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INFLUENCE OF SULFUR FUMIGATION ON VOLATILE OIL CONSTITUENTS OF CODONOPSIS RADIX (DANGSHEN) BY GC-MS METHOD

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Abstract

Background: Sulfur fumigation is one of the processing methods of Chinese medicinal herbs. This method can be used in bleaching herbs and controlling microorganisms, parasites and insects. Codonopsis Radix (*Dangshen* in Chinese), dried roots of *Codonopsis pilosula*, is one of the herbs commonly processed by sulfur fumigation. This study reports the influence of sulfur fumigation on the chemical constituents of the volatile oil of *Dangshen*.
Materials and Methods: The volatile oil of air-dried or sulfur-fumigated *Dangshen* was extracted by water-steam distillation and separated by GC capillary column chromatography. The components in the individual volatile oil were identified and quantitatively determined by GC-MS.
Results: The results showed that 45 compounds were separated and identified from the volatile oil of *Dangshen* samples. Among them, the contents of 23 compounds in sulfur-fumigated *Dangshen* were much lower than that in air-dried *Dangshen*, such as 4-bromo-1-cyclohexene (6), 3,5-dimethylcyclopentene (7), phenylethyl alcohol (11), 2,3-diazabicyclo[2.2.1]hepy-2-ene,4-methyl-1-(pent-4-en-1-yl)- (22) and cedren-13-ol, 8- (35). On the contrary, the contents of 12 compounds were increased after sulfur fumigation, such as (+)-sativene (25), thujopsene (27), chamigren (30), *α*-guaiene (32) and 3-buten-2-one, 3-methyl-4-[(1*R*,2*R*,6*S*)-1,3,3-trimethyl-7-oxabicyclo[4.1.0]hept-2-yl]-, (3*E*)-*rel*- (43). Furthermore, two peaks [bicyclo[3.2.0]heptan-2-one,6-hydroxy-5-methyl-6-vinyl- (17) and bornyl bromide (20)] disappeared and four peaks [3-(decyloxy)-2-hydroxy-1-propanesulfonic acid (18), *α*-lonone (19) tetradecanoic acid (42) and methyl *cis*-9,10-epoxystearate (45)] newly appeared in the chromatogram of the volatile oil of sulfur-fumigated *Dangshen*.
Conclusions: Sulfur fumigation caused significant variations in the constituents of volatile oil of *Dangshen*. Further explorations are needed to investigate how these chemical variations occurred and whether these variations would affect the clinical efficacy and safety of *Dangshen* in human use.

Key words: Sulfur fumigation; volatile oil; *Dangshen*; *Codonopsis pilosula*; GC-MS

Introduction

Post-harvest processing is necessary to turn raw herbal materials into ready-to-use herbs. Sulfur fumigation, one of the processing methods, utilizes sulfur dioxide generated during sulfur combustion to bleach herbs and control microorganisms, parasites and insects. Unfortunately, sulfur fumigation is often excessively used by farmers and traders to keep the artificially bright color of the herbs and to extend their shelf life. According to the recommendations from the Chinese Food and Drug Administration, 11 Chinese medicinal herbs, including Codonopsis Radix (*Dangshen* in Chinese), are allowed to be processed by sulfur fumigation, but the amount of sulfur dioxide residue should be less than 400 ppm. However, the rational of such residue limitation has not been evaluated by scientific research. Moreover, some reports showed that sulfur fumigation might cause chemical transformation of original components in the herbs, and consequently affect the clinical efficacy and safety of the herbs (Li et al. 2012, Cao et al. 2014, Jiang et al. 2013, Wang et al. 2014). *Dangshen*, a well-known traditional Chinese medicinal herb with *Qi* (氣) and *Yin* (陰) tonifying properties (Ma et al. 2014), is usually processed by sulfur fumigation after harvest. Recently, some studies have demonstrated that sulfur fumigation causes dramatic variations in the chemical profiles of *Dangshen*. One report showed that lobetyolin content in sulfur-fumigated *Dangshen* was lower than that in air-dried *Dangshen* (Li et al. 2010a). In our previous research, significant chemical differences between the organic solvent extracts of sulfur-fumigated and air-dried *Dangshen* samples were observed (Ma et al. 2014). Components of the volatile oil from *Dangshen* exhibit various bioactivities (Sun et al. 2008, Li et al. 2010b, Jiang 2001). However, until now, there is no study on the influence of sulfur fumigation on the constituents of the volatile oil of *Dangshen*. In this paper, we for the first time compared the volatile oil compositions of sulfur-fumigated and air-dried *Dangshen* by the GC-MS method.

Materials and Methods
Chemicals, Reference Compounds and Samples

Acetic ether was purchased from E. Merck (Darmstadt, Germany). Ultra-pure water was prepared using a Milli-Q Plus water purification system (Millipore, Billerica, MA, USA). Fresh *Dangshen* (FX-01) was collected from Gansu Province, China. Commercial *Dangshen* samples (SS-03, SS-04 and SS-05) were purchased from different herbal shops in Sichuan and Gansu provinces, China (Table 1). The herbal materials were morphological and histological authenticated by Prof. Zhi-ling Yu to be the dried root of *Codonopsis pilosula* (Franch.) Nannf., according to the monograph in Chinese Pharmacopoeia (version 2010). The voucher specimens were deposited in the Technology Development Division, School of Chinese Medicine, Hong Kong Baptist University, Hong Kong, China.

Volatile Oil Preparation

Sulfur-fumigated *Dangshen* (ZX-02) were prepared from fresh *Dangshen* (FX-01) (Table 1) following the procedures similar to that performed by the farmers: 10 g of sulfur powder was heated until it burnt; the burning sulfur was put into the lower layer of desiccator, and 100 g of fresh *Dangshen* slices (2-4 mm in thickness) were put into upper layer of desiccator. The desiccator was then kept closed for 12 h. The sulfur fumigation was repeated twice. After fumigation, the sulfur-fumigated *Dangshen* slices and its corresponding fresh *Dangshen* slices were dried in a fume cupboard for 6 days.

Table 1: The code numbers and sources of *Dangshen* samples

Code no.	Source	Description
FX-01	Gansu	Air-dried sample
ZX-02	Gansu	Sulfur-fumigated product of sample FX-01
SS-03	Sichuan	Commercial sample
SS-04	Gansu	Commercial sample
SS-05	Gansu	Commercial sample

The volatile oil was prepared according to Chinese Pharmacopoeia (2010). Fresh *Dangshen* (45 g) and distilled water (350 mL) were placed into an extraction apparatus and subjected to hydro-distillation for 5 h at 373.15 K.

GC-MS Analysis

The volatile oils were analyzed using a Thermo Quest Trace GC/ESD, and a HP-5MS capillary column (30.0 m × 250 μm × 0.25 μm). The column was kept at 333.15 K for 3 min, then the temperature was increased to 383.15 K at a rate of 277.15 K/min and held for 8 min, then to 433.15 K at a rate of 275.15 K/min and held for 1 min, and finally to 513.15 K at a rate of 283.15 K/min. Split injection was conducted at a split ratio of 25:1 and high purity helium was used as carrier gas at a flow rate of 1.0 mL/min. The spectrometers were operated in electron-impact (EI) mode, the scan range was 30-650 amu, the ionization energy was 70 eV, and the scan rate was 0.35 s per scan. The ionization source temperature and accelerating voltage were 493.15 K and 200 eV, respectively. The injector and detector temperatures were 473.15 and 523.15 K, respectively.

Results and Discussion

The total ion chromatograms (TIC) of the volatile oils from air-dried and sulfur-fumigated *Dangshen* are shown in Figure 1. The samples were separated in 58 min. Forty five compounds and their relative contents are listed in Table 2. They were tentatively identified on the basis of GC-MS library (Agilent Chemstation software NIST 02 database) and literature data (Tan et al. 1991, Xie et al. 2000). The volatile oil of *Dangshen* contains various constituents, including aldehyde, alcohol, fatty acid, fatty acid ester, alkanes and olefin. Using the developed GC-MS-based volatile oil profiling approach, chemical variations in *Dangshen* caused by sulfur fumigation were revealed. Figure 1 and Table 2 show that after sulfur fumigation, the contents of 23 volatile compounds of *Dangshen* decreased. These compounds were 2-methyl-4-pentenal (**1**), 13-tetradece-11-yn-1-ol (**2**), ethanone, 2,2-bis(acetyloxy)-1-phenyl- (**4**), 2-amylfuran (**5**), 4-bromo-1-cyclohexene (**6**), 3,5-dimethylcyclopentene (**7**), 3-(5-benzyloxy-3-methylpent-3-enyl)-2,2-dimethyloxirane (**8**), 2-octyn-1-ol (**9**), 1-undecyne (**10**), phenylethyl alcohol (**11**), 2,4-pentadien-1-ol,3-pentyl-,(2*Z*)- (**12**), 4-methyloctanoic acid (**13**), 2-propyl-2-heptenal (**16**), 2,3-diazabicyclo[2.2.1]hepy-2-ene,4-methyl-1-(pent-4-en-1-yl)- (**22**), bicycol[5.2.0]nonane,2-methylene-4,8,8-trimethyl-4-vinyl- (**26**), paeonol (**28**), pentanoic acid,10-undecenyl ester (**31**), 3,5-dimethoxybenzyl alcohol (**33**), cedren-13-ol, 8- (**35**), 8*S*,14-cedrandiol (**36**), 2,2',5,5'-tetramethyl-1,1'-biphenyl (**38**), 3,4-diethyl-1,1'-biphenyl (**40**), phenanthrene (**41**) and cyclohexyl butyl phthalate (**44**). Moreover, peaks of bicyclo[3.2.0]heptan-2-one,6-hydroxy-5-methyl-6-vinyl- (**17**) and bornyl bromide (**20**) disappeared from the chromatogram after sulfur fumigation. On the contrary, contents of 12 volatile compounds of *Dangshen* increased after sulfur fumigation, including 8-heptadecyne,1-bromo- (**14**), 6-(1-adamantylamino)-2,4,5-trichloronicotinonitrile (**15**), sulfurous acid, 5,8,11-heptadecatrien-1-yl methyl ester (**23**), (+)-sativene (**25**), thujopsene (**27**), cyclohexane, 1-ethenyl-1-methyl-2, 4-bis(1-methylethenyl)- (**29**), chamigren (**30**), *α*-guaiene (**32**), 2-naphthaleneethanol, 1,2,3,4,4a,5,6,8a-octahydro-6-hydroxy-4a,8-dimethyl-*β*-methylene- (**34**), 3,4-Diethyl-1,1'-biphenyl (**37**), 9-hexadecenoic acid (**39**) and 3-buten-2-one, 3-methyl-4-[(1*R*,2*R*,6*S*)-1, 3,3-trimethyl-7-oxabicyclo[4.1.0]hept-2-yl]-, (3*E*)-*rel*- (**43**). Furthermore, five volatile compounds only appeared in the sulfur-fumigated *Dangshen*, including 3-(decyloxy)-2-hydroxy-1-propanesulfonic acid (**18**), *α*-lonone (**19**), 2-myristynoyl pantetheine (**24**), tetradecanoic acid (**42**) and methyl *cis*-9, 10-epoxystearate (**45**).

Commercial *Dangshen* samples (SS-03, SS-04, SS-05) and sulfur-fumigated sample ZX-02 showed similar chemical contents (Figure 1). We speculated that all commercial *Dangshen* samples were sulfur-fumigated and the chemical variations were partly caused by sulfur-fumigation.There are several possible causes for the chemical variations of volatile oil of *Dangshen* during sulfur fumigation. Some of the components of the volatile oil might be lost during the heating process of sulfur fumigation. 3-(Decyloxy)-2-hydroxy-1-propanesulfonic acid (**18**) and 2-myristynoyl pantetheine (**24**), which contain sulfur atoms and appeared only in sulfur-fumigated samples, might be the transformation products of the sulfur fumigation. Moreover, the volatile oil might be oxidised during sulfur fumigation, which may explain the oxygen atom in some of the newly appeared compounds [*α*-lonone (**19**),

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Table 2: Analysis of the main chemical components of volatile oils from air-dried and sulfur-fumigated *Dangshen* and their relative percentages by GC-MS

No	Retention time/min	Compounds	Molecular weight	CAS No.	Molecular formula	Relative contents（%）				
						FX-01	ZX-02	SS-01	SS-02	SS-03
1	5.46	2-Methyl-4-pentenal	98	5187-71-3	$C_6H_{10}O$	1.77	0.69	0.22	1.18	0.51
2	6.75	13-Tetradece-11-yn-1-ol	208	-	$C_{14}H_{24}O$	0.34	0.16	0.03	0.13	0.06
3	8.36	5-Furfuryl-5-methylfuran-2(5H)-one	178	31969-27-4	$C_{10}H_{10}O_3$	0.17	0.15	0.2	0.01	0.08
4	9.27	Ethanone, 2,2-bis(acetyloxy)-1-phenyl-	236	5062-30-6	$C_{12}H_{12}O_5$	1.23	0.64	0.28	2.06	0.94
5	10.47	2-Amylfuran	138	3777-69-3	$C_9H_{14}O$	3.36	2.78	0.11	0.05	0.04
6	10.78	4-Bromo-1-cyclohexene	160	3540-84-9	C_6H_9Br	1.59	0.23	-	-	-
7	11.42	3,5-Dimethylcyclopentene	96	-	C_7H_{12}	0.98	0.11	0.03	-	-
8	12.77	3-(5-Benzyloxy-3-methylpent-3-enyl)-2,2-dimethyloxirane	260	-	$C_{17}H_{24}O_2$	0.72	0.44	0.35	0.21	0.54
9	13.40	2-Octyn-1-ol	126	20739-58-6	$C_8H_{14}O$	0.62	0.22	0.02	-	-
10	15.34	1-Undecyne	152	2243-98-3	$C_{11}H_{20}$	0.56	0.30	0.1	0.57	0.24
11	15.58	Phenylethyl alcohol	122	60-12-8	$C_8H_{10}O$	0.30	0.10	0.05	0.04	0.02
12	17.52	2,4-Pentadien-1-ol,3-pentyl-,(2*Z*)-	154	-	$C_{10}H_{18}O$	0.96	0.69	0.22	0.01	-
13	18.16	4-Methyloctanoic acid	158	54947-74-9	$C_9H_{18}O_2$	1.44	0.90	0.02	-	-
14	19.45	8-Heptadecyne,1-bromo-	314	56599-94-1	$C_{17}H_{31}Br$	0.31	0.82	0.4	-	-
15	21.61	6-(1-Adamantylamino)-2,4,5-trichloronicotinonitrile	355	339165-88-7	$C_{16}H_{16}C_{13}N_3$	0.22	0.26	-	0.06	0.02
16	22.08	2-Propyl-2-heptenal	154	34880-43-8	$C_{10}H_{18}O$	0.36	0.10	-	-	-
17	22.47	Bicyclo[3.2.0]heptan-2-one,6-hydroxy-5-methyl-6-vinyl-	166	-	$C_{10}H_{14}O_2$	0.54	-	-	0.09	-
18	22.57	3-(Decyloxy)-2-hydroxy-1-propanesulfonic acid	296	-	$C_{13}H_{28}O_5S$	-	0.65	-	-	-
19	22.94	*α*-Lonone	192	127-41-3	$C_{13}H_{20}O$	-	0.53	0.35	0.06	0.03
20	24.07	Bornyl bromide	216	4443-48-5	$C_{10}H_{17}Br$	1.83	-	-	-	-
21	24.97	2-Methoxy-4-vinylphenol	150	7786-61-0	$C_9H_{10}O_2$	0.20	0.24	0.05	0.06	0.04
22	25.75	2,3-Diazabicyclo[2.2.1]hepy-2-ene,4-methyl-1-(pent-4-en-1-yl)-	178	150667-99-5	$C_{11}H_{18}N_2$	5.67	0.20	-	-	-
23	29.06	Sulfurous acid, 5,8,11-heptadecatrien-1-yl methyl ester	328	56554-67-7	$C_{18}H_{32}O_3S$	0.26	0.28	0.37	0.44	0.34
24	29.68	2-Myristynoyl pantetheine	484	959100-16-4	$C_{25}H_{44}N_2O_5S$	-	0.33	-	-	-
25	32.47	(+)-Sativene	204	3650-28-0	$C_{15}H_{24}$	1.24	2.16	133	1.77	1.59
26	32.86	Bicycol[5.2.0]nonane,2-methylene-4,8,8-trimethyl-4-vinyl-	204	-	$C_{15}H_{24}$	2.2	0.92	0.6	0.31	0.36
27	33.9	(−)-Thujopsene	204	470-40-6	$C_{15}H_{24}$	0.32	0.38	0.35	0.41	0.53
28	34.4	Paeonol	166	552-41-0	$C_9H_{10}O_3$	4.28	2.15	0.07	0.13	0.02
29	34.77	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-	204	110823-68-2	$C_{15}H_{24}$	0.99	1.11	1.32	2.73	1.95
30	37.05	Chamigren	204	18431-82-8	$C_{15}H_{24}$	0.67	0.90	0.78	1.71	1.63
31	37.35	Pentanoic acid,10-undecenyl ester	254	-	$C_{16}H_{30}O_2$	1.56	1.13	-	-	-
32	38.15	*α*-Guaiene	204	3691-12-1	$C_{15}H_{24}$	0.55	0.79	0.13	0.19	0.11
33	39.25	3,5-Dimethoxybenzyl alcohol	168	705-76-0	$C_9H_{12}O_3$	1.10	0.39	-	-	-

34	41.12	2-Naphthaleneethanol, 1,2,3,4,4a,5,6,8a-octahydro-6-hydroxy-4a,8-dimethyl-*β*-methylene-	236	1005276-32-3	$C_{15}H_{24}O_2$	0.11	0.28	-	0.07	-
35	43.57	Cedren-13-ol, 8-	220	18319-35-2	$C_{15}H_{24}O$	1.04	0.24	-	0.18	0.04
36	45.17	8*S*,14-Cedrandiol	238	62600-05-9	$C_{15}H_{26}O_2$	0.51	0.27	0.07	0.13	0.07
37	48.06	3,4-Diethyl-1,1'-biphenyl	210	61141-66-0	$C_{16}H_{18}$	0.56	0.69	0.35	-	-
38	49.21	2,2',5,5'-Tetramethyl-1,1'-biphenyl	210	3075-84-1	$C_{16}H_{18}$	0.83	0.55	0.24	0.44	0.23
39	50.02	9-Hexadecenoic acid	254	2091-29-4	$C_{16}H_{30}O_2$	0.39	0.64	0.07	0.11	0.02
40	51.06	3,4-Diethyl-1,1'-biphenyl	210	61141-66-0	$C_{16}H_{18}$	0.45	0.31	0.12	-	0.11
41	54.20	Phenanthrene	178	85-01-8	$C_{14}H_{10}$	1.90	1.08	-	-	-
42	54.34	Tetradecanoic acid	228	544-63-8	$C_{14}H_{28}O_2$	-	1.28	1.9	1.67	1.24
43	56.42	3-Buten-2-one, 3-methyl-4-[(1*R*,2*R*,6*S*)-1,3,3-trimethyl-7-oxabicyclo[4.1.0]hept-2-yl]-, (3*E*)-*rel*-	222	97371-44-3	$C_{14}H_{22}O_2$	0.32	1.12	0.45	0.65	0.39
44	56.73	Cyclohexyl butyl phthalate	304	84-64-0	$C_{18}H_{24}O_4$	0.66	0.46	0.24	0.4	0.13
45	56.85	Methyl *cis*-9,10-epoxystearate	312	2566-91-8	$C_{19}H_{36}O_3$	-	0.62	0.62	0.52	0.49

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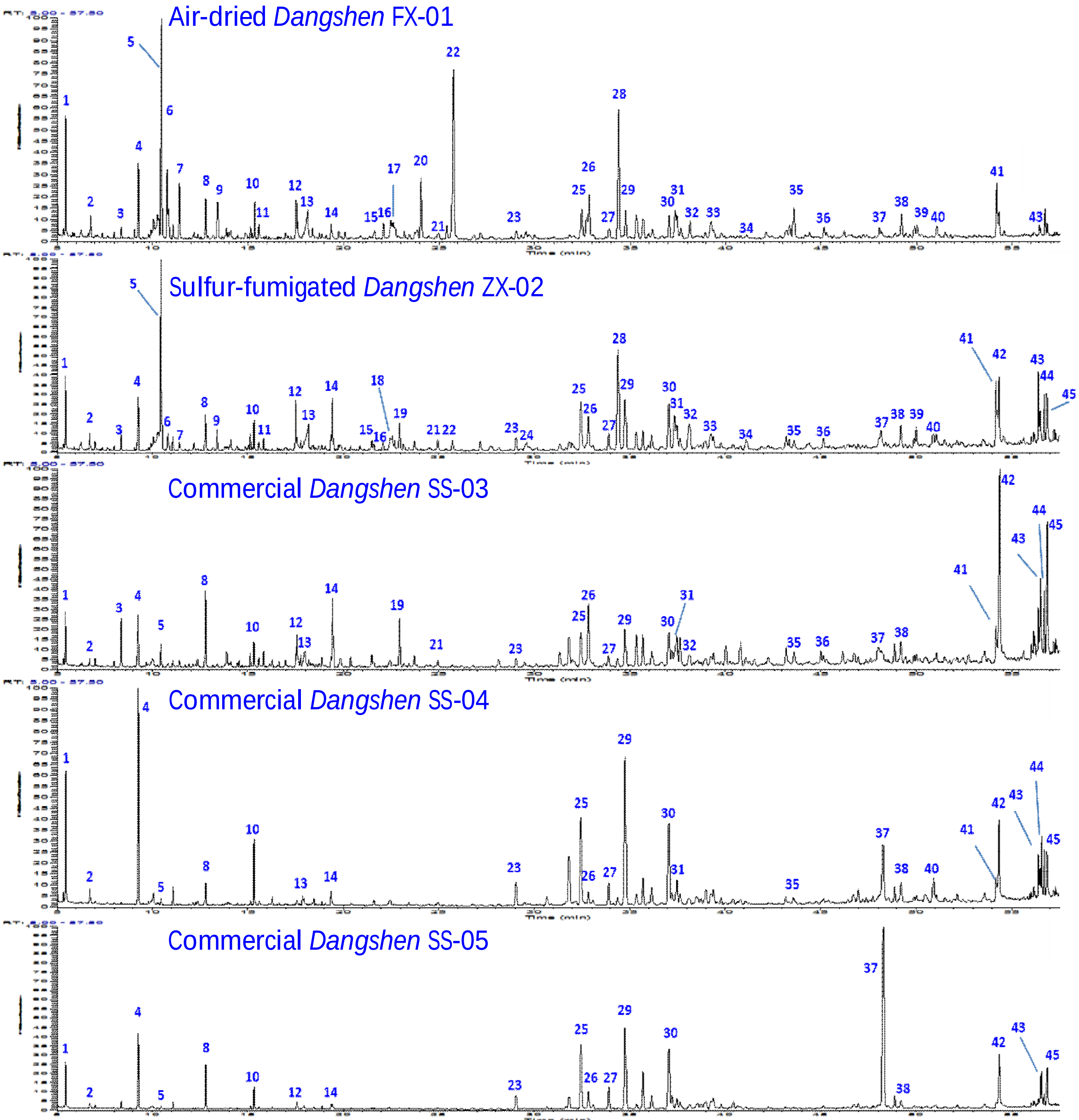

Figure 1: GC-MS results of the volatile constituents from air-dried, sulfur fumigated and commercial *Dangshen* samples, respectively.

tetradecanoic acid (**42**) and methyl *cis*-9,10-epoxystearate (**45**)] in sulfur-fumigated *Dangshen* samples.

Studies on the bioactivities of the identified compounds are very few. Only three compounds have relevant reports. Phenylethyl alcohol (**11**) exerts potent inhibitory action on Gram-negative bacteria (Lilley & Brewer 1953). Paeonol (**28**) has anti-tumor activity (Sun et al. 2008), and can be used for treating eczema (Deng et al. 2006) and diabetic encephalopathy (Liu *et al.* 2013). Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)- (**29**, Synonym: β-elemene) showed anticancer activities (Chen et al. 2012, Li et al. 2013, Zou et al. 2013).

For the toxicities of the constituents of volatile oil, it was reported that the LD_{50} of 2-amylfuran (**5**) on mouse by oral is 1200 mg/kg (Moran et al. 1980). Phenylethyl alcohol (**11**) is not a developmental toxicity hazard for humans (Politano et al. 2013). Bornyl bromide (**20**) may cause skin irritation and/or dermatitis (Material Safety Data Sheet, 2006). 2-Methoxy-4-vinylphenol (**21**) is irritating to eyes, respiratory system and skin (Lookchema, 2011). (+)-Sativene (**25**) is not dangerous (Lookchemb, 2011). (−)-Thujopsene (**27**) is toxic against *Dermatophagoides farinae* and *Tyrophagus putrescentiae* (Kim et al. 2015). Phenanthrene (**41**) did not show toxicity to human, but was recorded as a toxic pollutant to water (Irwin et al. 1997). The oral LD_{50} of tetradecanoic acid (**42**) for rat is more than 10 g/kg. This compound can cause skin and eye irritation, and can also cause mutation (Safety Data Sheet, 2015). Cyclohexyl butyl phthalate (**44**) can cause skin and eye irritation (Toxicology Data Network, 2009).

How did the chemical changes occur, and whether these changes would affect the efficacy and safety of *Dangshen* remain unknown. Further investigations are necessary to explore the mechanisms of chemical changes caused by sulfur fumigation and to determine the impacts of the chemical changes on the efficacy and safety of *Dangshen*.

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