Chemical Composition and Characteristic Odor Compounds in Essential Oil from *Alismatis Rhizoma* (Tubers of *Alisma orientale*)

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Abstract: Chemical composition and potent odorants that contribute to the characteristic odor of essential oil from *Alismatis Rhizoma* (tubers of *Alisma orientale*) were investigated by gas chromatography-mass spectrometry (GC-MS), GC-olfactometry (GC-O), aroma extract dilution analysis (AEDA) and relative flavor activity (RFA) methods. Fifty components, representing 94.5% of the total oil, were identified. In this study, we newly identified thirty-nine compounds in the oil from tubers of *A. orientale*. The major constituents of the essential oil were khusinol (36.2%), δ -elemene (12.4%), germacron (4.1%), alismol (3.8%), β -elemene (3.1%), and α -bisabolol (1.9%). Through sensory analysis, sixteen aroma-active compounds were detected and the key contributing aroma-active compounds were δ -elemene (woody, flavor dilution (FD)-factor = 4, RFA = 0.3) β -elemene (spicy, FD = 5, RFA = 0.7), spathulenol (green, FD = 5, RFA = 1.0), γ -eudesmol (woody, FD = 6, RFA = 1.5), and γ -cadinol (woody, FD = 5, RFA = 1.0). These compounds are thought to contribute to the odor from tubers of *A. orientale*. These results imply that the essential oil from the tubers of *A. orientale* deserve further investigations in the phytochemical and medicinal fields.

Key words: *Alismatis Rhizoma*, *Alisma orientale*, essential oil, aroma extract dilution analysis (AEDA), relative flavor activity (RFA)

1 INTRODUCTION

Crude drugs or herbal medicines ("Kampo" in Japanese) have been used for more than 2,000 years to treat various diseases in Japan, China, and Korea owing to their natural healing power. The pharmacological activities of crude drugs are attributed to their active ingredients such as volatile oils and volatile compounds, which impart aroma and flavors. We have been involved in the study of these compounds, in several crude drugs for a long time¹⁻⁹⁾.

Alisma orientale belongs to family Alismataceae and is widely cultivated in China and Japan. *Alismatis Rhizoma* (the dried tubers of *A. orientale*), termed Ze Xie in Chinese Pharmacopoeia, is a traditional Chinese medicine for the treatment of hyperlipidemia and diabetes and is used as a diuretic¹⁰⁾. Previous phytochemical investigation has shown that the main chemical constituents of the dried tubers of *A. orientale* ("takusha" in Japanese) are various terpenoids, including sesquiterpenoids, diterpenoids, and triterpenoids¹⁰⁻¹²⁾. In the supercritical CO₂ extract and

headspace extraction, twenty-seven and seventeen compounds were reported, respectively^{13, 14)}. However, there are no detailed reports on the aroma-active compounds of essential oil from the tubers of *A. orientale*.

In flavour analysis, GC-olfactometry (GC-O) is the most commonly used method for the evaluation of odorants, GC-O coupled with aroma extract dilution analysis (AEDA) is a particularly useful method for estimating the contributions of the most aroma-active compounds¹⁵⁾. The significant contribution of each odorant to the characteristic flavor can be determined by relative flavor activity (RFA). The higher flavor dilution (FD)-factors are often related to the aroma-active compounds, but a high FD-factor for the compounds may be caused by their high oil content. Therefore, RFA is calculated, which takes into consideration both the FD factor and percentage weight of each compound^{16, 17)}.

The aim of this study is to investigate the chemical composition of essential oil extracted from the tubers of *A. ori-*

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entale, and to evaluate its characteristic aroma-active compounds by AEDA and RFA methods.

2 EXPERIMENTAL

2.1 Plant material

Dried tubers of *A. orientale* (Lot. 517111) were obtained from Ohsugi Pharmaceutical Co., Ltd. (Osaka, Japan) and used for extracting the essential oil.

2.2 Isolation of the volatile oil

Dried tubers of *A. orientale* (100 g) were subjected to hydrodistillation for 3 h using a Likens-Nickerson type apparatus with diethyl ether as the extraction solvent to yield 0.17% (w/w) of yellowish oil. This oil was dried over anhydrous sodium sulfate and stored at 4°C prior to analysis.

2.3 GC-MS

An Agilent 6890N gas chromatography-5973 MSD mass spectrometer was used for the GC-MS analysis. The samples were analyzed using a fused-silica capillary columns, HP-5MS (5% phenyl 95% polydimethylsiloxane, 30 m × 0.25 mm i.d., film thickness = 0.25 µm) and DB-WAX (polyethylene glycol, 15 m × 0.25 mm i.d., film thickness = 0.25 µm). The oven temperature was programmed to increase from 40 to 260°C at the rate of 4°C/min and held at 260°C for 5 min. The flow rate of the carrier gas (He) was 1.5 mL/min. On the DB-WAX column, the oven temperature was increased from 40°C to 240°C at the rate of 4°C/min and held at 240°C for 5 min. The injector and transfer line temperatures were 230°C, and the ionization energy was 70 eV. The mass range was 39–450 amu. One µL of the sample was injected, and the split ratio maintained was 1:40.

2.4 Sniffing test

A trained panel of sensory evaluation specialists measured the odor intensities of the main aromatic constituents of dried tubers from *A. orientale*. Twelve panelists, aged 21–26 years (10 males and 2 females, members of Kinki University, Japan), participated in this study. Sensory analysis sessions were performed only after suitable training (>30 h). The sniffing test by GC-O was carried out using an Agilent Technologies-6890N gas chromatograph equipped with an Agilent 5973 MSD mass spectrometer and sniffing port ODP2 (Olfactory Detector Port 2, Gerstel). The GC instrument was equipped with an HP-5MS column (5% phenyl 95% polydimethylsiloxane, 30 m × 0.25 mm i.d., film thickness = 0.25 µm). The samples were injected into the GC in splitless mode. The GC eluent from the capillary column was split in a 1:1 (v/v) ratio between the mass spectrometer and the sniffing port. The oven conditions, injector and transfer line temperatures,

carrier gas, flow rate, and ionization mode were the same as those described above for the GC-MS.

2.5 AEDA

The results were expressed in terms of the FD factor, which is the ratio of the concentration of the odorant in the initial volatile oil to its concentration in the most diluted volatile oil in which the odor can be still detected by GC-O. The highest sample concentration (1 mg/mL) was assigned an FD factor of 1. The volatile oil was diluted stepwise with diethyl ether (1:1, v/v), and aliquots of the dilutions (1 mL) were evaluated. The process was stopped when the evaluators detected no aroma for a certain dilution.

2.6 RFA

An odorant with a high FD-factor can be considered as an important contributor to the characteristic flavor. Based on the AEDA results, RFA was calculated using the following equation:

$$\text{RFA} = \log \text{FD-factor} (2^n) / S^{0.5}$$

where 2^n is the FD-factor and S is the weight percentage of a compound^{16, 17}.

2.7 Identification and quantification of compounds

The compounds were identified by comparing their Retention indices (RIs) and mass spectra with the published data^{18–26}, digital libraries (Mass Finder 4 and NIST02), and Aroma Office version 3.0 (Nishikawa Keisoku Co. Ltd.), which includes 72,120 RI entries of compounds from literature sources. The RIs were calculated using a homologous series of *n*-alkanes (C₅–C₂₈) for the HP-5MS column. The quantitative analysis was performed by the internal standard addition method (alkanes C₁₂ and C₁₉). The essential oils were diluted 100× using Et₂O to achieve a 1 mL volume, and then 5 µL of C₁₂–C₁₉ solution (1 mg/mL) was added to the diluted oil. These prepared samples were subjected to GC-MS and GC-FID determinations. The quantitative analysis of the aroma-active compounds of the essential oil were based on the calibration curves for 2-pentyl furan (peak 1), menthol (peak 3), δ-elemene (peak 6), α-cubebene (peak 7), α-copaene (peak 9), β-elemene (peak 13), β-caryophyllene (peak 15), γ-elemene (peak 17), γ-murolene (peak 18), calamenene (peak 20), α-selinene (peak 22), δ-selinene (peak 23), spathulenol (peak 30), γ-eudesmol (peak 34), γ-cadinol (peak 36) and β-cadinol (peak 40) within the concentration range 0.5–1000 µg/mL. The weight-percent of each compound was calculated with response factors to the FID. The results were calculated as the mean values of the injection of the essential oil without correction factors. All determinations were performed in duplicate and averaged.

3 RESULTS AND DISCUSSION

In this study, 0.17% (w/w) yellowish oil with a woody, spicy, and green odor was obtained as the essential oil. Fifty compounds were identified, representing about 94.5% of the oil. In this study, we newly identified further thirty-nine compounds in addition to those identified in a previous study^{13, 14)}. The identified volatile compositions, along with their concentrations and the RIs on the HP-5MS and DB-WAX columns are listed in **Table 1**.

Sesquiterpene with a cadinane skeleton, namely, khusinol (peak **37**; 36.2%), is the most abundant compound, followed by δ -elemene (peak **6**; 12.4%), germacrone (peak **19**; 4.1%), alismol (peak **33**; 3.8%), and β -elemene (peak **13**; 3.1%) (**Fig. 1**). Khusinol has been reported to be the main compound of the essential oils from vetiver²⁷⁾. In addition, alismol is an unusual sesquiterpene, which is isolated as a characteristic compound from *Alismataceae*. It is identified as a compound in the oil from

tubers of *A. orientale* for the first time²⁸⁾. Alismol mainly inhibits Ca^{2+} influx through a voltage-dependent Ca^{2+} channels²⁹⁾, acting primarily on nerve terminals and inhibiting their responses to electrical stimulation by interfering with NAD release³⁰⁾. Monoterpenes and sesquiterpenes constituted 0.2% and 89.1% in the oil, respectively. In particular, a cadinane and an elemene type were rich in the oil, accounting for 39.0% and 14.8%, respectively.

In order to gain insight into the potent odorants contributing to the characteristic aroma of *A. orientale*, AEDA method was performed through GC-O analysis³¹⁾. The aroma-active compounds and their odor properties are shown in **Table 2**. A comparison of the gas chromatogram obtained by GC using an HP-5MS column and the corresponding FD chromatogram of the odor-contributing compounds is shown in **Fig. 1**. Based on the FD-factors, it was found that γ -eudesmol (peak **34**; FD = 6, woody) is the most intense aroma-active compound among sixteen aroma-active com-

Table 1 Chemical composition in the essential oils from tubers of *Alisma orientale*.

No.	Compounds ^a	RI ^b		peak area (w/w)	Identification method ^d	Reference source ^c
		HP-5MS	DB-WAX			
*1	2-pentyl furan	990	1129	0.8	RI, MS	Wako
2	allyl hexanoate	1064	1371	0.7	RI, MS	Ardrich
3	menthol	1173	1636	0.2	RI, MS	Fluka
4	2-decanone	1200	1521	0.2	RI, MS	Wako
5	cyclohexanone	1268	1452	0.3	RI, MS	Wako
6	δ -elemene	1327	1613	12.4	RI, MS	Wako
7	α -cubebene	1351	1546	1.1	RI, MS	Wako
8	1,2-dimethyl-cyclohexene	1355	—	0.3	RI, MS	TCI
*9	α -copaene	1372	1498	0.8	RI, MS	Wako
10	isodene	1379	1481	0.6	RI, MS	Wako
11	2-butyl-2,7-octadien-1-ol	1384	—	0.5	RI, MS	TCI
12	β -patchoulene	1387	1460	0.3	RI, MS	Wako
*13	β -elemene	1391	1580	3.1	RI, MS	Wako
14	cedrene	1408	1588	0.4	RI, MS	Wako
*15	β -caryophyllene	1420	1636	0.7	RI, MS	Wako
16	γ -patchoulene	1440	1684	0.6	RI, MS	Wako
*17	γ -elemene	1470	1722	1.1	RI, MS	Wako
18	γ -muurolene	1481	1671	1.0	RI, MS	Wako
19	germacrone	1482	1729	4.1	RI, MS	Wako
20	calamenene	1492	1808	0.5	RI, MS	Wako
*21	α -muurolene	1500	1725	1.3	RI, MS	Wako
*22	α -selinene	1507	1750	0.5	RI, MS	Wako
23	δ -selinene	1509	1756	0.9	RI, MS	Wako
24	γ -cadinene	1513	1758	0.8	RI, MS	Wako
*25	β -cadinene	1518	1769	1.3	RI, MS	Wako

Table 1 Continued.

No.	Compounds ^a	RI ^b		peak area (w/w)	Identification method ^d	Reference source ^e
		HP-5MS	DB-WAX			
26	2-cyclohexyl-2-phenyl-propane	1528	—	0.3	RI, MS	Wako
27	α -bisabolol	1532	1683	1.9	RI, MS	Ardrich
28	8,9-dehydro-neoisolongifolene	1545	—	0.7	RI, MS	Wako
29	aromadendrene oxide	1577	1833	0.6	RI, MS	Wako
*30	spathulenol	1581	2108	1.6	RI, MS	Wako
31	cadala-1(10),3,8-triene	1591	1712	0.3	RI, MS	TCI
32	chiloscyphone	1614	1594	0.9	RI, MS	Wako
33	alismol	1622	2136	3.8	RI, MS	Ardrich
34	γ -eudesmol	1628	1855	1.0	RI, MS	Wako
*35	α -copaene-8-ol	1655	—	1.1	RI, MS	Wako
36	γ -cadinol	1658	2210	1.5	RI, MS	Wako
37	khusinol	1666	2213	36.2	RI, MS	Wako
38	<i>epi</i> -globulol	1671	2221	1.3	RI, MS	Wako
39	germacra-4(15),5,10(14)-trien-1a-ol	1678	2226	0.9	RI, MS	TCI
40	β -cadinol	1689	2250	1.4	RI, MS	Wako
41	(<i>Z</i>)-nuciferol	1717	2241	1.2	RI, MS	Wako
42	β -(<i>Z</i>)-curcumen-12-ol	1751	2232	0.5	RI, MS	Wako
43	5 β -eudesma-1,3-dien-11-ol	1763	2236	1.3	RI, MS	TCI
44	2,2,6-trimethyl-1-(3-oxo-but-1-enyl)- 7-oxa-bicyclo-[4.1.0]-hept-4-en-3-one	1783	—	0.5	RI, MS	Wako
45	3-buten-2-one, 4-(5,5-dimethyl- 1-oxaspiro-[2.5]-oct-4-yl)	1818	—	0.4	RI, MS	Wako
46	1,4-dimethyl-7-(1-methylethyl)-azulene	1825	—	0.5	RI, MS	TCI
47	isolongifolen-9-one	1837	2321	0.4	RI, MS	Wako
48	(<i>Z</i>)-7-dodecenyl acetate	1847	2153	0.6	RI, MS	Ardrich
*49	ethyl palmitate	2059	2258	0.8	RI, MS	Wako
50	ethyl linolate	2244	2522	tr ^c	RI, MS	Wako
	hydrocarbons			34.3		
	alcohols			51.6		
	ketones			1.3		
	ethers			1.4		
	esters			5.9		
	total identified			94.5		

^a Compounds are listed in order of their elution time from a HP-5MS column. Presence of compound is indicated by its GC/FID percentage.

^b RI, retention indices determined on HP-5MS and DB-WAX columns, using the homologous series of *n*-alkanes (C₅–C₂₈).

^c tr = trace (< 0.1%)

^d Identification methods: RI, retention indice; MS, mass spectrum.

^e Reference materials were obtained from commercial source and our previous studies: Wako, Wako Pure Chemical Industries Ltd. (Osaka, Japan); TCI, Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan); Aldrich, Sigma-Aldrich, St. Louis, MO.

* = previous study

Fig. 1 Gas chromatogram using HP-5MS column and aromagram (FD-factor and RFA) of the essential oil from the tubers of *Alisma orientale*.

pounds detected by AEDA in the essential oil, followed by δ -elemene (peak 6; FD = 4, woody), γ -cadinol (peak 36; FD = 5, woody), β -elemene (peak 13; FD = 5, spicy), and spathulenol (peak 30; FD = 5, green) (Fig. 1). Compounds that are also somewhat involved in the aroma included β -cadinol (peak 40; FD = 4, woody), calamenene (peak 20; FD = 4, spicy), and α -copaene (peak 9; FD = 4, spicy) were also involved in the aroma. As a main compound, khusinol was less important among the aroma-active compounds of *A. orientale* as its FD-factor was not detected. Generally, FD-factors can be considered as an indication of aroma contribution with compounds having a high FD-factor being considered important aroma compounds. However, the high FD-factor of the compounds might be caused by their high content in the oil. They do not always significantly contribute to the aroma because they depend on the compound concentration. Therefore, RFA was calculated which takes into consideration both the FD-factor and the weight percentage of each compound³². The RFA values of the aroma-active compounds in *A. orientale* oil are shown in Table 2. As shown in Fig. 1, the RFA value of δ -elemene is not high in spite of its high FD-factor of 4. Consequently, it can be assumed that the high FD-factor of δ -elemene

might be due to its high concentration in the sample. α -Copaene, calamenene, spathulenol, γ -eudesmol, and γ -cadinol have a high FD-factor (≥ 4) as well as a high RFA (≥ 1.0). These compounds are suggested to be the characteristic aroma-active compounds in the oil from *A. orientale*.

Thus, the highest contribution compounds in the oil were δ -elemene (peak 6; RFA = 0.3), followed by γ -eudesmol (peak 34; RFA = 1.5), and γ -cadinol (peak 36; RFA = 1.0). Moreover, β -elemene (peak 13; RFA = 0.7) and spathulenol (peak 30; RFA = 1.0) also contributed essentially to the aroma of the oil at some level. As shown in Table 2, δ -elemene, γ -eudesmol, γ -cadinol, β -elemene, and spathulenol are ascribed woody, spicy, and green aromas, respectively, by the sniffing test (Fig. 2).

To the best of our knowledge, this is the first study that provides data on the key aroma compounds present in the tubers of *A. orientale*. A total of sixteen aroma-active compounds were determined by the AEDA and RFA methods. Thirty-nine compounds were newly identified in the oil. These results signify that the oil derived from *A. orientale* deserves further investigations in the phytochemical and medicinal fields.

Table 2 Aroma-active compounds in essential oils from tubers of *Alisma orientale*.

No.	Compounds ^a	Odor description ^b	peak area (w/w) ^c	FD-factor ^d (2 ⁿ)	RFA ^e
1	2-pentyl furan	sweet	0.8	2	0.7
3	menthol	mint	0.2	1	0.7
6	δ-elemene	woody	12.4	4	0.3
7	α-cubebene	green	1.1	1	0.3
9	α-copaene	spicy	0.8	4	1.0
13	β-elemene	spicy	3.1	5	0.7
15	β-caryophyllene	spicy	0.7	2	0.7
17	γ-elemene	woody	1.1	2	0.6
18	γ-murolene	herbal	1.0	2	1.9
20	calamenene	spicy	0.5	4	1.3
22	α-selinene	spicy	0.5	2	0.9
23	δ-selinene	woody	0.9	2	0.7
30	spathulenol	green	1.6	5	1.0
34	γ-eudesmol	woody	1.0	6	1.5
36	γ-cadinol	woody	1.5	5	1.0
40	β-cadinol	woody	1.4	4	0.8

^a Compounds are listed in order of their elution time from a HP-5MS column.^b Odour quality perceived through the sniffing port.^c These values were computed from the volatile oil yield and GC peak area.^d The sample concentration (1 mg/mL) was assigned an FD-factor of 1.^e RFA = log FD-factor (2ⁿ) / S^{0.5}, where S is the weight percentage. **Fig. 2** Key aroma-active compounds of the essential oil from tubers of *Alisma orientale*.

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