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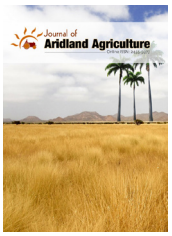

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Analysis of volatile compounds from three species of *Atractylodes* by Gas Chromatography-Mass Spectrometry

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ABSTRACT

A total of 99 different volatile compounds were detected through Gas Chromatography-Mass Spectrometry (GC-MS) from three species of *Atractylodes*, namely *Atractylodes lancea*, *Atractylodes japonica*, and *Atractylodes chinensis*. Thirteen-volatile flavor compounds i.e. acid, alcohol, aldehyde, alkane, alkene, alkyne, ester, ketone, monoterpene, oxygenated monoterpene, sesquiterpene, oxygenated sesquiterpene, and oxygenated triterpenoid detected from different species of *Atractylodes*. It was observed that all the species contained 38 common compounds, while *A. lancea* contained 7 unique compounds, *A. japonica* has 4 unique compounds, and *A. chinensis* hold 6 compounds not detected in the other extracts. In addition, essential oils from *A. lancea* and *A. japonica* possessed 11 compounds in common, and *A. lancea* and *A. chinensis* possessed 19 compounds in common. The remaining 14 compounds were detected only in *A. japonica* and *A. chinensis*. The total content of all components in the species was comparable, with 82.528%, 81.766%, and 81.799% of volatile components being detected for *A. lancea*, *A. japonica*, and *A. chinensis*, respectively. Curzerene was found to be the most predominant compound in both *A. lancea* (14.1%) and *A. chinensis* (16.7%), while murolan-3,9(11)-diene-10-peroxy was found predominantly in *A. japonica* (16.8%). The present study suggests that the identified volatile compounds may possess important biological properties, and could be suitable for application in both oriental medicines and the pharmaceutical industry.

KEYWORDS: Volatile compounds, medicinal plants, *Atractylodes chinensis*, *Atractylodes japonica*, *Atractylodes lancea*, essential oil, GC-MS analysis

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INTRODUCTION

Volatile organic compounds (VOC), generally lipophilic liquids with high steam pressures, symbolize the largest group of natural products in plants. These types of compounds cover multiple effects on both floral and vegetative tissues in many plant species (Pichersky *et al*., 2006). Usually, much floral volatiles provides to attract pollinators and also do something as guards for precious reproductive parts of plants against harmful pathogens, parasites, and herbivores (Dudareva et al., 2004). In most cases, vegetative volatiles engages in the signaling of inter-plant or inner plant organs and plant defense against pathogens, heat, and oxidative stress (Unsicker *et al*., 2009). In addition, numerous aromatic plants have been used as flavorings, preservatives, and herbal remedies (Pichersky et al., 2006).

Atractylodes is one of an important genus belongs to the family *Asteraceae* and is composed of eight species of perennial medicinal plants widely distributed in East Asia (Willis, 1966). Some species of the *Asteraceae* family, including *Atractylodes lancea*, *A. japonica*, and *A. chinensis*, are well known for their use in traditional Chinese medicine. Essential oils are the main active constituents of *Atractylodes* spp., with previous studies examining volatile oil biosynthesis in some species of *Atractylodes*. It was reported that one of the endophytic *Acinetobacter* sp. ALEB16A enhanced the biosynthesis of volatile components in *A. lancea* (Wang *et al*., 2015). Inoculation with the endophytic fungus of *Gilmaniella* sp. AL12 boosted the actions of total protein phosphorylation needed for endophyte-induced volatile oil production in *A. lancea* (Ren and Dai, 2012). It proclaimed that jasmonic acid performs in NO- and H_2O_2-

mediated volatile oil accumulation as a downstream signaling molecule in A. *lancea* provoked by endophytic fungi (Ren and Dai, 2012). The existence of a fungal elicitor might therefore considerably enhance the content of volatile oil in A. *lancea* (Zhang *et al*., 2008). Furthermore, it was reported that the geographical disparity for the active components in rhizome essential oils of A. *lancea* and A. *chinensis* imitates mainly for genetic variability (Takeda *et al*., 1996).

It was also reported that essential oil from *Atractylodes* spp. showed an insecticidal activity and postponed gastric emptying in stress-induced rats (Zhang *et al*., 2008). The governing actions on delayed gastric emptying from the essential oil in *Atractylodes lancea* are mostly owing to the reduction of the release of the central corticotropin-releasing factor (Zhang et al., 2008). Besides, these essential oil from *Atractylodes chinensis* (DC.) Koidz showed strong insecticidal activity which works well against *Drosophila melanogaster* L. (Chu *et al*., 2011). It has been previously reported that plants belonging to the *Atractylodis* genus are rich in volatile compounds and essential oils, including sesquiterpenes and polyacetylenes. In particular, A. *lancea* is used in Chinese patent medicines (Chen *et al*., 2007; Xie *et al*., 2008). These essential oils and volatile compounds can be used in anti-inflammatory, anticonvulsant, sedative, analgesic, antianoxic, antiviral, and anti-hepatotoxic treatments (Guo *et al*., 2006).

As these species showed lots of inevitable biological properties, therefore this study was undertaken to identify different volatile components present in A. *lancea*, A. *japonica*, and A. *chinensis*, and compared the characteristics of each component using gas chromatography-mass spectrometry.

MATERIALS AND METHODS

Identification of Different Volatile Compounds Through Headspace Solid-phase Microextraction Technique

Three species of *Atractylodes*were collected from a different area of China i.e. *Atractylodes lancea* Thunb. was collected from Wuhan, Hubei, *Atractylodes japonica* Koidz was from Yanji, Jilin, and *Atractylodes chinensis* Koidz was from Zhangjiajie, Hunan. After collection, samples were weighed and immediately taken into a vial containing a headspace of 25mL. For the absorption of volatile compounds, a fused-silica fiber coated having a 75μm layer of carboxen/poly dimethyl siloxane (CAR/PDMS) was utilized. The fiber was opened to the headspace of the vial maintaining 25 °C for about 20 min, after that it was eliminated from the vial and initiated directly into the GC injector. Here thermal desorption analysis was conducted at 250 °C for 3 min. The compounds were identified from their mass spectra and the quantitative detection was calculated by utilizing peak areas of the compounds. Standards for GC-MS were collected from the National Institute of Standards and Technology (NIST).

GC and GC-MS Analysis

Herewith an Agilent 6890N GC mainframe connected with an HP-5 fused-silica capillary column (30 m × 0.32mmID, 0.25 μm film thickness), as well as the flame ionization detector (FID) (Agilent, USA) the GC analysis was done. Temperatures of both injector and detector for each analysis were placed at 250 °C and 280 °C, respectively. As the carrier gas, here nitrogen was used maintaining a flow rate of 1.0 ml/min. The column temperature was retained at 50 °C for 5min and was then modified as follows: 1) Ramp from 50 °C to 260 °C at a rate of 3 °C min^{-1}; 2) Ramp from 260 °C to 280 °C at a rate of 10 °C min^{-1}; 3) Hold at 280 °C for 5 min.

GC-MS analysis was conducted on GC/MSD Polaris Q (Thermo Finnigan, USA) prepared with an HP-5 fused-silica capillary column (30 m × 0.32mm ID, 0.25μm film thickness) (Agilent, USA). Here as the carrier gas, Helium was utilized giving a flow rate of 1.0mLmin^{-1}. An electron ionization system having a 70eV system energy, a 250μA trap current, and an ion source at 200 °C was used for GC-MS detection.

Samples Identification

The respective compounds were identified by contrasting the mass spectra also with the data of NIST and WILLY library of the GC-MS system as well as the data from the literature. Total ion current chromatograms were measured following the mass range 40–400amu.

RESULTS

Composition of Essential Oil

GC-MS analysis assisted to detect 99 volatile compounds from three different species of *Atractylodes* by evaluating their GC-MS spectra with standard compounds as well as with previous literature reports (Yosioka *et al*., 1976; Chen *et al*., 2009; Wang *et al*., 2012). A total of 99 volatile compounds were detected in A. *lancea*, A. *japonica*, and A. *chinensis* (Table 1). Among the 99 volatile compounds, six compounds, i.e. 3-octen-5-yne, 5-butyl-1,3-cyclohexadiene, caryophyllene, cubenol, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-[1R-(1α,3aβ,4α,7β)]-azulene, and (3aS,6S,6aS,9aR,9bR)-azuleno [4,5-b]furan-2,9-dione, were detected only in A. *lancea*. In the case of A. *japonica*, four unique compounds were detected i.e. β-curcumene, decahydro-1,5,5,8a-tetramethyl-[1s-(1α,3aβ,4aα,7β,8aβ)]-1,4-methanoazulen-7-ol, 3-hydroxy-7-isopropenyl-1,4a-dimethyl-2,3,4,4a,5,6,7,8-octahydronaphthalen-2-yl acetate, and methyl 9,11-octadecadienoate. In addition, six volatile compounds, namely α-phellandrene, 1-methyl-3-(1-methylethyl)-benzene, 6-isopropylidene-1-methyl-bicyclo[3.1.0]hexane, β-copaene, ledene oxide-(II), and longiverbenone were found only in A. *chinensis*. Furthermore, 11 of the 99 compounds, namely 2,5-dimethyl-3-methylene-1,5-heptadiene, α-ethyl-α-2,5,7-octatrienyl-benzenemethanol, decahydro-1,1,3a-trimethyl-7-methylene-[1aS-(1aα,3aα,7aβ,7b)]-1*H*-cyclopropa[a] naphthalene, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-[1S-(1α,2β,4β)]-cyclohexane, α-acorenol, γ-eudesmol, 1,2,3,4,4a,5,6,8a-octahydro-α,α,4a,8-tetramethyl-[2R-(2α,4Aα,8Aβ)]-2-naphthalenemethanol, neocurdione,

Table 1: Volatile compounds and their quantities in *Atractylodes* spp., determined by solid-phase microextraction (SPME).

No	Compounds	Retention time (min)	Peak area (%)			Class of chemical	Chemical formula
			Atractylodes lancea Thunb	*Atractylodes japonica* Koidz	*Atractylodes chinensis* Koidz		
1	3-Octen-5-yne	11.392	9.007	-	-	Alkyne	C_8H_{12}
2	γ-Terpinene	13.613	-	0.053	10.334	Monoterpene	$C_{10}H_{16}$
3	D-Limonene	13.801	0.585	-	0.198	Monoterpene	$C_{10}H_{16}$
4	α-Phellandrene	14.447	-	-	0.211	Monoterpene	$C_{10}H_{16}$
5	5-Butyl-1,3-cyclohexadiene	14.515	0.509	-	-	Alkene	$C_{10}H_{16}$
6	2-Carene	15.615	2.273	0.426	0.113	Monoterpene	$C_{10}H_{16}$
7	1-Methyl-3-(1-methylethyl)benzene	15.761	-	-	0.086	Monoterpene	$C_{10}H_{14}$
8	α-Ylangene	16	0.748	-	0.534	Sesquiterpene	$C_{15}H_{24}$
9	2,5-Dimethyl-3-methylene-1,5-heptadiene	16.483	0.091	0.239	-	Monoterpene	$C_{10}H_{16}$
10	4-Terpinenyl acetate	17.028	0.463	-	8.205	Oxygenated monoterpene	$C_{12}H_{20}O_2$
11	6-Isopropylidene-1-methylbicyclo[3.1.0]hexane	17.218	-	-	1.698	Monoterpene	$C_{10}H_{16}$
12	1,5,5,6-Tetramethyl-1,3-cyclohexadiene	17.502	0.245	-	0.156	Monoterpene	$C_{10}H_{16}$
13	*m*-Phenethylbenzonitrile	18.24	0.643	0.028	0.711	Other	$C_{15}H_{13}N$
14	1,2-Ethanediol monobenzoate	18.498	0.086	-	1.318	Ester	$C_9H_{10}O_3$
15	2-Bromoethyl benzoate	18.696	0.096	0.953	0.115	Ester	$C_9H_9BrO_2$
16	α-Ethyl-α-(2,5,7-octatrienyl)benzenemethanol	18.811	0.171	0.135	-	Alcohol	$C_{17}H_{22}O$
17	2,6-Pyridinedicarboxaldehyde	19.202	0.155	-	0.302	Aldehyde	C_6H_5NO
18	3,6-Diethyl-3,6-dimethyl-*trans*-tricyclo[3.1.0.0(2.4)] hexane	21.164	0.193	-	0.647	Alkane	$C_{12}H_{20}$
19	Longifolene-(V4)	26.417	0.232	-	0.359	Sesquiterpene	$C_{15}H_{24}$
20	1R,4R,7R,11R-1,3,4,7-Tetramethyltricyclo[5.3.1.0(4.11)] undec-2-ene	32	0.171	0.312	0.386	Sesquiterpene	$C_{15}H_{24}$
21	Decahydro-1,1,3a-trimethyl-7-methylene-[1aS-(1aα,3aα,7aα,7b)]-1*H*-cyclopropa[a]naphthalene	32.153	0.37	0.034	-	Sesquiterpene	$C_{15}H_{24}$
22	2,2-Bis-(3,5-dimethoxybenzyl)-5,7-dimethoxyindan-1-one	32.353	0.277	0.143	0.125	Ketone	$C_{29}H_{32}O_7$
23	4-Ethenyl-4-methyl-3-(1-methylethenyl)-1-(methylethyl)-(3R-*trans*)-cyclohexene	32.636	1.306	-	0.27	Sesquiterpene	$C_{15}H_{24}$
24	α-Guaiene	32.847	0.092	0.959	0.65	Sesquiterpene	$C_{15}H_{24}$
25	[(2,4,6-Triethylbenzoyl)thio]acetic acid	33.096	-	0.033	0.286	Acid	$C_{15}H_{20}O_3S$
26	(-)-Isoaromadendrene-(V)	34.045	0.189	0.118	0.108	Sesquiterpene	$C_{15}H_{24}$
27	(3Z,8Z)-4,8,11,11-Tetramethylbicyclo[7.2.0]undeca-3,8-diene	34.262	2.193	1.586	1.159	Sesquiterpene	$C_{15}H_{24}$
28	1,2,3,3a,4,5,6,7-Octahydro-1,4-dimethyl-7-(1-methylethenyl)-[1R-(1α,3aβ,4α,7β)]-azulene	34.513	2.862	-	-	Sesquiterpene	$C_{15}H_{24}$
29	α-Eudesmol	34.673	0.846	2.004	1.696	Oxygenated sesquiterpene	$C_{15}H_{24}O$
30	Isocomene	34.796	-	0.215	0.062	Sesquiterpene	$C_{15}H_{24}$
31	1-Ethenyl-1-methyl-2,4-bis(1-methylethenyl)-[1S-(1α,2β,4β)]-cyclohexane	34.942	0.076	0.029	-	Sesquiterpene	$C_{15}H_{24}$
32	δ-Selinene	35.332	2.859	-	0.06	Sesquiterpene	$C_{15}H_{24}$
33	1,5,9-Trimethyl-1,5,9-cyclododecatriene	35.488	-	1.733	1.238	Sesquiterpene	$C_{15}H_{24}$
34	3-Methyl-2-(2,4-pentadienyl)-(*Z*)-2-cyclopenten-1-one	35.888	3.328	3.039	1.372	Ketone	$C_{11}H_{14}O$
35	Caryophyllene	36.104	0.072	-	-	Sesquiterpene	$C_{15}H_{24}$
36	1-Ethenyl-1-methyl-2-(1-methylethenyl)-4-(1-methylethyldene)-cyclohexane	36.191	3.558	0.081	0.039	Sesquiterpene	$C_{15}H_{24}$
37	γ-Elemene	36.509	0.096	4.336	1.883	Sesquiterpene	$C_{15}H_{24}$
38	α-Acorenol	37.072	1.103	0.853	-	Oxygenated sesquiterpene	$C_{15}H_{26}O$
39	2,6,6,9-Tetramethyl-(1R,2S,7R,8R)-tricyclo[5,4,0,0(2,8)] undec-9-ene	37.36	-	1.012	0.553	Sesquiterpene	$C_{15}H_{24}$
40	[1aR-(1aα,4aα,7β,7aβ,7bα)]-decahydro-1,1,7-trimethyl-4-methylene-, 1*H*-cycloprop[e]azulen-7-ol	37.759	0.438	0.033	0.037	Oxygenated sesquiterpene	$C_{15}H_{24}O$
41	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene	38.313	0.186	0.901	3.871	Sesquiterpene	$C_{15}H_{24}$
42	1-Methyl-5-methylene-8-(1-methylethyl)-[S-(E.E)]-1.6-cyclodecadiene	38.437	1.259	0.125	0.531	Sesquiterpene	$C_{15}H_{24}$
43	11-Isopropylidenetricyclo[4.3.1.1(2,5)]undec-3-en-10-one	38.656	0.659	2.049	0.73	Oxygenated sesquiterpene	$C_{14}H_{18}O$
44	1,5,5,8a-Tetramethyl-decahydro-[1S-(1α,2α,3aβ,4α,8aβ,9R)]-1,2,4-methenoazulne	38.935	-	0.318	0.356	Sesquiterpene	$C_{15}H_{24}$
45	β-Curcumene	39.314	-	0.078	-	Sesquiterpene	$C_{15}H_{24}$

(Contd...)

Table 1: *(Continued)*

No	Compounds	Retention time (min)	Peak area (%)			Class of chemical	Chemical formula
			Atractylodes lancea Thunb	*Atractylodes japonica* Koidz	*Atractylodes chinensis* Koidz		
46	β-Bisabolene	39.438	0.141	0.055	0.112	Sesquiterpene	$C_{15}H_{24}$
47	β-Copaene	39.56	-	-	0.309	Sesquiterpene	$C_{15}H_{24}$
48	Isocaryophillene	39.831	-	0.054	0.032	Sesquiterpene	$C_{15}H_{24}$
49	3-(1,5-Dimethyl-4-hexenyl)-6-methylene cyclohexene	39.923	-	1.238	0.566	Sesquiterpene	$C_{15}H_{24}$
50	Cubedol	40.103	0.993	-	-	Oxygenated sesquiterpene	$C_{15}H_{26}O$
51	Guaia-3,9-diene	40.434	2.785	-	2.937	Sesquiterpene	$C_{15}H_{24}$
52	Selina-3,7(11)-diene	40.524	-	6.62	0.803	Sesquiterpene	$C_{15}H_{24}$
53	2-(3-Isopropyl-4-methyl-pent-3-en-1-ynyl)-2-methyl-cyclobutanone	40.766	-	0.123	0.053	Oxygenated sesquiterpene	$C_{14}H_{20}O$
54	γ-Himachalene	40.832	0.343	0.605	0.157	Sesquiterpene	$C_{15}H_{24}$
55	Dehydroaromadendrene	41.318	0.373	1.478	1.425	Sesquiterpene	$C_{15}H_{24}$
56	Calarene epoxide	41.593	0.582	1.036	0.396	Oxygenated sesquiterpene	$C_{15}H_{24}O$
57	1-Hydroxy-1,7,7-dimethyl-4-isopropyl-2,7-cyclodecadiene	42.3	0.576	0.224	0.265	Oxygenated sesquiterpene	$C_{15}H_{26}O$
58	Decahydro-1,5,5,8a-tetramethyl-[1s-(1α,3aβ,4aα,7β,8aβ)]-1,4-methanoazulen-7-ol	42.521	-	0.084	-	Oxygenated sesquiterpene	$C_{15}H_{26}O$
59	Dihydrocucurbitacin B	42.706	0.331	0.176	0.312	Oxygenated Triterpenoids	$C_{32}H_{48}O_8$
60	Epiglobulol	43.201	0.296	0.06	0.196	Oxygenated sesquiterpene	$C_{15}H_{26}O$
61	2,2,6-Trimethyl-1-(3-methyl-1,3-butadienyl)-5-methylene-7-oxabicyclo[4.1.0]heptane	43.464	-	0.191	0.104	Oxygenated sesquiterpene	$C_{15}H_{22}O$
62	Cubenol	43.558	0.092	-	-	Oxygenated sesquiterpene	$C_{15}H_{26}O$
63	4-epi-Cubedol	43.863	0.118	-	0.081	Oxygenated sesquiterpene	$C_{15}H_{26}O$
64	Guaiol	44.138	5.006	-	0.075	Oxygenated sesquiterpene	$C_{15}H_{26}O$
65	γ-Eudesmol	44.354	0.325	0.33	-	Oxygenated sesquiterpene	$C_{15}H_{26}O$
66	Decahydro-α,α,4a-trimethyl-8-methylene-[2R-(2α,4aα,8aβ)]-2-naphthalenemethanol	44.608	3.62	0.153	2.515	Oxygenated sesquiterpene	$C_{15}H_{26}O$
67	Curzerene	44.991	14.179	3.558	16.729	Oxygenated sesquiterpene	$C_{15}H_{20}O$
68	1,2,3,4,4a,5,6,8a-Octahydro-α,α,4a,8-tetramethyl-[2R-(2α,4Aα,8Aβ)]-2-naphthalenemethanol	45.199	1.225	0.288	-	Oxygenated sesquiterpene	$C_{15}H_{26}O$
69	3-Hydroxy-7-isopropenyl-1,4a-dimethyl-2,3,4,4a,5,6,7,8-octahydronaphthalen-2-yl acetate	45.454	-	0.568	-	Ester	$C_{17}H_{26}O_3$
70	Fenretinide	45.713	-	0.214	0.762	Other	$C_{26}H_{33}NO_2$
71	Ambrosin	45.795	0.412	0.079	1.271	Oxygenated sesquiterpene	$C_{15}H_{18}O_3$
72	Diepicedrene-1-oxide	46.31	-	0.091	0.137	Oxygenated sesquiterpene	$C_{15}H_{24}O$
73	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalene-2,3-diol	46.482	1.817	-	1.216	Oxygenated sesquiterpene	$C_{15}H_{24}O_2$
74	[1,1′-Biphenyl]-4-carbonyl chloride	46.916	0.079	0.028	0.148	Other	$C_{13}H_9ClO$
75	Ledene oxide-(II)	47.443	-	-	0.185	Oxygenated sesquiterpene	$C_{15}H_{24}O$
76	Murolan-3,9(11)-diene-10-peroxy	47.604	0.658	16.883	0.992	Oxygenated sesquiterpene	$C_{15}H_{24}O_2$
77	Longiverbenone	47.747	-	-	0.031	Oxygenated sesquiterpene	$C_{15}H_{22}O$
78	Neocurdione	48.044	0.239	0.778	-	Oxygenated sesquiterpene	$C_{15}H_{24}O_2$
79	[1,1′-Biphenyl]-4-carboxaldehyde	48.798	0.51	-	2.647	Aldehyde	$C_{13}H_{10}O$
80	(3β,5Z,7E)-9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol	49.388	0.227	-	0.937	Other	$C_{27}H_{44}O_3$
81	2-Methyl-4-(1,3,3-trimethyl-7-oxabicyclo[4.1.0]hept-2-yl)-3-buten-2-ol	49.676	0.275	0.066	0.056	Oxygenated sesquiterpene	$C_{14}H_{24}O_2$

(Contd...)

Table 1: *(Continued)*

No	Compounds	Retention time (min)	Peak area (%)			Class of chemical	Chemical formula
			Atractylodes lancea Thunb	*Atractylodes japonica* Koidz	*Atractylodes chinensis* Koidz		
82	Decahydro-6,9a-dimethyl-3-methylene-, (3aS,6S,6aS,9aR,9bR)-azuleno[4,5-b]furan-2,9-dione	50.251	0.385	-	-	Oxygenated sesquiterpene	$C_{15}H_{20}O_4$
83	*trans*-Longipinocarveol	50.734	0.163	0.032	0.024	Oxygenated sesquiterpene	$C_{15}H_{24}O$
84	7,8-Di(hydroxymethyl)-5-methyl-2-isopropyl-spiro-6-(bicyclo[3.2.1]octane)-2′-(oxirane)	51.739	0.132	0.109	-	Oxygenated sesquiterpene	$C_{15}H_{26}O_3$
85	(10a-Hydroxy-3a-methoxy-2,10-dimethyl-3,8-dioxo-4,6a,7,9,10,10b-hexahydrobenzo[e]azulen-5-yl)methyl acetate	52	2.803	-	0.355	Ester	$C_{20}H_{26}O_6$
86	7,8,15,16-Tetramethyl-1,9-dioxacyclohexadeca-4,13-diene-2-10-dione	52.709	0.126	0.394	0.72	Ketone	$C_{18}H_{28}O_4$
87	5-Methyl-1-[2,6,6-trimethyl-2,4-cyclohexadien-1-yl]-1,4-hexadien-3-one	52.955	3.01	0.024	0.017	Ketone	$C_{20}H_{30}O_5$
88	*n*-Hexadecanoic acid	53.577	0.289	0.036	0.039	Acid	$C_{16}H_{32}O_2$
89	Methyl Retinoate	55.155	0.197	0.086	-	Ester	$C_{21}H_{30}O_2$
90	Andrographolide	55.555	0.278	-	0.016	Ketone	$C_{16}H_{22}O$
91	4,4′-Dimethyl-2,2′-dimethylenebicyclohexyl-3,3′-diene	56.561	0.248	0.374	0.083	Alkene	$C_{16}H_{22}$
92	(6a,9-Dihydroxy-6-methyl-3-methylidene-2-oxo-3a,4,5,6,7,8,9,9b-octahydroazuleno[4,5-b]furan-9a-yl) methyl acetate	57.98	0.067	0.149	0.037	Ester	$C_{17}H_{24}O_6$
93	Propoxyphene	61.099	1.077	-	0.019	Ketone	C22H2906
94	9,10-dihydro-9,10[1′,2′]-benzenoanthracene	64.218	0.042	4.39	0.12	Alkene	$C_{20}H_{14}$
95	3-(4-Methoxyphenyl)-2-ethylhexyl-2-propenoate	64.58	0.249	1.622	1.079	Ester	$C_{18}H_{26}O_3$
96	2-(4-Diethylaminophenyliminomethyl)phenol	66.929	0.154	1.272	3.264	Acid	$C_{17}H_{20}N_2$
97	9-Cycloheptatrienylidene-9,10-dihydro-10-oxoanthracene	74.656	-	1.87	0.178	Ketone	$C_{21}H_{14}O$
98	Methyl 9,11-octadecadienoate	78.213	-	5.221	-	Ester	$C_{19}H_{30}O_2$
99	*N*-[[3,6-dichloro-2,7-bis(2-diethylaminoethyloxy)fluoren-9-ylidene]amino]-2,2-dimethylpropanamide	78.587	0.669	9.382	-	Other	$C_{30}H_{42}C_{l2}N_4O_3$
		Total	82.528	81.766	81.799		

7,8-di(hydroxymethyl)-5-methyl-2-isopropyl-spiro-6-(bicyclo[3.2.1]octane)-2'-(oxirane), methyl retinoate, and N-[[3,6-dichloro-2,7-bis(2-diethylaminoethyloxy) fluoren-9-ylidene]amino]-2,2-dimethylpropanamide were observed both in A. *lancea* and A. *japonica* samples. Both in A. *japonica* and A. *chinensis* further 14 compounds were detected, namely γ-terpinene, [(2,4,6-triethylbenzoyl) thio]acetic acid, isocomene, 1,5,9-trimethyl-1,5,9-cyclododecatriene, 2,6,6,9-tetramethyl-(1R,2S,7R,8R)-tricyclop[5,4,0,0(2,8)]undec-9-ene, 1,5,5,8a-tetramethyl-decahydro-[1S-(1α,2α,3aβ,4α,8aβ,9R)]-1,2,4-methenoazulne, isocaryophillene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-cyclohexene, selina-3,7(11)-diene, 2-(3-isopropyl-4-methyl-pent-3-en-1-ynyl)-2-methyl-cyclobutanone, 2,2,6-trimethyl-1-(3-methyl-1,3-butadienyl)-5-methylene-7-oxabicyclo[4,1,0] heptane, fenretinide, diepicedrene-1-oxide, and 9-cycloheptatrienylidene-9,10-dihydro-10-oxoanthracene. The remaining compounds, i.e. D-limonene, α-ylangene, 4-terpinenyl acetate, 1,5,5,6-tetramethyl-1,3-cyclohexadiene, 1,2-ethanediol monobenzoate, 2,6-pyridinedicarboxaldehyde, 3,6-diethyl-3,6-dimethyl-*trans*-tricyclo[3.1.0.0(2.4)] hexane, longifolene-(V4), 4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(methylethyl)-(3R-*trans*)-cyclohexene, δ-selinene, guaia-3,9-diene, 4-epi-cubedol, guaiol, 6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalene-2,3-diol, [1,1'-biphenyl]-4-carboxaldehyde, (3β,5Z,7E)-9,10-secocholesta-5,7,10(19)-triene-3,24,25-triol, (10a-hydroxy-3a-methoxy-2,10-dimethyl-3,8-dioxo-4,6a,7,9,10,10b-hexahydrobenzo[e]azulen-5-yl) methyl acetate, andrographolide, and propoxyphene, were detected both in A. *lancea* and A. *chinensis*. Considering all 99 volatile compounds, the total amounts contained in each of the three A*tractylode* species were comparable, with values of 82.528%, 81.766%, and 81.799% calculated for A. *lancea*, A. *japonica*, and the most abundant volatile and A. *chinesis*, respectively. Among these compounds detected in A. *lancea* were curzerene (14.1%) and 3-octen-5-yne (9.01%), while murolan-3,9(11)-diene-10-peroxy (16.8%) was the most abundant volatile in A. *japonica*. The most abundant compounds in A. *chinensis* were curzerene (16.7%), γ-terpinene (10.3%), N-[[3,6-dichloro-2,7-bis(2-diethylaminoethyloxy)fluoren-9-ylidene]amino]-2,2-dimethylpropanamide (9.4%), and 4-terpinenyl acetate (8.2%).

Numbers of Volatile Flavor Compounds and their Quantities (%) in Different species of *Atractylodes*

There are 13 volatile flavor compounds i.e. acid, alcohol, aldehyde, alkane, alkene, alkyne, ester, ketone, monoterpene, oxygenated monoterpene, sesquiterpene, oxygenated sesquiterpene, and oxygenated triterpenoid detected from different species of A*tractylodes* (Table 2). The amount of alcohol was too low within the species where no alcohol was found in A. *chinensis*. The highest amount of acid, aldehyde, alkane, monoterpene, and oxygenated monoterpene types volatile flavor compounds were

Table 2: Numbers of volatile flavor compounds in *Atractylodes* spp.

Class of chemical components	*Atractylodes lancea* Thunb		*Atractylodes japonica* Koidz		*Atractylodes chinensis* Koidz	
	Number	%	Number	%	Number	%
Acid	2	0.443	3	1.341	3	3.589
Alcohol	1	0.171	1	0.135	1	-
Aldehyde	2	0.665	0	-	0	2.949
Alkane	1	0.193	0	-	1	0.647
Alkene	3	0.799	2	4.764	2	0.203
Alkyne	1	9.007	0	-	0	-
Ester	6	3.498	6	8.599	5	2.904
Ketone	6	8.096	5	5.470	7	2.447
Monoterpene	4	3.194	3	0.718	7	12.796
Oxygenated monoterpene	1	0.463	0	-	1	8.205
Sesquiterpene	20	19.911	21	21.887	24	18.400
Oxygenated sesquiterpene	23	34.139	21	29.024	20	26.789
Oxygenated triterpenoid	1	0.331	1	0.176	1	0.312
Other	4	1.618	4	9.652	4	2.558
Total	75	82.528	67	81.766	76	81.799

found in A. *chinensis*. The levels of accumulation of alcohol, alkyne, ketone, and oxygenated sesquiterpenes were found to be the highest amount in A. *lancea*. The species A. *japonica* contained the highest amount of alkene, ester and sesquiterpene. Among the volatile flavor compounds oxygenated sesquiterpene dominated over other volatile flavors compounds irrespective of species. The species A. *lancea*, A. *japonica*, and A. *chinensis* contained 23, 21, and 20 oxygenated sesquiterpenes, having 41.37%, 35.50%, and 32.75% of total volatile flavor compounds, respectively. After oxygenated sesquiterpene, the second-largest accumulated volatile flavor compounds were sesquiterpene. Here the contained of sesquiterpene was 26.77%, 24.13%, and 22.49% in the A. *japonica*, A. *lancea*, and A. *chinensis*, respectively. Volatile flavor compounds alkyne was detected only in the species of A. *lancea* having 10.91% of total volatile flavor compounds. The amount of ester was 10.52%, 4.24%, and 3.55% in the A. *japonica*, A. *lancea*, and A. *chinensis*, respectively.

DISCUSSION

The essential oils and volatile compounds derived from the A. *lancea*, A. *japonica*, and A. *chinensis* species have been reported to have therapeutic value in Chinese medicine. The present study identified 99 volatile compounds from these plants using GC-MS, by quantifying each volatile compound in the three species. Interestingly, it was found that although all extracts contained 38 common compounds, each extract also contained volatiles unique to that particular species. It was found that the variation in the quantity of these compounds depends on the location of the collected samples as well as the differences of species. A total of 77 volatile compounds were detected in total having 13 monoterpenoids, 19 sesquiterpenoids, and others in *Mentha* species (Park et al., 2016). In an another study (Zouaoui *et al*., 2020) reported that a total of 91 volatile organic compounds (VOC): 39 VOC were identified in *Thymusalgeriensis* (with dominance of β-myrcene = 13.78%, camphor = 12.29%, linalyl acetate = 9.11%); 37 VOC in *Artemisia*campestris (β-farnesene = 14.17%, β-myrcene = 13.84%); 50 VOC in *Juniperusphoenicea* (α-pinene = 27.18%); 42 VOC in *Teucrium polium* (α- guaiene = 11.33%, trans-caryophyllene = 9.49%, γ-elemene = 9.25%), 45 VOC in *Rosmarinus officinalis* (camphor = 17.46%, trans-caryophyllene = 14.83%); and 41 in *Artemisia herba-alba* (α-thujone = 24.59%, β-thujone = 13.73%). In *Artemisia herba-alba* growing in the region of biskra, α-thujone (24.59%) and β-thujone (13.73%) were the major compounds, followed by verbenene (8.30%), sabinol (7.51%), carvone (5.05%), and p-cineole (4.81%). These results are partially similar to those reported by (Belhattab *et al*., 2014) that used plant samples from different regions (Benifouda, Bougaa, Boussaada, and Boutaleb) of Algeria; and from the region of Buseirah (Jordan) (Abu-Darwish *et al*., 2015). Here in this study, a total of 99 different volatile compounds have been detected which indicated variation of volatile compounds might vary with the variation of region. The nature of volatile compounds varied from species to species. α-pinene (27.18%) was the major compound *in Juniperus phoenicea* growing in drylands of Algeria. It is followed by β-citronellol (6.13%), δ-3-carene (4.78%), β-farnesene (4.71%), α-terpineol (4.12%), germacrene D (3.50%), δ-cadinene (3.26%), and geranyl acetone (3.01%). These results are in agreement with those described by (Mazari *et al*., 2010) in the region of Sidi Safi (Tlemcen, Algeria) and in the region of Angad (Oujda, Morocco) (Ait-Ouazzou et al., 2012).The major VOC in *Teucriumpolium* were α-guaiene (11.33%), trans-caryophyllene (9.49%), and γ-elemene (9.25%). These are followed by β-farnesene (7.56%), farnesol (6.14%), allo-aromadendrene (4.34%), δ-guaiene (4.21%), geranyl acetone (3.65%) and α-gurjunene (3.36%). Octyl acetate (24.22 to 33.16 %), 2-undecanone (12.43 to 23.82%), and 2-nonanone (14.11 to 41.69%) were found to be major components of the volatiles extracted by hydro distillation or head-space method of two populations of *Ruta chalepensis* L. (Rutaceae) (Fakhfakh *et al*., 2012), whereas in this study curzerene was found to be the most predominant compound in both A. *lancea* (14.1%) and A. *chinensis* (16.7%), while murolan-3,9(11)-diene-10-peroxy was found predominantly in A. *japonica* (16.8%). Our findings are in agreement with many previous studies that applied the same procedures for the extraction and detection of volatile compounds. For instance, when GC–MS was used, the number of VOC was 61 compounds in *Teucrium polium* (Gholivand *et al*., 2013) and 42 compounds in *Rhaponticum acaule* roots (Benyelles *et al*., 2014). The chemical composition of *Thymus algeriensis* is marked by the presence of β-myrcene (13.78%), camphor (12.29%), and linalyl acetate (9.11%) as the major constituents, followed by p-cineole (6.31%), β-farnesene (5.23%), terpineol (5.07%), bornyl acetate (4.79%), α-pinene (4.65%) and camphene (4.61%). These results are partially in line with those reported by (Zouari *et al*., 2011) and (Ali *et al*., 2012). According to (Ali *et al*., 2015), there is a large quantitative and qualitative variation in VOC between leaves, stems, and roots of the same plant species. In *Artemisia campestris* growing in Algerian drylands, the major VOC are β-farnesene (14.17%) and β-myrcene (13.84%) followed by α-cedrene (7.88%), germacrene D (7.29%), α-pinene (4.63%),

and β-pinene (4.21%). These results are partially in line with those reported by (Ghorab *et al*., 2013) and (Al Jahid *et al*., 2016). The slight quantitative difference in contents of major VOC may be due to genetic variation and geographical origin of plant material; knowing that (Al Jahid *et al*., 2016) collected samples from Saharan zones of Morocco, whereas (Ghorab *et al*., 2013) harvested plants from semi-arid areas of Algeria. Besides, differences in VOC contents between studies can be related to differences in extraction method, analysis conditions or even the vegetal organ analyzed 'leaves in (Al Jahid *et al*., 2016)' or the freshness of plant materials, as (Ghorab *et al*., 2013) used fresh plants in VOC screening while most studies use dried plant materials.

The present study suggests that the identified volatile compounds may possess important biological properties, and could be suitable for application in both oriental medicines and the pharmaceutical industry. This report, therefore, presents further information regarding the quantification and abundance of these volatile compounds, which are expected to possess a range of important biological properties, and could there be useful for application in oriental medicine in countries such as Korea and China.

CONCLUSION

Based on these results, it is suggested that the *Atractylodes* species and their identified volatile compounds may possess important biological properties, and could be suitable for application in both oriental medicines and in the pharmaceutical industry. Appropriate separation of the components within these essential oils may lead to the development of new drug targets or therapeutic treatments.

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