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, alkenol, ester, ketone, monoterpen, oxygenated monoterpenes, sesquiterpen, oxygenated sesquiterpene, and oxygenated interpenoid 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)-dien-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

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 provide 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 interplant 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 H₂O₂-
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 5 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 5 min 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 × 0.32nmID, 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-(1S,2R)-azulene, and (3S,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,3β,4α,7β,8α)]-1,4-methanoazulen-7-ol, 3-hydroxy-7-isopropenyl-1,4a-dimethyl-2,3,4,4a,5,6,7,8-octahydrophthalan-2-yl acetate, and methyl 9,11-octadecadienoate. In addition, six volatile compounds, namely α-phellandrene, 1-methyl-3-(1-methylthyl)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-benzemethanol, decahydro-1,1,3a-trimethyl-7-methylene-[1aS-(1α,3α,7α,7b,7a)]-1H-cyclopropa[a]naphthalene, 1-ethenyl-1-methyl-2,4-bis(1-methylene)-15S-(1α,2β,4β)-cyclohexane, α-acorenol, γ-eudesmol, 1,2,3,4,4a,5,6,8a-octahydro-α,α,4a,8-tetramethyl-[2R-(2α,4α,8αβ)]-2-naphthalenemethanol, neocurdiene,
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 |
|----|-----------|----------------------|---------------|-----------------------------------|
| 1  | 3-Octen-5-yne | 11.392 | 9.007 | - | Alkyne C8H16 |
| 2  | γ-Terpinene | 13.613 | - | 0.053 | Monoterpene C10H16 |
| 3  | D-Limonene | 13.801 | 0.585 | - | Monoterpene C10H16 |
| 4  | α-Phellandrene | 14.447 | - | 0.211 | Monoterpene C15H24 |
| 5  | 5-Butyl-1,3-cyclohexadiene | 14.515 | 0.509 | - | Alkene C15H18 |
| 6  | 2-Carene | 15.615 | 2.273 | 0.426 | Monoterpene C15H24 |
| 7  | 1-Methyl-3-(1-methylethyl)benzene | 15.761 | - | - | Monoterpene C16H16 |
| 8  | α-Ylangene | 16 | 0.748 | - | Monoterpene C16H20 |
| 9  | 2,5-Dimethyl-3-methylene-1,5-heptadiene | 16.483 | 0.091 | 0.239 | Monoterpene C16H20 |
| 10 | 4-Terpinyl acetate | 17.028 | 0.463 | - | 8.205 | Oxygenated monoterpenes C20H30O2 |
| 11 | 6-Isopropyliden-1-methylbicyclo[3.1.0]hexane | 17.218 | - | - | Monoterpenes C16H20 |
| 12 | 1,5,5,6-Tetramethyl-1,3-cyclohexadiene | 17.502 | 0.245 | - | Monoterpenes C16H20 |
| 13 | m-Phenethylbenzonitrile | 18.24 | 0.643 | 0.028 | Other C10H13N |
| 14 | 1,2-Ethandiol monobenzoate | 18.498 | 0.086 | - | 1.318 | Ester C10H13O2 |
| 15 | 2-Bromomethyl benzoate | 18.969 | 0.096 | 0.953 | 0.115 | Ester C10H13BrO |
| 16 | α-Ethyl-α-(2,5,7-octatrienyl)benzenemethanol | 18.811 | 0.171 | 0.135 | Alcohol C16H20O |
| 17 | 2,6-Pyrindinedicarboxaldehyde | 19.202 | 0.155 | - | 0.302 | Alddehyde C7H8O2 |
| 18 | 3,6-Diethyl-3,6-dimethyl-trans-tricyclo[3.1.0.0(2,4)]hexane | 21.164 | 0.193 | - | 0.647 | Alkane C15H20 |
| 19 | Longifolene-(V4) | 26.417 | 0.232 | - | 0.359 | Sesquiterpenes C20H30 |
| 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 | Sesquiterpenes C20H30 |
| 21 | Decahydo-1,1a,3a-trimethyl-7-methylene-[1aS-(1aα,3aα,7aβ)]:1 H-cyclopenta[α]naphthalene | 32.153 | 0.37 | 0.034 | - | Sesquiterpenes C20H30 |
| 22 | 2,2-Bis-(3,5-dimethoxybenzyl)-5,7-dimethyloxindan-1-one | 32.353 | 0.277 | 0.143 | 0.125 | Ketone C20H20O2 |
| 23 | 4-Ethenyl-4-methyl-3-(1-methylethyl)-1-(methylethyl)- (3R)-trans-cyclohexene | 32.636 | 1.306 | - | 0.27 | Sesquiterpenes C20H30 |
| 24 | α-Guaiae | 32.847 | 0.092 | 0.959 | 0.65 | Sesquiterpenes C20H30 |
| 25 | [(2,4,6-Triethylbenzyl)thio]acetic acid | 33.096 | - | 0.033 | 0.286 | Acid C15H22O2S |
| 26 | (-)-Isoromandendrene-(V) | 34.045 | 0.189 | 0.118 | 0.108 | Sesquiterpenes C20H30 |
| 27 | (32,82)-4,8,11,11-Tetramethylbicyclo[7.2.0]undeca-3,8-diene | 34.262 | 2.193 | 1.586 | 1.159 | Sesquiterpenes C20H30 |
| 28 | 1,2,3,3a,4,4,6,7-Octahydro-1,4-dimethyl-7-(1- (1-methylallyl)-(1R-(1a,3aα,4α,7β)]-azulene | 34.513 | 2.862 | - | - | Sesquiterpenes C20H30 |
| 29 | α-Eudesmol | 34.673 | 0.846 | 2.004 | 1.696 | Oxygenated sesquiterpenes C20H30O |
| 30 | Isoeudesmol | 34.796 | - | 0.215 | 0.062 | Sesquiterpenes C20H30 |
| 31 | 1-Ethenyl-1-methyl-2,4-bis(1-methylethyl)-[15-(1a,2b,4b)]-cyclohexane | 34.942 | 0.076 | 0.029 | - | Sesquiterpenes C20H30 |
| 32 | δ-Selinene | 35.323 | 2.859 | - | 0.06 | Sesquiterpenes C20H30 |
| 33 | 1,5,9-Trimethyl-1,5,9-cyclodecatriene | 35.488 | - | 1.733 | 1.238 | Sesquiterpenes C20H30 |
| 34 | 3-Methyl-2-(2,4-pentadienyl)-(Z)-2-cyclopenten-1-one | 35.888 | 3.328 | 3.039 | 1.372 | Ketone C14H20O |
| 35 | Caryophyllene | 36.104 | 0.072 | - | - | Sesquiterpenes C20H30 |
| 36 | 1-Ethenyl-1-methyl-2-(1-methylethyl)-4-(1- (1-methylallyl)-cyclohexene | 36.191 | 3.558 | 0.081 | 0.039 | Sesquiterpenes C20H30 |
| 37 | γ-Elemene | 36.509 | 0.096 | 4.336 | 1.883 | Sesquiterpenes C20H30 |
| 38 | α-Acoranol | 37.072 | 1.103 | 0.853 | - | Oxygenated sesquiterpenes C20H30O |
| 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 | Sesquiterpenes C20H30 |
| 40 | (1αR-1αa,4αa,7αβ,7β)trans-decahydro-1,1,7-trimethyl- 4-methylene, 1H-cyclopenta[α]azulen-7-ol | 37.759 | 0.438 | 0.033 | 0.027 | Oxygenated sesquiterpenes C20H30 |
| 41 | 2-Isopropenyl-4α,8-dimethyl-1,2,3,4a,5,6,7-octahydroanthracene | 38.313 | 0.186 | 0.901 | 3.871 | Sesquiterpenes C20H30 |
| 42 | 1-Methyl-5-methoxy-8-(1-methylethyl)-[5-(E,E)]-1,6-cycloaddene | 38.437 | 1.259 | - | 0.125 | 0.531 | Sesquiterpenes C20H30 |
| 43 | 11-Isopropylidenetricyclo[4.3.1.1(2,5)]undec-3-ene-10-one | 38.656 | 0.659 | 2.049 | 0.73 | Oxygenated sesquiterpenes C20H30 |
| 44 | 1,5,5,8a-Tetramethyl-decahydro-(1S-(1α,2a,3α,4a,8a,9β)]-1,2,4-methanoazulene | 38.935 | - | 0.318 | 0.356 | Sesquiterpenes C20H30 |
| 45 | β-Curcumene | 39.314 | - | 0.078 | - | Sesquiterpenes C20H30 |

(Contd...)
| No | Compounds                                                      | Retention time (min) | Peak area (%) | Class of chemical | Chemical formula |
|----|---------------------------------------------------------------|----------------------|---------------|-------------------|------------------|
|    |                                                               | Atractylodes lancea  | Atractylodes japonica | Atractylodes chinensis |                 |
|    |                                                               | (Thunb)              | (Koidz)       | (Koidz)           |                  |
| 46 | β-Bisabolene                                                  | 39.438               | 0.141         | 0.055             | 0.112            | Sesquiterpene    | C_{25}H_{30}O    |
| 47 | β-Copaene                                                     | 39.56                | -             | -                 | 0.309            | Sesquiterpene    | C_{25}H_{30}O    |
| 48 | Isocaryophyllene                                              | 39.831               | -             | 0.054             | 0.032            | Sesquiterpene    | C_{25}H_{30}O    |
| 49 | 3-(1,5-Dimethyl-4-hexenyl)-6-methylene cyclohexene            | 39.923               | 1.238         | 0.566             |                  | Sesquiterpene    | C_{25}H_{30}O    |
| 50 | Cubedol                                                       | 40.103               | 0.993         | -                 | 0.396            | Oxygenated sesquiterpene | C_{25}H_{30}O    |
| 51 | Guaia-3,9-diene                                               | 40.434               | 2.785         | -                 | 2.937            | Sesquiterpene    | C_{15}H_{20}O    |
| 52 | Selina-3,7(11)-diene                                          | 40.524               | -             | 6.62              | 0.803            | Sesquiterpene    | C_{15}H_{20}O    |
| 53 | 2-(3-Isopropyl-4-methyl-pent-3-en-1-ynyl)-2-methyl-cyclobutanone | 40.766               | 0.123         | 0.053             |                  | Oxygenated sesquiterpene | C_{15}H_{20}O    |
| 54 | γ-Himachalene                                                 | 40.832               | 0.343         | 0.605             | 0.157            | Sesquiterpene    | C_{25}H_{30}O    |
| 55 | Dehydroaromadendrene                                          | 41.318               | 0.373         | 1.478             | 1.425            | Sesquiterpene    | C_{25}H_{30}O    |
| 56 | Calarene epoxide                                              | 41.593               | 0.582         | 1.036             | 0.396            | Oxygenated sesquiterpene | C_{25}H_{30}O    |
| 57 | 1-Hydroxy-1,7,7-dimethyl-4-isopropyl-2,7-cyclododecadiene     | 42.3                 | 0.576         | 0.224             | 0.265            | Oxygenated sesquiterpene | C_{25}H_{30}O    |
| 58 | Decahydro-1,5,5,8a-tetramethyl-[1s-(1α,3αj,4αa,7β,8βj)]-1,4-methanoazulene-7-ol | 42.521 | - | 0.084 | - | Oxygenated sesquiterpene | C_{15}H_{20}O |
| 59 | Dihydrocurcurbitacin B                                        | 42.706               | 0.331         | 0.176             | 0.312            | Oxygenated Triterpenoids | C_{25}H_{48}O |
| 60 | Epiglobulol                                                   | 43.201               | 0.296         | 0.06              | 0.196            | Oxygenated sesquiterpene | C_{25}H_{30}O |
| 61 | 2,2,6-Trimethyl-1-(3-methyl-1,3-butadienyl)-5-methylene-octahydro-1-(4,1.0)heptane | 43.464 | - | 0.191 | 0.104 | Oxygenated sesquiterpene | C_{25}H_{30}O |
| 62 | Cubenol                                                       | 43.558               | 0.092         | -                 | -                | Oxygenated sesquiterpene | C_{25}H_{30}O |
| 63 | 4-epi-Cubedol                                                 | 43.863               | 0.118         | -                 | 0.081            | Oxygenated sesquiterpene | C_{25}H_{30}O |
| 64 | Guaiol                                                        | 44.138               | 5.006         | -                 | 0.075            | Oxygenated sesquiterpene | C_{25}H_{30}O |
| 65 | γ-Eudesmol                                                    | 44.354               | 0.325         | 0.33              | -                | Oxygenated sesquiterpene | C_{25}H_{30}O |
| 66 | Decahydro-α,α,4a-trimethyl-8-methylene-[2R-(2a,4a,6aβ)-2-naphthalenemethanol | 44.608 | 3.62 | 0.153 | 2.515 | Oxygenated sesquiterpene | C_{25}H_{30}O |
| 67 | Curzerene                                                     | 44.991               | 14.179        | 3.558             | 16.729           | Oxygenated sesquiterpene | C_{25}H_{30}O |
| 68 | 1,2,3,4,4a,5,6b,8a-Octahydro-α,α,4a,8-tetramethyl-[2R-(2a,4a,6aβ)-2-naphthalenemethanol | 45.199 | 1.225 | 0.288 | - | Oxygenated sesquiterpene | C_{25}H_{30}O |
| 69 | 3-Hydroxy-7-isopropenyl-1,4a-dimethyl-2,3,4,4a,5,6,7,8-octahydroxynaphthalen-2-yl acetate | 45.454 | - | 0.568 | - | Ester | C_{25}H_{30}O |
| 70 | Fenretinide                                                   | 45.713               | -             | 0.214             | 0.762            | Other Oxygenated sesquiterpene | C_{25}H_{30}O NO |
| 71 | Ambrosin                                                      | 45.795               | 0.412         | 0.079             | 1.271            | Other Oxygenated sesquiterpene | C_{25}H_{30}O OX |
| 72 | Diepicedrene-1-oxide                                          | 46.31                | -             | 0.091             | 0.137            | Oxygenated sesquiterpene | C_{25}H_{30}O |
| 73 | 6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8a-octahydroxynaphthalen-2,3-diol | 46.482 | 1.817 | - | 1.216 | Oxygenated sesquiterpene | C_{25}H_{30}O |
| 74 | [1,1’-Biphenyl]-4-carboxylic acid                             | 46.916               | 0.079         | 0.028             | 0.148            | Other Oxygenated sesquiterpene | C_{25}H_{30}O ClO |
| 75 | Ledene oxide-(II)                                             | 47.443               | -             | -                 | 0.185            | Oxygenated sesquiterpene | C_{25}H_{30}O |
| 76 | Murolan-3,9(11)-diene-10-peroxy                              | 47.604               | 0.658         | 16.883            | 0.992            | Oxygenated sesquiterpene | C_{25}H_{30}O |
| 77 | Longiverbenone                                                | 47.747               | -             | -                 | 0.031            | Oxygenated sesquiterpene | C_{25}H_{30}O |
| 78 | Neocurdiine                                                   | 48.044               | 0.239         | 0.778             | -                | Oxygenated sesquiterpene | C_{25}H_{30}O |
| 79 | [1,1’-Biphenyl]-4-carboxaldehyde                             | 48.798               | 0.51          | -                 | 2.647            | Aldehyde | C_{15}H_{10}O |
| 80 | (3F,5Z,7E)-9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol | 49.388               | 0.227         | -                 | 0.937            | Other | C_{25}H_{30}O |
| 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_{25}H_{30}O |

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Table 1: (Continued)

| No | Compounds                                                                 | Retention time (min) | Peak area (%) | Class of chemical | Chemical formula |
|----|---------------------------------------------------------------------------|----------------------|--------------|-------------------|-----------------|
| 82 | Decahydro-6,9a-dimethyl-3-methylene-, (3α,6S,6aS,9αR,9bR)-azulenol(4,5-b)furan-2,9-dione | 50.251              | 0.385        | -                 | Oxygenated      | C₂₉H₂₅O₄       |
| 83 | trans-Longipinocarveol                                                     | 50.734              | 0.163        | -                 | Oxygenated      | C₂₉H₂₃O       |
| 84 | 7,8-Dihydroxymethyl)-5-methyl-2-isopropyl-spiro-6-(bicyclo[3.2.1]octane)-2'-oxirane | 51.739              | 0.132        | -                 | Oxygenated      | C₂₉H₂₃O       |
| 85 | (10α-Hydroxy-3α-methoxy-2,10-dimethyl-3,8-dioxo-4,6a,7,9,10,10b-hexahydrobenzo(e)azulen-5-yl)methyl acetate | 52                  | 2.803        | -                 | Ester           | C₂₅H₂₄O₄       |
| 86 | 7,8,15,16-Tetramethyl-1,9-dioxacyclohexadeca-4,13-diene-2,10-dione           | 52.709              | 0.126        | -                 | Ketone          | C₂₇H₂₄O₄       |
| 87 | 5-Methyl-1,2,6,6-trimethyl-2,4-cyclohexadien-1-yl)-1,4-hexadien-3-one       | 52.955              | 3.01         | 0.024             | Ketone          | C₂₇H₂₄O₆       |
| 88 | n-Hexadecanoic acid                                                        | 53.577              | 0.289        | 0.036             | Acid            | C₁₈H₃₄O₂       |
| 89 | Methyl Retinoate                                                          | 55.155              | 0.197        | 0.086             | Ester           | C₁₈H₂₀O₂       |
| 90 | Androgapholide                                                            | 55.555              | 0.278        | -                 | Ketone          | C₂₁H₁₄O       |
| 91 | 4,4'-Dimethyl-2,2'-dimethylenecyclohexyl-3,3'-diene                        | 56.561              | 0.248        | 0.374             | Alkene          | C₂₇H₃₄O       |
| 92 | (6α,9-Dihydroxy-6-methyl-3-methylidene-2-oxo-3a,4,5,6,7,8,9,9b-octahydroazulen-4,5-bifuran-9-yl)methyl acetate | 57.98               | 0.067        | 0.149             | Ester           | C₂₃H₂₄O₆       |
| 93 | Propoxyphene                                                               | 61.099              | 1.077        | -                 | Ketone          | C₂₂H₂₉O₆       |
| 94 | 9,10-dihydro-9,10(1′,1′)-benzenoanthracene                                 | 64.218              | 0.042        | 4.39              | Alkene          | C₂₃H₂₄O        |
| 95 | 3-(4-Methoxyphenyl)-2-ethylhexyl-2-propanoate                              | 64.58               | 0.249        | 1.622             | Ester           | C₂₃H₂₄O₂       |
| 96 | 2-(4-Diethylaminophenyliminophenyl)methanol                                 | 66.929              | 0.154        | 1.272             | Acid            | C₂₃H₂₄N₂       |
| 97 | 9-Cycloheptatrienylidene-9,10-dihydro-10-oxygenated triterpenoid           | 74.656              | -            | 1.87              | Ketone          | C₂₃H₂₄O       |
| 98 | Methyl 9,11-decadienoate                                                   | 78.213              | -            | 5.221             | Ketone          | C₂₃H₂₄O       |
| 99 | N-(3,6-dichloro-2,7-bis(2-diethylnitrooxy)fluoren-9-ylidene)(amino)-2,2-dimethylpropanamide | 78.587              | 0.669        | 9.382             | Other           | C₂₃H₂₃N₃O₄    |

| 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-diethylnitrooxy)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 α-terpinene, [(2,4,6-trithylenzoil)thio]acetic acid, isocarophyllene, 1,5,9-trimethyl-1,5,9-cyclododecatriene, 2,6,6,9-tetramethyl-(1R,2S,7R,8R)-tricyclo-[5,4,0.0(2,8)]undec-9-ene, 1,5,5,5a-tetramethyldecahydro-[1S-(1α,2α,3αβ,4α,5αβ,9βR)]-1,2,4-methanoazulene, isocarophyllene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-cyclohexene, selina-3,7(11)-diene, 2-(3-isopropyl-4-methylpent-3-en-1-ynyl)-2-methyl-cyclobutane, 2,2,6-trimethyl-1-(3-methyl-1,3-buta dienyl)-5-methylene-7-oxabicyclo[4,1,0]heptane, fenretine, diisopropenyl-1-oxide, and 9-cycloheptatrienylidene-9,10-dihydro-10-oxygenated triterpenoid. The remaining compounds, i.e. D-limonene, α-ylangene, 4-terpinenyl acetate, 1,5,5,6-tetramethyl-1,3-cyclohexadiene, 1,2-ethanedion mono benzoxazole, 2,6-pyridinedicarboxaldehyde, 3,6-diethyl-3,6-dimethyl-trans-tricyclo-[3,1.0.0(2,4)] hexane, longifolene-(V4), 4-ethyl-4-methyl-1-(1-methylethyl)-1-(methylthyl)-(3R-trans)-cyclohexene, δ-selinene, guaia-3,9-diene, 4-epi-cubedol, guaiol, 6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8-octahydroanthalene-2,3-diol, [1,1'-biphenyl]-4-carboxaldehyde, (3β,5Z,7E)-9,10-secocholesta-5,7,10(19)-triene-3,24,25-triol, (10α-hydroxy-3α-methoxy-2,10-dimethyl-3,8-dioxo-4,6a,7,9,10,10b-hexahydrobenzo(e)azulen-5-yl)methyl acetate, androgapholide, 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 Atractylode 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. chinensis, respectively. Among these compounds detected in A. lancea were curzerene (14.1%) and 3-octen-5-yn-1-one (9.01%), while murolan-3(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-diethylnitrooxy)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, monoterpen, oxygenated monoterpen, sesquiterpene, oxygenated sesquiterpene, and oxygenated triterpenoid detected from different species of Atractylodes (Table 2). The Table 2. The highest amount of acid was too low within the species where no alcohol was found in A. chinensis. The highest amount of oxygenated sesquiterpe, alkane, monoterpe, and oxygenated monoterpe 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 |
|-----------------------------|---------------------------|-----------------------------|-----------------------------|
| Acid                        | 2                         | 3                           | 3                           |
| Alcohol                     | 1                         | 1                            | 1                           |
| Aldehydes                   | 2                         | 0                            | 0                           |
| Alkane                      | 1                         | 0                            | 0                           |
| Alkene                      | 3                         | 2                            | 2                           |
| Alkylene                    | 1                         | 0                            | 0                           |
| Ester                       | 6                         | 6                            | 5                           |
| Ketone                      | 6                         | 5                            | 5                           |
| Monoterpenes                | 4                         | 3                            | 4                           |
| Oxygenated monoterpenes     | 1                         | 1                            | 0                           |
| Sesquiterpenes              | 20                        | 21                           | 24                          |
| Oxygenated sesquiterpenes   | 23                        | 21                           | 20                          |
| Oxygenated triterpenoids    | 1                         | 1                            | 1                           |
| Other                       | 4                         | 4                            | 4                           |
| Total                       | 75                        | 82                           | 76                          |

found in A. chinensis. The levels of accumulation of alcohol, alkene, ketone, and oxygenated sesquiterpenes were found to be the highest amount in A. lancea. The species A. japonica contained the highest amount of alkane, 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 alkene was detected only in the species of A. lancea having 10.91% of total volatile flavor compounds. The amount of ester was 10.32%, 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 monoterpensoids, 19 sesquiterpenoids, and others in Mentha species (Park et al., 2016). In 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 = 12.92%, linalyl acetate = 9.11%), 37 VOC in *Artemisiscampestris* (β-farnesene = 14.17%, β-myrcene = 13.84%); 50 VOC in *Juniperusphoenixica* (α-pinene = 27.18%); 42 VOC in *Teucriumpolium* (α-guaiene = 11.53%, 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 verbene (8.50%), sabdol (7.51%), carvone (5.05%), and p-cineole (4.81%). These results are partially similar to those reported by (Belhadj et al., 2014) that used plant samples from different regions (Benifouda, Bougaa, Boussaada, and Bouteahl) of Algeria; and from the region of Busirah (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 phoenixica* growing in drylands of Algeria. It is followed by β-citronellol (6.15%), 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 (11.35%), trans-caryophyllene (9.49%), and γ-elemene (9.25%). These are followed by β-farnesene (7.56%), farnesol (6.14%), allo-aromandendrene (4.34%), δ-guaiene (4.21%), geranyl acetone (3.65%) and α-gurjene (3.56%). Octyl acetate (24.22 to 33.16%), 2-undecanone (12.43 to 23.82%), and 2-nonanone (11.41 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-poyroxy 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 *Teucriumpolium* (Gholivand et al., 2015) and 42 compounds in *Rhaponticumacaule* roots (Benyelles et al., 2014). The chemical composition of *Thymusalgeriensis* is marked by the presence of β-myrcene (13.78%), camphor (12.92%), 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 (Zouaoui 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 *Artemisiscampestris* 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 (7.25%), α-pinene (4.63%);
and β-pinene (4.21%). These results are partially in line with those reported by (Ghorab et al., 2015) 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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