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Seasonal variations in the content and composition of essential oil from *Zanthoxylum piperitum*

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Abstract

Seasonal variations in the profile and concentrations of essential oil in *Zanthoxylum piperitum* were investigated by gas chromatography-mass spectrometry. Seasonal changes in the percentages of the main constituents of the essential oil of both leaves and fruits from *Z. piperitum* varied. Variations in essential oil yield and the amount of monoterpenes and sesquiterpenes in leaves and fruits at different developmental stages were significant. The characteristic content of essential oil in leaves was determined mainly due to the content of monoterpenes, and that in fruits was determined largely due to the sesquiterpenes. Twenty-nine compounds in the oil from *Z. piperitum* leaves were detected; the major compounds were β-phellandrene (26.90%), citronella (15.32%), β-myrcene (3.24%), α-pinene (2.79%), trans-caryophyllene (2.66%), and fanesyl acetate (2.30%). The highest yield of oil (43.89%) in *Z. piperitum* leaves was obtained in May but decreased gradually beginning in June. The yield of essential oil from *Z. piperitum* leaves during early periods was higher than that during later periods and usually decreased from early maturation stages to subsequent stages. However, in contrast to leaves, the oil yield in *Z. piperitum* fruit increased in June, and oil yield later in the season was higher than that earlier in the season. These results indicate that the essential oil produced from *Z. piperitum* leaves at the early developmental stages was stored in leaves, and might be transferred to fruit at the final developmental stages.

Key words: β-phellandrene, essential oil, gas chromatography-mass spectrometry, seasonal variation, *Zanthoxylum piperitum*

INTRODUCTION

Zanthoxylum piperitum belongs to the Rutaceae family, is distributed in northeast Asia (Korea, China, and Japan), and is commonly used in Korean dishes as a spice and as a traditional medicinal plant. In particular, the *Z. piperitum* fruit pericarp has been used as an anthelmintic and for treating disorders of the digestive organs in Korea (Epple et al. 2001, Setzer et al. 2005, Kim et al. 2006b, Lee and Lim 2008). The extracts from the fruits and leaves of *Z. piperitum* are chemically complex (Yasuda et al. 1982, Kashiwada et al. 1997, Hur et al. 2003). This plant produce an essential oil, and many of its constituents are volatile. The essential oil is mainly comprised of monoterpenes and sesquiterpenes, which characterize the aroma and belong to a structurally diverse group of natural products known as isoprenes. Mixtures of volatile essential oils lend a characteristic odor to plant foliage. Large variations in the concentrations of constituent compounds are found due to plant parts, season, location, and individual differences (Jiang and Kubota 2004). Several studies (Pfänder and Frohne 1987, Kamsuk et al. 2007, Chang and Kim 2008, Hwang and Kim 2012) have reported the composition of *Z. piperitum* essential oil. In addition to

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these compounds, a number of non-volatile alkylamides (Hatano et al. 2004, Jang et al. 2008, Machmudah et al. 2009) and phenolic compounds (Chon et al. 2009, Jeong et al. 2011) are present. At least one of the alkylamides, sanshool, causes a strong tingling sensation, similar to a mild electric shock, as well as cooling, when applied to the human tongue (Bryant and Mezine 1999). Pfänder and Frohne (1987) determined that the main constituents of the essential oil from *Z. piperitum* are limone, α-terpineol, linalool, citral, citronellal, cineol dipentene, and geraniol. Chang and Kim (2008) determined that the essential oil of Korean *Z. piperitum* consists of limonene, geranyl acetate, cryptone, citronellal, cuminal, and phellandral, whereas the most abundant compound in Chinese *Z. piperitum* is β-phellandrene, followed by sabinene, terpinen-4-ol, and linalool. Hwang and Kim (2012) also reported that the percentages of *Z. piperitum* essential oil constituents were *dl*-limonene (18%), geranyl acetate (15.3%), cryptone (8.5%), citronellial (7.1%), cuminal (6.2%), and phellandral (5.2%). Chung (2005) found that the largest constituent components of *Z. piperitum* oil were hydrocarbons and alcohols, primarily *dl*-limonene (37.9%), sabinene (13.3%), and β-myrcene (7.17%). According to the essential oil composition in the literature, *Z. piperitum* can be divided into two groups such as limonene-rich and β-phellandrene rich, as different results can occur depending on sampling season and location. Thus, a more complete study of the *Z. piperitum* essential oil profile in Korea is needed, as well as seasonal variations in its composition.

The first aim of this study was to determine seasonal variations in the yield and composition of essential oil from *Z. piperitum* leaves. The second aim was to elucidate the composition of essential oil in each different organ of *Z. piperitum* (i.e., leaves, green fruit, and ripe fruit) using gas chromatography-mass spectrometry (GC-MS).

MATERIALS AND METHODS

Locations and oil extraction

Z. piperitum leaves were collected from three sites; an abandoned area on Mt. Muhak (35°11′15″ N and 128°32′30″ E, 665 m elevation, 1,400.3 mm precipitation, temperature range 9.8-27.7°C) from April 5-October 30, 2010. Three plants in a similar development stage were collected at each site at approximate 2-week intervals. Plant samples were sealed in plastic bags and transported to the laboratory. All materials (leaves, green fruit, and fruit) were removed from each plant, and 3 g samples were immediately ground with pure sand and extracted with n-pentane and 1 mL of 1% tetradecane as an internal standard. The plant extracts were filtered with sodium sulfate and concentrated by evaporation with a gentle stream of nitrogen gas (Kim et al. 2006a).

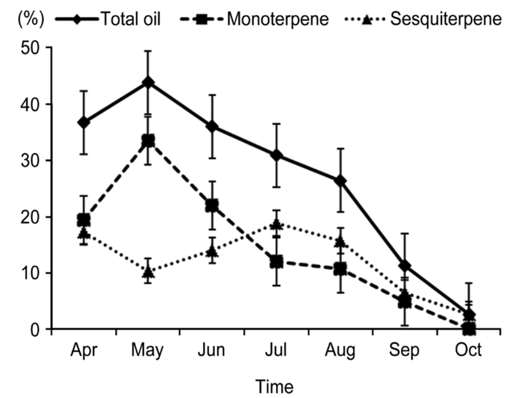

Fig. 1. Essential oil, monoterpenes, and sesquiterpenes yields in *Zanthoxylum piperitum* leaves during differential growing periods. Percentage means peak area from gas chromatography-mass spectrometry.

Essential oil analysis

Samples were analyzed by GC-MS (Hewlett Packard 5890; Hewlett Packard, Dallas, TX, USA) using a 30 m long HP5 (i.d. 0.25 mm, flame ionization detector) capillary column. Helium gas was used as the carrier gas. The oven temperature for terpene was initially 37°C for 5 min, increased to 180°C at a rate of 5°C per min, then increased by 20°C per min until a final temperature of 320°C was reached. One μL of the extract was injected for the GC-MS analysis.

Individual terpenes were identified by comparison with the internal spectral library of the instrument (Wiley library ver. 7.0) and retention times based on references. The peak concentrations at selected retention times were estimated from the peak area using the internal standard curve of tetradecane. All compounds were measured in triplicate.

RESULTS AND DISCUSSION

Variation in oil yield

The amounts of monoterpenes and sesquiterpenes measured at different developmental stages were significantly different ($P < 0.05$) (Fig. 1). Maximum oil content

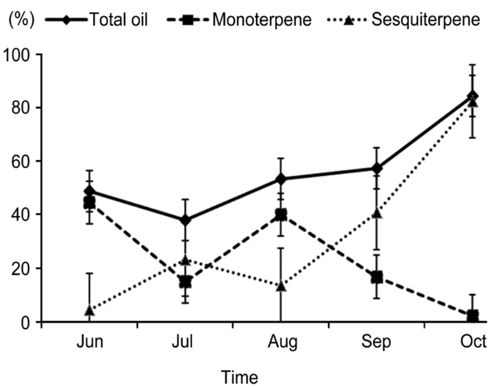

Fig. 2. Essential oil, monoterpenes, and sesquiterpenes in fruits of *Zanthoxylum piperitum*. Samples of June and July were green fruits, and others were ripe fruits. Percentage means peak area from gas chromatography-mass spectrometry.

(43.89%) in *Z. piperitum* leaves was obtained in May and then decreased gradually beginning in June. The highest content of monoterpenes occurred in May, and decreased starting in June. Therefore, the characteristic content of the essential oil can be determined mainly based on the content of monoterpenes. The monoterpenes in the essential oil from three sampling seasons (April, May, and June) were abundant as well as the content of sesquiterpenes obtained from leaves collected in July, August, and September. Previous studies on essential oil content of some plants such as coriander (Telci et al. 2006) and dill (Callan et al. 2007) confirmed that the essential oil yield during early periods was higher than that during later periods and usually decreased from early maturation stages to subsequent stages. These results are similar to the results of the present study.

The yields and composition of essential oils from *Z. piperitum* fruit varied with maturation stage (Fig. 2). In contrast to the essential oil in *Z. piperitum* leaves, the yield of essential oil in *Z. piperitum* fruit increased beginning in June, when fruits were unripe. The highest yield of fruit essential oil was detected at October, and that was determined largely due to the content of sesquiterpenes. As shown in Fig. 2, the essential oil composition in *Z. piperitum* fruit was dominated by the sesquiterpene fraction, but the amounts were quite different; unripe fruits were rich in monoterpenes, whereas ripe fruits were rich in sesquiterpenes.

These results (Figs. 1 and 2) seemed to indicate that the yield of essential oil in leaves of *Z. piperitum* moved to fruit, and that the composition of the essential oil changed from rich in monoterpenes at the early stage to rich in sesquiterpenes at the maturation stages. Telci et al. (2009) reported similar findings in sweet fennel (*Foeniculum vulgare* Mill.), in which maximum monoterpene content was obtained during the prematuration periods. These changes in essential oil composition could result from chemical modifications of terpenes including genetic features and the environment such as temperature and light. Transformation of mono- and sesquiterpenes hydrocarbons may result from their bioconversion by some microorganisms (Miyazawa and Wada 2000) and enzymatic transformation. However, these transformations are poorly understood, so the differences we found in oil composition provide only a partial explanation based on studies of individual terpenes. These results agree with those of previous studies (Silvestre et al. 1997, Rodilla et al. 2008, Mediouni et al. 2012), in which the essential oils obtained from aromatic plants showed qualitative and quantitative differences in chemical composition. Those studies reported that the variations in essential oil composition and yield of some plants such as *Eucalyptus golbulus* Labill (Silvestre et al. 1997), *Lippia citriodora* (Argyropoulou et al. 2007), and *Laurus novocanariensis* (Rodilla et al. 2008) were due to seasonal and leaf age influences. Moreover, seasonal influences were detected in the essential oil yield with higher values in May and lower values in December.

Constituents of essential oil

The chemical compositions of the essential oil from *Z. piperitum* leaves during the developmental stages (early April to October) are summarized in Table 1. Twenty-nine compounds were identified, representing 97.8% of the total oil. β-phellandrene (26.90 ± 8.43%), citronella (15.32 ± 6.77%), β-myrcene (3.24 ± 0.97%), α-pinene (2.79 ± 0.99%), and trans-caryophyllene (2.66 ± 1.73%) were the main components, constituting 50.91% of the total oil, followed by citronellyl acetate (1.31 ± 0.71%) and farnesyl acetate (2.30 ± 2.44%). Irrespective of the plant's growth phase, β-phellandrene, α-pinene, citronella, and β-myrcene were the dominant compounds in the essential oil of *Z. piperitum* leaves. These results agreed with those of previous reports on *Z. piperitum* leaves (Jiang and Kubota 2004, Chang and Kim 2008). In our observations, the percentages of the main components changed between the seasons studied. In May, β-phellandrene had a percentage of 37.53% and that of citronella was 25.12%, whereas in October, these values were 19.84 and 11.29%, respectively. However, some authors (Chung 2005, Hwang and Kim 2012), without stating the plant developmental stage, reported that *dl*-limonene is the major component in the essential oil of *Z. piperitum* leaves. Sampling site and season as well as GC-MS conditions can lead to different essential oil composition results. In addition, Batish et al. (2006) reported that there are many constraints such as inconsistency in the amount of essential oil, which vary with season, changing climate, species and even leaf age. Nevertheless, the chemical composition of aromatic plants may be influenced by seasonal changes, and mostly quantitative rather than qualitative variations in oil composition are observed (Kim 1997, Kamatou et al. 2008). Furthermore, essential oil composition depends upon internal factors affecting the plant such as genetic factors (Telci et al. 2006).

Few reports are available on the seasonal variation of essential oil composition in *Z. piperitum* in Korea. Moreover, if samples for previous studies were obtained from market places, there is no evidence to classify them as *Z. piperitum*. Because the shapes of the leaves and fruit of *Z. piperitum* are very similar to those of *Z. schinifolium*, most people use the plants interchangeably. We found that *dl*-limonene was the main component of *Z. schinifolium* essential oil (data not shown). If extracting techniques and GC-MS conditions were not optimized, identification of β-phellandrene and limonene could be confused, because their structures are very similar

Table 1. Variations in the essential oil (%) in *Zanthoxylum piperitum* leaves during different developmental stages

Compound	R.T.	Apr	May	Jun	Jul	Aug	Sep	Oct
Diethyl sidulfide		0.10	0.05	-	-	-	-	-
α-Pinene	**8.79**	**1.32**	**4.01**	**4.02**	**2.40**	**1.49**	**1.46**	**2.82**
Sabinene	10.7	0.11	0.40	-	0.30	0.15	0.17	-
β-Myrcene	**11.37**	**1.54**	**4.51**	**3.60**	**2.17**	**2.15**	**2.37**	**2.65**
β-Phellandrene	**12.72**	**15.04**	**37.53**	**29.42**	**17.51**	**16.20**	**19.43**	**19.84**
1,8-cineole	12.96	-	-	-	1.15	0.26	-	1.85
1,3,6-Octatriene	13.54	**1.17**	**0.17**	**0.00**	**0.00**	**0.15**	**0.37**	-
α-Terpinolene	14.89	0.20	0.63	0.49	0.30	0.41	0.30	-
Linalool	15.65	t	0.20	-	-	0.82	0.64	-
Citronella	**17.21**	**1.81**	**25.12**	**21.32**	**13.33**	**10.72**	**13.54**	**11.29**
α-Terpineol	18.66	-	0.18	-	-	0.10	0.07	-
β-Citronellol	19.87	-	-	0.91	0.49	0.31	0.91	0.71
3-Cyclohexen-1-one	20.49	-	0.11	0.00	0.30	0.10	t	-
2-Undecanone	21.53	0.38	0.32	0.49	-	0.05	-	-
Dodecane	22.66	-	t	-	-	0.05	0.10	-
Camphene	23.11	0.14	0.24	0.32	0.26	0.05	0.30	-
Citronellyl acetate	**23.21**	**0.79**	**1.29**	**1.23**	**0.85**	**0.05**	**2.20**	**0.79**
Tridecane	23.58	0.30	0.25	0.32	0.30	0.10	2.40	0.62
Geranyl acetate	24.08	0.25	0.57	0.29	0.16	0.21	0.44	-
Trans-caryophyllene	**25.04**	**2.94**	**2.85**	**2.30**	**1.51**	**1.03**	**0.71**	**1.50**
α-Humulene	25.95	0.32	0.30	0.39	0.33	0.15	0.30	-
Germacrene-D	26.66	0.06	0.11	-	-	-	0.07	-
2-Tridecane	27.01	0.89	0.80	0.36	0.20	0.21	0.24	-
Nerolidol	28.85	0.08	0.34	0.49	0.36	-	-	-
Cyclohexanol	30.65	0.29	0.20	-	0.16	0.21	-	-
Tetradecanoic acid	31.51	0.88	0.46	0.29	0.16	0.21	0.20	-
2-Pentadecanone	31.89	1.45	0.84	0.55	0.30	0.21	-	
Farnesol	32.55	0.25	0.53	0.71	0.66	0.67	0.20	-
Farnesyl acetate	35.18	1.06	2.28	2.04	8.80	0.46	0.88	-
Others		18.67	15.68	30.47	48.00	63.50	52.63	57.93

R.T., retention time (in min). t: (<0.05%).
Percentage composition was computed from the gas chromatography peak area.
"Others" represents all compounds that emerged at an R.T. of 35.18.

Table 2. Major compounds (>0.5%) of the essential oils obtained from of *Zanthoxylum piperitum* fruit collected during different seasons (%)

Jun	Jul	Aug	Sep	Oct
dl-Limonene (0.68)	β-Myrcene (4.05)	Sabinene (0.54)	β-Myrcene (13.21)	β-Phellandrene (5.3)
Sabinene (0.6)	β-Phellandrene (35.21)	β-Myrcene (10.57)	β-Phellandrene (23.15)	Octadecanoic acid (54.32)
β-Myrcene (10.31)	1,2-Benzenedicarboxylic acid (17.38)	β-Phellandrene (49.96))	1,3,6-Octatriene (0.57)	
β-Phellandrene (75.24)		α-Terpinolene (2.03)	α-Terpinolene (0.63)	
α-Terpinolene (1.41)		Citronella (1.94)	Citronella (1.18)	
Citronella (1.0)		2-Cyclohexen-1-one (0.5)	1,2-Benzenedicarboxylic acid (8.34)	
β-Citronellol (0.6)		1,2-Benzenedicarboxylic acid (7.53)	Octadecanoic acid (32.13)	
		Octadecanoic acid (29.04)		

Percentage, peak area from gas chromatography-mass spectrometry.

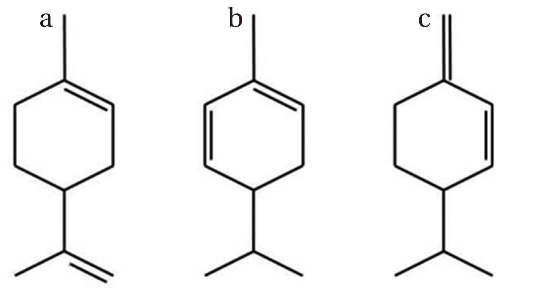

Fig. 3. The structure of limonene (a), α-phellandrene (b) and β-phellandrene (c).

(Fig. 3). Furthermore, a GC-MS capillary column can become easily contaminated, if it is used to analyze other compounds.

Analysis of the essential oil from fruit of *Z. piperitum* during the maturation period (late June to September) produced 33 compounds, which comprised 93.5% of the total oil. β-phellandrene (75.2-23.2%), β-myrcene (4.1-13.2%), α-terpinolene (0.6-2.3%), and citronella (1-1.94%) were the major components in *Z. piperitum* fruit, constituting 64.76% of the total oil. β-phellandrene (75-35%) was the most abundant compound found in immature fruit (green fruit in June and July); however, this compound decreased sharply in ripe fruit (5.3%). Compounds that emerged with a retention time of 35.18 (1, 2-benzenedicarboxylic acid and octadecanoic acid) were detected in major percentages in ripe *Z. piperitum* fruit (Table 2). In particular, β-phellandrene, β-myrcene, and α-terpinolene were higher in fruit than those in leaves of *Z. piperitum*. The sesquiterpene hydrocarbons, 1, 2-benzenedicarboxylic acid and octadecanoic acid occurred in fruits and were highest in October, but absent in leaves.

In conclusion, seasonal changes in the percentages of the main constituents in the essential oils of both leaves and fruit from *Z. piperitum* varied. Despite the available reports on *Z. piperitum* essential oil composition, no studies have evaluated the chemical composition of *Z. piperitum* during developmental and seasonal periods. Thus, it is not possible to compare data with those of previous studies. Nevertheless, recent studies performed on the essential oil composition of other plants, also indicated that their compositions were different. The essential oil composition in leaves and stems of *Schefflera heptaphylla* (Wang et al. 2012) and that of leaves and fruit from *Laurus novocanariensis* (Rodilla et al. 2008) were different. Msaada et al. (2009) and Telci et al. (2009) also reported that the main component of plant essential oil varies significantly during maturation.

ACKNOWLEDGMENTS

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