measured in other species (Perry et al., 1999). In our study, the amount of dihydrotagetone fluctuated significantly during the season, ranging from 40.6 to 81.6% ($F = 9.49$, $p < 0.0001$; Fig. 2). However, we found no significant differences in seasonal variations for the other volatile compounds examined here (for example, β-caryophyllene: $F = 1.59$, $p > 0.05$; germacrene-d: $F = 1.72$, $p > 0.05$; α-humulene: $F = 1.42$, $p > 0.05$). The excretion of dihydrotagetone began in early June, increasing to a higher level that was maintained from June to late September, in October. Ahn et al. (2003) have also reported that the major aromatic essential oils found in Hwangchil lacquer vary both seasonally and individually, with concentrations holding constant during the growing season, but, again, greatly decreasing in October.

The total amount of essential oils that consisted of monoterpenoids and sesquiterpenoids is shown in Figure 3. Levels of monoterpenoids were 3.17 to 7.03 times greater than for sesquiterpenoids, and were highly correlated with the level of dihydrotagetone ($r = 0.97$). However, variations in total sesquiterpenoids were not correlated to the same degree ($r = 0.60$). Therefore, because the essential oils in this species are dominated

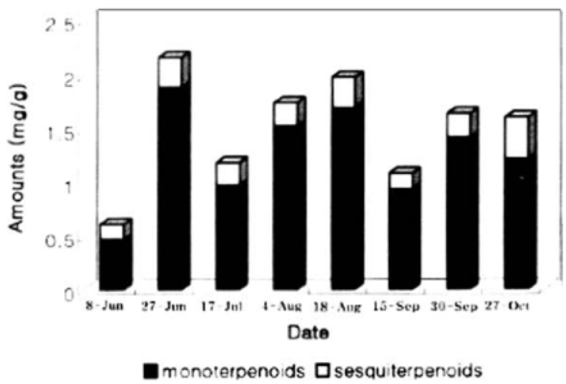

Figure 3. Composition of monoterpenes and sesquiterpenes in leaf extracts of *E. cilliata*. Seasonal variations were significantly different for total monoterpenoids ($F = 3.11$, $p < 0.05$) and sesquiterpenoids ($F = 5.56$, $p < 0.005$).

by the dihydrotagetone component (i.e., 40.5 to 81.6% of the total), any significant seasonal differences in compound yields depend on the overall production of dihydrotagetone. Based on these results, we suggest that, if the essential oils of *E. cilliata* are to be used for commercial production, the effects of this particular component on the final product should be further evaluated. Moreover, its generally sweet fragrance could make this species an important source of additive material for food, bath water, or other aromatic purposes.

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