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RESEARCH NOTE

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Volatile Compounds of *Zanthoxylum piperitum* A.P. DC.

Mi-Sook Chung*

Department of General Education, Duksung Women's University, Seoul 132-714, Korea

Abstract Volatile compounds, isolated from *Chopi* (*Zanthoxylum piperitum* A.P. DC.) using steam distillation, were analyzed by gas chromatography/mass spectrometry-olfactometry (GC-MS-O). Forty-six volatile compounds, consisting of 12 hydrocarbons, 8 aldehydes, 5 esters, 12 alcohols, 4 ketones, 4 oxides and 1 acid, were tentatively identified from the essential oil of *Chopi*. Unidentified compounds constituted 7.2% of the total peak area. Limonene was the most abundant compound, followed by geranyl acetate, citronellal, cryptone and β-myrcene. In addition, aroma-active compounds, in particular citronellal and limonene, which are related to the citrus and *Chopi* flavors of *Chopi* essential oil, were detected. The aroma of *Chopi* essential oil had a score of 4.8 on the preference test (neither like nor dislike) and a score of 5.97 on the intensity test (slightly strong) using the 9-point hedonic scale.

Keywords: *Zanthoxylum piperitum* A.P. DC., *Chopi*, aroma-active compounds, preference

Introduction

Chopi (*Zanthoxylum piperitum* A.P. DC.) is a perennial, aromatic, medicinal plant belonging to the Rutaceae family. The fruits of *Chopi*, grow wild in Korea, and are used for medicinal purposes. The fruits of *Chopi*, having a peculiar citrus-like flavor, have also been used as a traditional spice to mask fishy flavors in Korea (1-2). *Sancho* (*Zanthoxylum schinifolium*) and *Andaliman* (*Zanthoxylum acanthopodium*) also belong to the Rutaceae family, and have the same characteristic citrus-like flavor. *Andaliman* is a traditional spice used in Northern Sumatra, Indonesia (3). In Korea, two different *Zanthoxylum* species, *Chopi* and *Sancho*, are used as spices and plant medicines (1-2). *Chopi* has both antioxidative and DPPH radical-scavenging activities, and has been widely used to treat several pathological conditions since ancient times (4).

Plant essential oils are highly enriched with terpenoids. Terpenoids exert inhibitory action against microorganisms by disrupting their membranes (5, 6). The essential oils of *Chopi* have been studied by several researchers (7, 8), and have been shown to consist mainly of terpenoids (geraniol, limonene, β-phellandrene, phellandral, myrcene, citronellal, linalool, citronellyl acetate and geranyl acetate). Kim et al. (9) identified 3-hydroxy-2-butanone, γ-butyrolactone and 2,3-butanedione as the major flavor compounds of pectin-elicited *Z. piperitum* suspension cultures. However, research on the volatile compounds of *Chopi* has been limited. The objectives of this study were to evaluate volatile and aroma-active compounds, and to rate the preference and intensity of the essential oils in dried *Chopi*.

Materials and Methods

Samples Dried *Chopi*, harvested from the area around Yeongju-si in Geongsangbuk-do, was purchased at Geongdong market in Seoul, Korea, and was packaged in 2003. The sample was kept at -70°C in air-tight bags until the analysis was performed. Lemongrass and grapefruit essential oils were obtained from Dr. Eberhardt GmbH, Eisenstadt, Australia.

Extraction of essential oil Dried *Chopi* was crushed for 30 sec in a blender (HMC-400T, Hanil Electronics, Seoul, Korea), and 100 g samples were extracted by steam distillation using a Clavenger-type apparatus (Hanil Labtech Ltd, Incheon, Korea) for 2 hr. The yield of the essential oils was 1.3% (v/w), which was dried over anhydrous sodium sulfate, and after filtration, was stored at 4°C until it was tested and analyzed.

Gas chromatography-mass spectrometry (GC-MS) The volatile compounds were analyzed using an Agilent 6890 gas chromatograph/5973 mass selective detector (Agilent Co., Palo Alto, CA, USA). One microliter of the essential oil was injected into a HP-5MS column (30 m length × 0.25 mm i.d. × 0.25 μm film thickness; Agilent Co., Palo Alto, CA, USA) using a microsyringe. Helium was used as a carrier gas at a constant flow rate of 1.0 mL/min. The oven temperature was held constant at 40°C for 5 min, then increased to 220°C at 2°C/min, and held constant at 220°C for an additional 5 min. The injector and detector temperatures were 200 and 250°C, respectively. The ionization energy of the mass selective detector was 70 eV, with a scanning mass range of *m/z* 33-330.

GC-olfactometry (GC-O) The GC-O system consisted of a Varian 3800 (Varian Instrument Group, Walnut Creek, CA, USA), equipped with a flame ionization detector and sniffing port. One microliter of the essential oil was injected into a HP-5MS column (30 m length × 0.25 mm i.d. × 0.25 μm film thickness; Agilent Co., Palo Alto, CA, USA) using a microsyringe. The GC conditions were the same as described above. The GC-O was performed by three researchers familiar with the *Chopi* aroma.

Identification of volatile compounds The tentative

*Corresponding author: Tel: 82-2-901-8590; Fax: 82-2-901-8442
E-mail: mschung@duksung.ac.kr
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identification of the volatile compounds was based on their mass spectra compared with those in an on-line computer library (Wiley 275) (Agilent Co., Palo Alto, CA, USA). The retention indices (RIs) of the compounds, determined using n-paraffins C_8-C_{22} as external references (10), were compared with those of published in literature (11). The quantification of each volatile compound was performed based on the ratio of the peaks obtained from a MS total ion chromatogram.

Preference test of essential oils To evaluate the potency of natural spices of the essential oil of *Chopi*, a preference test was carried out. The 34 participants who participated in this study were students at Duksung Women's University, Seoul, Korea. Since the essential oil of *Chopi* has a characteristic citrus-like flavor (7, 8), the preference and intensity were compared with those of two popular citrus-like essential oils, lemongrass and grapefruit. The untrained participants smelled individual strips (1 cm × 10

Table 1. Volatile compounds of *Zanthoxylum piperitum* A.P. DC.

Peak No.	Compounds	RI[1)]	Relative peak area (%)[2)]	Identification[3)]
1	hexanal	799	0.08±0.01	MS/RI
2	heptanal	900	0.03±0.01	MS
3	α-thujene	925	0.05±0.01	MS/RI
4	α-pinene	934	0.26±0.03	MS/RI
5	sabinene	973	0.54±0.01	MS/RI
6	heptanol	977	0.02±0.01	MS/RI
7	β-myrcene	989	6.39±0.04	MS/RI
8	octanal	1004	0.33±0.03	MS/RI
9	limonene	1031	24.43±0.46	MS/RI
10	2,6-dimethyl-5-heptenal	1054	0.37±0.01	MS
11	isolimonene	1066	0.03±0.01	MS
12	cis-linalool oxide	1070	0.08±0.01	MS
13	undecanol	1078	0.11±0.02	MS
14	α-terpinolene	1091	0.10±0.02	MS/RI
15	linalool	1101	1.69±0.03	MS/RI
16	rose oxide	1109	0.61±0.06	MS
17	cis-limonene oxide	1124	0.33±0.04	MS
18	isopulegol	1142	2.71±0.12	MS
19	citronellal	1153	11.08±0.53	MS/RI[4)]
20	trans-3(10)-caren-2-ol	1172	0.20±0.03	MS
21	terpinen-4-ol	1182	0.33±0.04	MS/RI
22	cryptone	1189	6.59±0.13	MS/RI
23	cis-piperitol	1215	0.22±0.03	MS/RI
24	dihydrocarvone	1217	0.15±0.05	MS
25	trans-carveol	1222	0.59±0.11	MS
26	cuminal	1227	3.86±0.81	MS
27	citronellol	1228	0.65±0.06	MS/RI[4)]
28	carvone	1254	0.79±0.06	MS/RI
29	piperitone	1256	1.01±0.12	MS
30	linalyl acetate	1257	0.62±0.05	MS/RI[4)]
31	geraniol	1258	2.43±0.10	MS/RI
32	phellandral	1267	6.36±0.12	MS
33	safranal	1273	0.71±0.16	MS
34	*p*-cumic alcohol	1292	1.91±0.06	MS
35	nonanoic acid	1298	0.45±0.06	MS/RI
36	α-terpinyl acetate	1346	0.58±0.06	MS
37	citronellyl acetate	1354	2.59±0.20	MS/RI[4)]
38	geranyl acetate	1383	12.85±0.17	MS/RI[4)]
39	tetradecane	1400	0.07±0.01	MS/RI
40	β-caryophyllene	1430	0.06±0.01	MS/RI
41	cuminyl acetate	1437	0.02±0.01	MS
42	δ-cadinene	1528	0.05±0.01	MS/RI
43	caryophyllene oxide	1592	0.30±0.05	MS/RI
44	T-muurolol	1646	0.03±0.01	MS
45	8-heptadecene	1674	0.09±0.01	MS
46	heptadecane	1700	0.03±0.01	MS/RI

1)Retention indices were determined using n-paraffins C_8-C_{22} as external references.
2)Average of the relative percentage of the peak area in the MS total ion chromatogram (n=3) ± standard deviation.
3)Tentative identification was performed as follows: Mass spectrum (MS) was consistent with that of the Wiley mass spectrum database [2001, Hewlett Packard Co., Palo Alto, USA]; Retention index (RI) was consistent with that found in literature (11).
4)Identification based on reference no. 3.

cm; Whatman chromatography paper, Whatman International Ltd., Maidstone, UK), each containing one drop of the individual essential oil. Each strip of essential oil was presented randomly. The participants were asked to sniff each essential oil strip and evaluate the preference and intensity of aroma using a 9-point hedonic scale, with a score of 9 signifying "extremely like" and a score of 1 signifying "extremely dislike" in the preference test. In the intensity test, a score of 9 signified "extremely strong" and a score of 1 signified "extremely weak". After smelling one essential oil, the participants relaxed for 1 min (12). This test was performed in triplicate, with the data presented as the mean with standard deviation for the individual oils, which were analyzed by a one-way analysis of variance (ANOVA). Statistical significance was defined as $p<0.05$ for all analysis.

Results and Discussion

Volatile compounds of *Chopi* To identify the volatile compounds from *Chopi*, steam distillation was initially performed to extract the essential oils, which were then separated and analyzed as described earlier. As shown in Table 1, 46 volatile compounds, consisting of 12 hydrocarbons, 8 aldehydes, 5 esters, 12 alcohols, 4 ketones, 4 oxides and 1 acid, were tentatively identified from the essential oil of *Chopi*. Unidentified compounds constituted 7.22% of the total peak area. Limonene (24.43%) was the most abundant compound, followed by geranyl acetate (12.85%), citronellal (11.08%), cryptone (6.59%) and β-myrcene (6.39%). Of the hydrocarbons there were 9 terpene compounds, with limonene being the most abundant. Limonene is the most predominant compound, accounting for 68.5% in Rutaceae plants, such as *Citrus limon* Burmann (13). However, recent reports have revealed the most abundant compound of *Z. acanthopodium* DC and *Z. schinifolium,* which also belong to the Rutaceae family, to be geranyl acetate, accounting for 32.04 and 23.90%, respectively (3, 14). In Korea, two different *Zanthoxylum* species, *Chopi* and *Sancho*, are used as spices and plant medicines, but the predominant volatile compounds in these two species were found to be limonene and geranyl acetate, respectively.

Extraction of volatile compounds from the pectin-elicited suspension culture of *Z. piperitum* has been reported, and the flavor profile of the suspension culture of *Z. piperitum* is different from that of wild *Z. piperitum*, with a total of 17 flavor compounds formed in the pectin-elicited suspension. In particular, the carbonyl compounds, 3-hydroxy-2-butanone, γ-butyrolactone and 2,3-butanedione, were identified as the most abundant compounds (9).

In this study, 11.08% citronellal and 2.71% isopulegol were identified. Isopulegol, a monoterpenic alcohol, is widely used in the flavor industry for the production of fragrances with blossom compositions, and is an important ingredient in various pharmaceuticals. It has been established that isopulegol can be obtained from citronellal through cyclization (15).

The larger esters detected by GC-MS, such as linalyl, a-terpinyl, citronellyl, geranyl and cuminyl acetate, were consistent with the results of previously reported studies on the aroma of the Rutaceae family (3, 7, 8, 14). Acids and oxides were also detected, but these groups displayed relatively low proportions of 0.45 and 1.32%, respectively. In this study, 0.33% cis-limonene oxide was detected; this compound is usually detectable in trace amounts from fresh lemon oils (13). The ketones, cryptone (4-isopropyl-2-cyclohexen-1-one), dihydrocarvone, carvone (6, 8(9)-ρ-menthadien-2-one) and piperitone (ρ-menth-1-en-3-one), were tentatively identified, and are also aroma compounds in *Chopi*. Piperitone has a powerful fresh-minty-camphoraceous odor and is commonly used in flavor compositions, particularly in spice complexes with caraway and estragon (16).

Aroma-active compounds GC-O was performed to evaluate the aroma-active compounds from *Chopi*. As shown in Table 2, 14 aroma-active compounds were detected. According to the compound descriptions perceived at the sniffing port, both citronellal and limonene had citrus and *Chopi* flavors. The reported flavor dilution factors of citronellal and limonene were 128 and 32, respectively (3). In this study, citronellal and cryptone were described as citrus, cucumber and fatty. Moreover, the volatile compounds of *Chopi* contained a relatively high peak area (11.08%) of citronellal compared with other compounds. In this study, linalool was also identified as an aroma-active compound. β-Myrcene contributed grassy and spicy flavors to *Chopi* and *Cheongung* (17). Rose oxide, cuminal, citronellol, piperitone, linalyl acetate, geraniol, citronellyl acetate and geranyl acetate also contributed to *Chopi*'s citrusy and spicy aroma. Kim *et al.* (9) reported the aroma-active compounds of the pectin–elicited *Z. piperitum* suspension culture to be 2, 3-butanedione, ethyl 3-methylbutyrate and ethyl 2-methylpropanoate, which were quite different from those found in the intact plant in this study.

Preference test of essential oils Since the essential oil of *Chopi* has a characteristic citrus flavor, the preference and intensity of its aroma were compared with those of

Table 2. Aroma-active compounds of *Zanthoxylum piperitum* A.P. DC using GC-O

RI[1)]	Compounds	Aroma descriptions[2)]
989	β-myrcene	citrus, grassy, spicy
1004	octanal	lemon, green
1031	limonene	citrus, lemon, fresh *Chopi*
1101	linalool	citrus, floral
1109	rose oxide	floral, woody
1153	citronellal	*Chopi*, fatty, citrus, soapy
1189	cryptone	cucumber, fatty
1227	cuminal	citrus, sweet, pine
1228	citronellol	citrus, warm, herbal
1256	piperitone	minty, floral
1257	linalyl acetate	citrus, herbal, fruity
1258	geraniol	citrus, rose
1354	citronellyl actate	citrus, sweet, dust
1383	geranyl acetate	citrus, floral

[1)]Retention indices.
[2)]Aroma descriptions perceived at the sniffing port.

Table 3. Preference and intensity scores of the essential oils

	Chopi	Grapefruit	Lemongrass
Preference score[1)]	$4.80^{c,2)} \pm 1.08$	$6.57^{a} \pm 0.76$	$6.12^{b} \pm 0.84$
Intensity of essential oil	$5.97^{b} \pm 1.82$	$4.72^{c} \pm 1.47$	$6.99^{a} \pm 1.43$

[1)]Mean ± standard deviation. Means were from triplicate data of 34 participants.
[2)]Means with the same superscript within a row were not significantly different ($p<0.05$, Duncan's multiple range test).

two popular citrus-like essential oils, lemongrass and grapefruit (Table 3). The aroma of the essential oils from lemongrass and grapefruit are well known to be citrus-like, sweet and popular with people, particularly young women; however, the aroma of *Chopi* was not familiar to them. On the 9-point hedonic scale, a score of 4.8 in the preference test was a middle score. The aroma intensity for *Chopi* was 5.97, which was between the values obtained for grapefruit (4.72) and lemongrass (6.99). Essential oils have various effects when used in foods: not only do they impart flavor, they also have antioxidant and antimicrobial properties. However the use of essential oils is often limited by individual likes and dislikes towards certain flavors. This study revealed the aroma of *Chopi* essential oil to have a moderate (neither like nor dislike) score on the preference test and a slightly strong score on the intensity test.

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