NPC Natural Product Communications

2014
Vol. 9
No. 0
1 - 4

Volatile Compounds from Roots, Stems and Leaves of *Angelica acutiloba* growing in Taiwan

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Received: July 10th, 2013; Accepted: XX, 2014

The present study analyzed and compared the volatile compounds in fresh *Angelica acutiloba* roots, stems and leaves both qualitatively and quantitatively. The volatile compounds were isolated by either steam distillation (SD) or headspace-solid phase microextraction (HS-SPME). A total of 61 compounds were identified using gas chromatography/mass spectrometry (GC/MS). All 61 compounds were verified by SD, with 3*n*-butyl phthalide, γ-terpinene, *p*-cymene and *cis*-β-ocimene as the main compounds. Thirty-three compounds were verified by HS-SPME, with γ-terpinene and *p*-cymene as the main compounds. The leaf samples contained the highest essential oil content. Compared with SD, HS-SPME sampling resulted in relatively higher amounts of highly volatile monoterpenes and lower amounts of less volatile compounds such as 3*n*-butyl phthalide. These findings demonstrate that *A. acutiloba* roots, stems and leaves have high 3*n*-butyl phthalide contents; thus, all parts of *A. acutiloba* may be used for further application and development.

Keywords: *Angelica acutiloba*, Essential oil, Headspace-solid phase microextraction (SPME), 3*n*-Butyl phthalide

Several *Angelica* spp., family Umbelliferae, are widely grown in Asia, including *A. sinensis* (Oliv.) Diels in China, *A. gigas* Nakai in Korea and *A. acutiloba* Kitagawa in Japan. In Taiwan, *A. acutiloba* is known as 'Japan danggui', 'local danggui' or 'danggui'. It was first cultivated in Taiwan in the 19th century, and today, the species is commonly grown in Nantou and Hualien. Danggui is traditionally used for gynecological diseases such as menoxenia and anemia, and is recognized as a health food product for women in Asia, Europe and America [1]. Its pharmacological effects include anti-oxidative [2], anti-inflammatory [3], anti-tumor [4], antivirus and immune enhancement activities [5].

The most commonly used parts of danggui are the roots, while the leaves and stems are rarely used. The aim of the present study was to investigate the volatile constituents of *A. acutiloba* in different plant parts (roots, stems and leaves) and to compare different extraction methods. Because variations in geography, climate, and other environmental aspects result in different plant chemotypes, differences in aromatic notes attributable to plant chemotype are also discussed.

Constituents of* A. acutiloba *essential oils: The essential oils in the roots, stems and leaves of *A. acutiloba* grown in Taiwan were extracted by steam distillation (SD). The yields, on a wet weight basis, were 0.05 ± 0.01% (roots), 0.06 ± 0.01% (stems) and 0.12 ± 0.02% (leaves), with the roots having the lowest percentage of essential oils. Song *et al.* [6] previously reported a 0.2 mL yield of oil isolated by SD from dry *A. sinensis* root samples (50 g). Seo [7] reported a 0.31% yield when using a simultaneous steam distillation and extraction of oils in non-irradiated *A. gigas* samples, when irradiated with 1, 3, 5, 10, and 20 kGy, gave yields of 0.31%, 0.31%, 0.31%, 0.31%, and 0.29%, respectively. The difference in essential oil yield from previous studies in other parts of the world may reflect differences in extraction techniques, the cultivated varieties used, and growing conditions [8]. Considering the lower essential oil content in the present study and the use of fresh raw material, the water content of the samples may have also been a contributing factor.

The volatile compounds in the essential oils of *A. acutiloba* were analyzed by GC and GC/MS. Table 1 shows the 61 compounds that were identified, which included 17 monoterpene hydrocarbons, 10 terpene alcohols, 1 terpene ketone, 3 terpene esters, 12 sesquiterpene hydrocarbons, 2 terpene oxides, 5 aliphatic aldehydes, 1 aliphatic alcohol, 1 aliphatic ketone, 1 aliphatic ester, 1 hydrocarbon, 2 furans and 5 miscellaneous. The major volatile components of the essential oils were 3*n*-butyl phthalide (30.8-37.9%), γ-terpinene (21.1-27.2%), *p*-cymene (3.6-11.6%) and *cis*-β-ocimene (7.0-7.4%). However, ligustilides were not detectable in the samples, and *n*-butylidene phthalide was only present in trace amount, despite previous findings suggesting that the anti-endothelial properties could be attributed to these compounds [9]. Comparing the chemical composition of the essential oils of *A. acutiloba* roots and stems with that of the leaves revealed qualitative differences. The volatile composition of the root had the highest content of monterpenes, such as γ-terpinene, *p*-cymene and *cis*-β-ocimene, and the lowest content of 3*n*-butyl phthalide. The stems had a higher percentage of 3*n*-butyl phthalide than the roots and leaves, and the leaves had the highest content of *trans*-2-hexenal and *cis*-3-hexenol. C_6 aldehydes and C_6 alcohols have green notes, and *trans*-2-hexenal has a particularly strong green aroma [10]. In contrast to the present findings, Du [11] reported that the main compounds of the essential oils of *A. acutiloba* roots were ligustilide and butylidene phthalide. Using accelerated solvent extraction (ASE) to analyze three common danggui species in Asia, Cho [12] reported that the main components of *A. gigas* were

Table 1: Percentages of volatile compounds of roots, stems, and leaves of *Angelica acutiloba* analyzed by steam distillation (SD) /GC/GC-MS

Compound	RI[z]	SD Contents (%)[y]		
		Roots	Stems	Leaves
Butanal	609	<0.1	<0.1	0.3 ± 0.1
2-Ethylfuran	683	-[x]	-	0.3 ± 0.0
Hexanal	777	<0.1	<0.1	0.1 ± 0.0
2-Hexenal	830	-	0.1 ± 0.0	2.5 ± 0.7
3-Hexenol	839	-	0.4 ± 0.3	2.6 ± 1.3
Heptanal	879	<0.1	<0.1	-
Tricyclene	923	<0.1	0.1 ± 0.0	0.1 ± 0.0
α-Thujene	926	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
α-Pinene	941	0.3 ± 0.0	0.7 ± 0.1	1.2 ± 0.3
Camphene	949	0.5 ± 0.0	1.0 ± 0.1	1.7 ± 0.4
4-Octanone	956	<0.1	<0.1	-
Sabinene	969	0.3 ± 0.0	0.3 ± 0.1	0.6 ± 0.1
2-Pentylfuran	973	0.5 ± 0.3	<0.1	<0.1
β-Myrcene	982	1.1 ± 0.4	1.5 ± 0.6	1.2 ± 0.6
3-Hexenyl acetate	985	-	-	<0.1
α-Phellandrene	1002	<0.1	<0.1	<0.1
δ-3-Carene	1004	<0.1	<0.1	<0.1
α-Terpinene	1011	0.1 ± 0.0	<0.1	<0.1
p-Cymene	1014	11.6 ± 0.5	6.8 ± 1.5	3.6 ± 6.1
Limonene	1030	1.8 ± 0.6	2.1 ± 0.3	3.4 ± 0.9
cis-β-Ocimene	1037	7.4 ± 0.7	7.0 ± 1.4	7.2 ± 0.8
trans-β-Ocimene	1040	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
γ-Terpinene	1051	27.2 ± 0.6	21.1 ± 3.0	23.4 ± 4.9
α-Terpinolene	1085	0.1 ± 0.1	0.1 ± 0.0	0.4 ± 0.2
Linalool	1087	0.3 ± 0.0	0.2 ± 0.1	0.1 ± 0.1
Undecane	1093	0.1 ± 0.0	-	-
allo-Ocimene	1116	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
2-Nonenal	1129	0.1 ± 0.0	<0.1	-
Borneol	1158	<0.1	-	<0.1
4-Terpineol	1162	0.2 ± 0.01	0.1 ± 0.0	0.1 ± 0.0
2,4-Decadien-1-ol	1164	<0.1	<0.1	0.1 ± 0.0
α-Terpineol	1174	<0.1	<0.1	0.1 ± 0.0
Nerol	1203	<0.1	<0.1	<0.1
Geraniol	1227	<0.1	<0.1	<0.1
Safrole	1271	-	0.1 ± 0.1	0.1 ± 0.0
Borneol acetate	1278	<0.1	<0.1	-
Phthalic anhydride	1292	0.4 ± 0.1	<0.1	<0.1
Neryl acetate	1366	-	<0.1	0.1 ± 0.0
α-Copaene	1378	<0.1	<0.1	<0.1
β-Elemene	1382	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.0
β-Caryophyllene	1430	<0.1	2.0 ± 0.9	2.4 ± 0.4
α-Gurjunene	1438	<0.1	<0.1	-
trans-β-Farnesene	1448	0.6 ± 0.2	2.3 ± 0.6	2.6 ± 1.0
α-Humulene	1455	<0.1	0.4 ± 0.1	0.4 ± 0.0
β-Ionone	1461	-	0.2 ± 0.1	0.2 ± 0.1
α-Bergamotene	1480	0.6 ± 0.1	1.9 ± 0.4	1.0 ± 0.1
Germacrene D	1495	0.6 ± 0.1	1.0 ± 0.7	1.0 ± 0.1
α-Farnesene	1500	0.1 ± 0.0	0.6 ± 0.2	0.3 ± 0.0
α--Muurolene	1504	0.2 ± 0.1	<0.1	<0.1
γ-Cadinene	1505	-	0.4 ± 0.2	-
δ-Cadinene	1526	-	0.1 ± 0.0	0.1 ± 0.1
trans-Carveol	1539	0.1 ± 0.0	-	-
Nerolidol	1558	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
Spathulenol	1580	<0.1	-	-
Caryophyllene oxide	1585	2.0 ± 0.5	1.3 ± 0.4	1.5 ± 0.3
Carotol	1597	0.8 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Humulene oxide	1604	-	-	0.1 ± 0.0
Butylidene phthalide	1673	2.4 ± 0.1	1.1 ± 1.0	1.9 ± 0.2
3*n*-Butyl phthalide	1710	30.8 ± 1.1	37.9 ± 2.2	34.1 ± 3.4
Farnesyl acetate	1738	0.5 ± 0.0	<0.1	<0.1
Butylidene dihydrophthalide	1740	<0.1	<0.1	<0.1

[z]Retention indices, using paraffin (C_5-C_{25}) as references. [y]Values are means ± SD of triplicates. [x]undectable.

decursinol and angelate, of *A. sinensis* butylidene dihydrophthalide and 4-hydroxy-4-methyl-2-pentanone, and of *A. acutiloba* 9, 12-octadecanoic acid. Kim *et al*. [13] extracted volatile flavor components of danggui cultivars using solvent free injection (SFSI) and hydrodistillation. In their study, furfural and butylidene phthalide were the main components of Japanese Angelica, and butylidene dihydrophthalide the main component of Chinese danggui. In addition to the aforementioned reports that butylidene phthalide is an important component of Japanese angelica, Ko *et al*. [14] and Hsieh *et al.* [15] both proposed that this component may be beneficial for antispasmodic activity and blood flow improvement. Upton [16] reported that the most active compounds in *A. sinensis* were phthalides, polysaccharides and ferulic acid.

Peng *et al.* [17] indicated that 3*n*-butylphthalide (NBP) is a potentially beneficial drug for the treatment of ischemic stroke with multiple actions on different pathophysiological processes. Liu *et al.* [18] showed that dl-3*n*-butyl-phthalide prevents stroke via improvement of cerebral microvessels in stroke-prone renovascular hypertension (RHRSP). 3*n*-Butyl-phthalide was present in significant amounts in *A. acutiloba* leaves, stems, and roots. The constituents most often associated with the pharmacological activities of *Angelica* roots are ferulic acid and ligustilide. Zhao *et al.* [19] found that *A. sinensis* root yielded a greater amount of ferulic acid and ligustilide than *A. acutiloba* and *A. gigas*. However, these compounds were not detectable in our samples. As the plants used in the present study were cultivated in Taiwan, this difference may have been due to variations in climate and different regions of cultivation [19] and consequently variations in chemotype. Choi *et al.* [20] described the pharmacological activities of phthalide from *Angelica* species, including anticholinergic effects. Development and further study of *A. acutiloba* leaves in terms of its health benefits may enhance its economic value.

Volatile constituents of fresh* A. acutiloba*: The volatile compounds of fresh *A. acutiloba* were also analyzed using headspace-solid phase microextraction (HS-SPME) coupled with GC and GC/MS. Table 2 shows the 33 components that were identified, which included 15 monoterpenes, 2 terpene alcohols, 1 terpene ester, 8 sesquiterpene compounds, 1 aliphatic alcohol, 1 aliphatic aldehyde, 1 hydrocarbon and 3 miscellaneous. The principal components of all the fresh *A. acutiloba* samples (roots, stems and leaves) were γ-terpinene (41.2-52.1%), *p*-cymene (10.6-17.0%), β-myrcene (6.7-8.6%), *cis*-β-ocimene (4.9-7.4%) and *allo*-ocimene (4.2-5.3%) (Table 1). A comparison of the differences among the three plant parts revealed that the roots had a higher content of β-pinene, ρ-cymene, and γ-terpinene and that the leaves had a higher content of 3-hexenol and *cis*-3-hexeyl acetate. The various compound contents in the stems were intermediate between

Table 2: Percentages of volatile compounds of roots, stems, and leaves of *Angelica acutiloba* analyzed by headspace-solid phase microextraction (HS-SPME)/GC/GC-MS.

Compound	RI[z]	HS-SPME Contents (%)[y]		
		Roots	Stems	Leaves
3-Hexenol	839	<0.1	0.5 ± 0.2	3.4 ± 1.1
Tricyclene	923	<0.1	0.2 ± 0.0	0.2 ± 0.0
α-Thujene	926	0.6 ± 0.1	0.4 ± 0.1	0.5 ± 0.0
α-Pinene	941	0.5 ± 0.1	1.3 ± 0.4	1.9 ± 0.2
Camphene	949	0.5 ± 0.1	1.5 ± 0.8	2.1 ± 0.3
Sabinene	969	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.0
β-Myrcene	982	8.5 ± 3.4	8.6 ± 3.4	6.7 ± 1.2
3-Hexenyl acetate	985	-[x]	-	1.1 ± 0.8
α-Phellandrene	1002	0.4 ± 0.0	0.3 ± 0.1	0.2 ± 0.2
α-Terpinene	1011	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
p-Cymene	1014	17.0 ± 2.5	13.0 ± 3.5	10.6 ± 0.0
Limonene	1030	2.2 ± 0.6	2.9 ± 1.6	4.5 ± 0.3
cis-β-Ocimene	1037	4.9 ± 3.3	7.1 ± 4.1	7.4 ± 1.3
trans-β-Ocimene	1040	0.6 ± 0.0	0.6 ± 0.5	0.6 ± 0.2
γ-Terpinene	1051	52.1 ± 1.9	47.4 ± 10.0	41.2 ± 0.3
α-Terpinolene	1085	0.4 ± 0.1	0.5 ± 0.2	0.5 ± 0.1
Undecane	1093	0.2 ± 0.1	-	-
allo-Ocimene	1116	4.2 ± 2.3	5.3 ± 2.0	5.1 ± 0.1
2-Nonenal	1129	<0.1	-	-
4-Terpineol	1162	<0.1	<0.1	<0.1
α-Terpineol	1174	<0.1	<0.1	<0.1
Borneol acetate	1278	-	0.1 ± 0.1	-
β-Caryophyllene	1430	0.4 ± 0.0	2.5 ± 1.7	3.7 ± 0.4
α-Gurjunene	1438	<0.1	-	-
trans-β-Farnesene	1448	0.1 ± 0.0	1.0 ± 0.4	0.8 ± 0.4
α-Humulene	1455	-	0.2 ± 0.1	-
α-Bergamotene	1480	-	0.1 ± 0.0	0.2 ± 0.2
Germacrene D	1495	<0.1	0.3 ± 0.1	0.5 ± 0.2
α-Faenesene	1500	<0.1	<0.1	<0.1
δ-Cadinene	1526	<0.1	<0.1	0.4 ± 0.1
Butylidene phthalide	1673	<0.1	<0.1	<0.1
3*n*-Butyl phthalide	1710	<0.1	<0.1	<0.1
Butylidene dihydrophthalide	1740	<0.1	<0.1	<0.1

[z]Retention indices, using paraffin (C_5-C_{25}) as references. [y] Values are means ± SD of triplicates. [x] undetectable.

those observed for the roots and leaves. Ruberto & Baratta [21] reported that among 100 pure compounds, terpinolene, α-terpinene, and γ-terpinene possessed antioxidative activity. The findings of the present study are consistent with those of Kim *et al.* [22], who analyzed the components of Korean, Chinese, and Japanese Angelicas using the SPME method and reported that γ-terpinene, β-myrcene, and β-ocimene are the main components of Japanese Angelica. While Choi [20] found that the essential oil of fresh *A. gigas* leaves primarily consisted of β-phellandrene (51.9%), the main components of *Angelica* stems from the present study were β-phellandrene, dodecanoic acid, and isobutylidene phthalide. Choi [20] also found that the *p*-cymene content of fresh leaves is higher than that of the stems. Although low amounts of α-phellandrene were recorded, β-phellandrene was not detectable in our samples. The isobutylidene phthalide and 3-butyl phthalide contents we measured in *Angelica* stems were also low due to the use of different species, and the resulting difference in volatile components. 3*n*-Butyl phthalide, an important danggui component mentioned in several studies, was detected at low levels in the roots, leaves, and stems using SPME. Consistently, Kim *et al*. [22] reported that butylidene dihydro-phthalide was detectable in Chinese and Japanese Angelica using supercritical fluid extraction (SFE), but not using SPME.

Comparison of steam distillation and HS-SPME analyses: As shown in Tables 1 and 2, SPME analysis of *cis*-3-hexenol and the monoterpene components of fresh *A. acutiloba* samples revealed that γ-terpinene, *p*-cymene and *allo*-ocimene were present in higher amounts than in steam distilled samples. However, the steam distilled samples had higher terpene alcohols and 3*n*-butyl phthalide contents. Furan components were not detected in the essential oils extracted from fresh leaves and were assumed to be generated through heat. SD is an exhaustive process that isolates the essential oil. Because SPME requires less time for extraction and is solvent free, oxidation and hydroxylation are less likely to occur. In an analysis of peppermint oil, Rohloff [23] found that SPME resulted in relatively higher amounts of highly volatile monoterpenes. The detection of lower amounts of less volatile compounds was similar to the finding of the present study. Yang *et al.* [24] described the SPME method as an excellent tool for analysis of herbs because it is simple, fast, and does not leave any residues. The results of the present study demonstrate that although SPME could extract the aroma of the samples, particularly the highly volatile components, very little 3*n*-butyl phthalide, an important *Angelica* component, was extracted. It is, therefore, recommended that both extraction methods be used concurrently to complement each other in the analysis of herbs.

The volatile compounds of *A. acutiloba* roots, stems and leaves were compared. The findings demonstrate that although the roots are traditionally used, the leaves and stems also had high amounts of 3*n*-butyl phthalide, a compound whose potential health benefits warrant further development and study.

Experimental

Plant material: Fresh *A. acutiloba* was purchased from the Fruithouse Farm in Nantou, Taiwan. A herbarium sample (No. AC 0331) lodged in the flavor and fragrance research laboratory, China Medical University, Taiwan. The samples were washed under running water, drip dried, and separated into 3 parts (leaves, stems and roots).

Steam distillation (SD): The roots, stems and leaves of *A. acutiloba* (400 g) were homogenized for 2 min in 1600 mL of deionized water then placed into a 5 L round bottom flask. The homogenate was steam distilled for 5 h to obtain the essential oils and the prepared samples were immediately stored in brown flasks at 4°C prior to analysis by gas chromatography. One μL of each essential oil sample was injected into the gas chromatograph in split mode with a 1: 100 ratio. This experiment and all other experiments in the present study were performed in triplicate.

HS-SPME: Fresh roots, stems and leaves of *A. acutiloba* were homogenized for 2 min, and A 50/30 μm divinylbenzene/carboxen/ polydimethylsiloxane fiber (Supelco, Inc., Bellefonte, PA) was used for aroma extraction. Each sample (3 g) was placed in a 10 mL vial (Supelco 10 mL clear vial, screw top with hole, PTFE/Silicone Septa). The SPME fiber was exposed to each sample for 20 min at 25°C. Each sample was then injected into a gas chromatograph injection unit in splitless mode.

Analysis of volatile compounds

(1) Qualitative and quantitative analyses of the volatile compounds were conducted using an Agilent 6890 GC apparatus equipped with a 60 m × 0.25 mm i.d. DB-1 fused-silica capillary column with a film thickness of 0.25 μm and a flame ionization detector. The injector and detector temperatures were maintained at 250 and 300°C, respectively. The oven temperature was held at 40°C for 1 min and then raised to 200°C at 2°C /min and held for 9 min. The carrier gas (nitrogen) flow rate was 1 mL/min. Kovats indices were calculated for the separated components relative to a C_5-C_{25} *n*-alkanes mixture [25].

(2) Identification of the volatile compounds was conducted using an Agilent 6890 GC apparatus equipped with a 60 m × 0.25 mm i.d. DB-1 fused-silica column, with a film thickness of 0.25 μm, and with the temperature maintained at 250°C. The chromatograph was connected to an Agilent model 5973 N MSD mass spectrometer (MS). The injector GC−MS analyses were the same as those described for the GC analysis. The carrier gas (helium) flow rate was 1 mL/min, and the electron energy was 70 eV at 230°C. The constituents were identified by matching their spectra with those recorded in the MS library (Wiley 7n).

Acknowledgment - This research was supported by a research grant from China Medical University (CMU100-TC-15) in Taiwan.

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