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Volatile constituents in the flowers of *Elsholtzia argyi* and their variation: a possible utilization of plant resources after phytoremediation*

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Abstract: Phytoremediation effectiveness and remediation costs are driving factors of this project. Full utilization of plant resources after their being used for phytoremediation is an unsolved problem. GC/MS technique was used to investigate the volatiles of the flowers from *Elsholtzia argyi* (PFE1: Purple Flower Elsholtzia) and their variation (WFE: White Flower Elsholtzia), naturally growing in Pb/Zn mined area, and *Elsholtzia argyi* (PFE2: Purple Flower Elsholtzia), naturally growing in Jiuxi uncontaminated agriculture soil. Seventeen compounds constituting 86.88% of total essential oils were identified in PFE1, with 2,6-octadienoic acid,3,7-dimethyl-methyl ester being the main constituent (63.30%). Sixteen compounds accounting for 95.32% of total essential oils were identified in WFE, with caryophyllene being the main component (55.02%). Compared to PFE1, PFE2 contained lower level of 2,6-octadienoic acid,3,7-dimethyl-methyl ester (31.76%), which was the main constituent in the total essential oils of PFE2. Caryophyllene is the main ingredient of flavor. Elsholtzia ketone was identified in all the three Elsholtzia plants. It can be concluded that the selected *Elsholtzia argyi* plants can be exploited on their versatile uses as fragrances and antiseptics due to the perfume ingredient and antibacterial components existing in their essential oils.

Key words: *Elsholtzia argyi*, Essential oils, GC/MS, Phytoremediation
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INTRODUCTION

Some plant species endemic to metalliferous soil accumulate large amounts of heavy metals in their shoot parts so that they show great potential for phytoremediation of metal contaminated soil. *Elsholtzia argyi* exists widely in Southeast China and grows in some ancient Pb/Zn-mining areas. In the old Pb/Zn mined area, healthy growth of *Elsholtzia argyi* (PFE1: Purple Flower Elsholtzia) was observed in the soil with extractable Zn, Pb and Cu of approximately 90, 160, and 2.5 mg/kg, shoot concentrations of Zn, Pb and Cu were 180~480, 73~74, and 12~17 mg/kg, respectively (Jiang *et al*., 2004). Variation of *E. argyi* (WFE: White Flower Elsholtzia) was also found at the same Pb/Zn contaminated soil, and can accumulate more metal in its shoot parts than PFE1. Both plants grow well at the contaminated soil with high levels of Zn, Pb, but low levels of Cu. With the large-scale phytoremediation for the metal contaminated soil by *Elsholtzia argyi*, the disposition and utilization of these recourses of plants raise a severe environmental problem.

E. argyi is an endemic, herbaceous, annual plant, whose essential oils are very fragrant and have broad-spectrum antibacterial and antiseptic properties to resist Bacillus, Coccus, and so on (Ke, 1980; Zhu *et al*., 1992). But little information is available on *E. argyi* and its variation naturally growing in the metal contaminated soil. In this study, the volatiles components of different *E. argyi* plants–Purple Flower Elsholtzia (PFE1) and White Flower Elsholtzia (WFE) from the old Pb/Zn mined area, and Purple Flower

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Elsholtzia (PFE2) from Jiuxi uncontaminated agriculture soil were compared to make full use of these plant resources, especially after their being used for phytoremediation.

MATERIALS AND METHODS

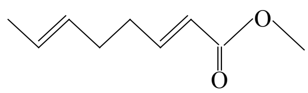
E. argyi (PFE1) and their variation (WFE) were harvested in November 2003 from Pb/Zn mined area in Sanmen County of Zhejiang Province, and the uncontaminated *E. argyi* (PFE2) from Jiuxi agriculture soil in Hangzhou County of Zhejiang Province.

A simple laboratory quickfit apparatus with a 2000 ml steam generator flask, a distilling flask, a condenser, and a receiving vessel, was used for the steam distillation. Eighty gram air-dried and chipped flower parts of *Elsholtzia* plants were subjected to steam distillation in the assembly described above. The volatile components were collected into the receiving flask during 1.5 h of steam distillation, dehydrated by Na_2SO_4, and identified by GC/MS (TRACE2000 series, ThermoQuest) technique with a DB-5MS 30 m×0.25 mm quartz capillary column, current gas was N_2, inject temperature was 280 °C, and 0.2 μl was injected (split ratio of 10:1). MS conditions were as follows: 200 °C EI source temperature, 70 eV electron energy, 250 °C interface temperature, 250 eV detector voltage and 2.0 s scan rate. The relative proportions of the essential oil components were expressed as percentages obtained by GC peak area normalization. The identification of their components was based on comparisons of their relative retention times and mass spectra with those of GC/MS library data.

RESULTS AND DISCUSSION

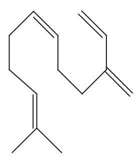
The total ion chromatograms (TIC) of essential oils from the flowers of the Elsholtzia plants are presented in Fig.1. The results of GC/MS analyses are listed in Table 1. Seventeen compounds identified in PFE1 constituted 86.7% of total essential oils. The essential oils of the flowers of PFE1 contained a high level of 2,6-octadienoic acid,3,7-dimethyl-methyl ester (1) (63.3%). Dehydroelsholtzia ketone (5.5%), caryophyllene (4.5%) and 7,11-dimethyl-3-methylene-1,6,10-dodecatriene (Z)- (2) (7.9%) were identified. Sixteen compounds amounting to 95.3% were identified in the volatile oils of WFE; caryophyllene (3) (55.0%) was the main ingredient of fragrances. 2,6-octadienoic acid,3,7-dimethyl-methyl ester (1) (10.2%), dehydroelsholtzia ketone (2.8%) and 7,11-dimethyl-3-methylene-1,6,10-dodecatriene (Z)- (6.5%) were obtained. 7,11-dimethyl-3-methylene-1,6,10-dodecatriene (E)- (4) (10.6%) was identified in WFE but not in PFE1 and PFE2. PFE1 and WFE have evolved to survive in the same Pb/Zn mined area, but showed different phytochemical properties. These compounds are shown as follows:

2,6-octadienoic acid,3,7-dimethyl-methyl ester



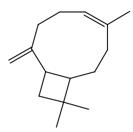
(1)

7,11-dimethyl-3-methylene-1,6,10-dodecatriene (Z)-



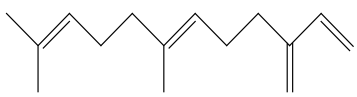
(2)

Caryophyllene



(3)

7,11-dimethyl-3-methylene-1,6,10-dodecatriene (E)-



(4)

Compared to PFE1, PFE2 contained lower level of 2,6-octadienoic acid,3,7-dimethyl-methyl ester (1) (31.8%) and 7,11-dimethyl-3-methylene-1,6,10-dod ecatriene (Z)- (2) (5.9%), but exhibited greater level of dehydroelsholtzia ketone (10.3%), 3,7-dimethyl-2,6-octadienoal (Z)- (5.4%), 3,7-dimethyl-2,6-octadienoal (E)- (7.8%) and caryophyllene (3) (5.9%), indicating that the difference in geographical areas where the plants of Purple Flower Elsholzta grow resulted in distinct difference in essential oil components in the flower of the both plants.

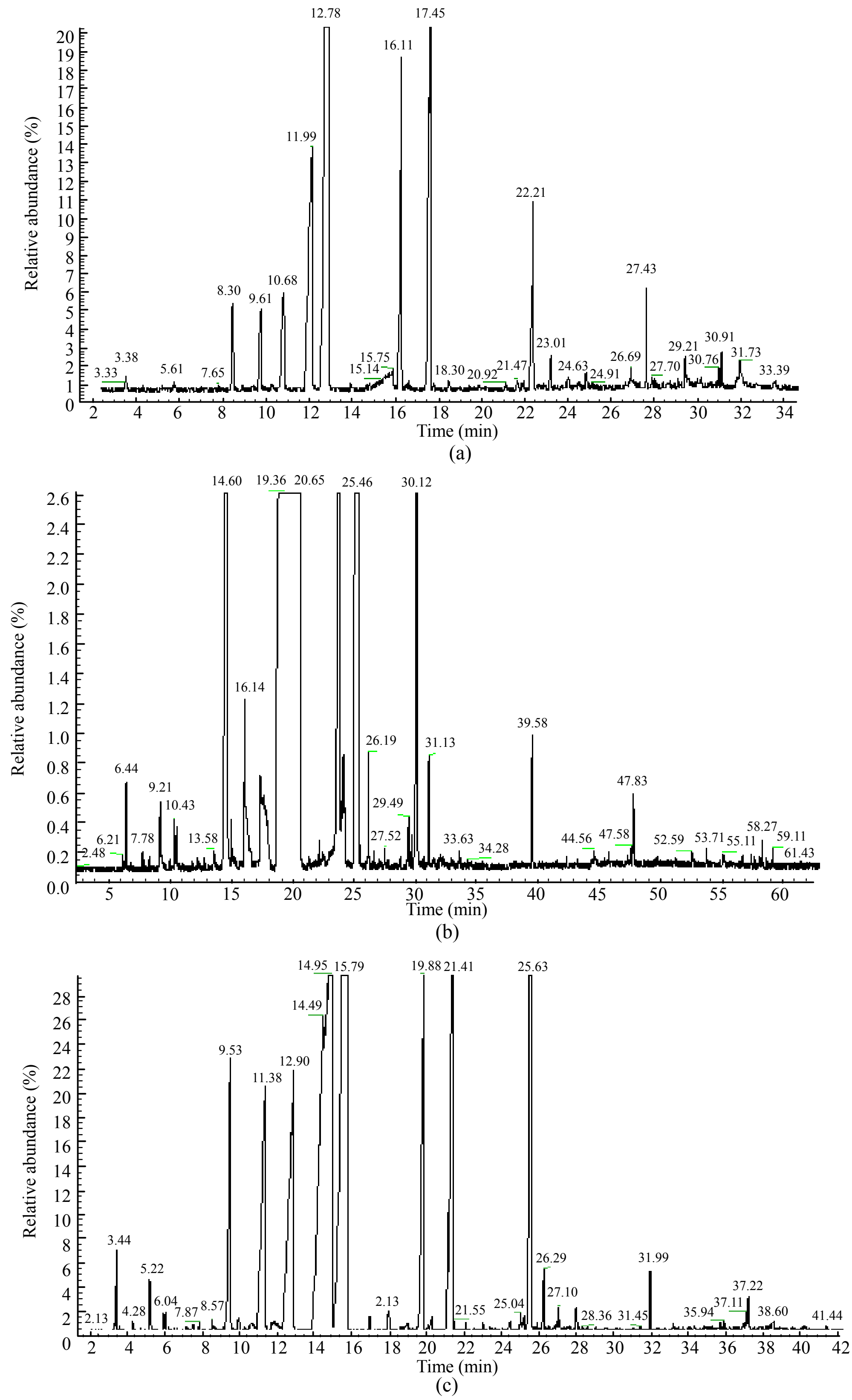


Fig.1 The total ion chromatograms (TIC) obtained from the volatile oil in the flower of (a) the variation of *E. argyi* naturally growing in Pb/Zn mined area; (b) *E. argyi* naturally growing in Pb/Zn mined area; (c) *E. argyi* naturally growing in the uncontaminated agricultural soil

Table 1 The main components of the essential oils from *Elsholtzia argyi*

No.	Compounds	Molecular formula	*MW*	Relative level (%)		
				WFE*	PFE1*	PFE2*
1	Acetophenone	C_8H_8O	120	–	0.07	0.29
2	6-methyl-5-hepten-2-one	$C_8H_{14}O$	126	0.37	0.32	0.02
3	1-octen-3-ol	$C_8H_{16}O$	128	–	0.09	0.39
4	3-octanol	$C_8H_{18}O$	130	–	–	–
5	1-methyl-5-(1-methylethenyl)-cyclohexene	$C_{10}H_{16}$	136	–	0.01	–
6	(1,3-dimethyl-2-methylene-cyclopentyl)-methanol	$C_9H_{16}O$	140	–	–	0.06
7	Nonanal	$C_9H_{18}O$	142	–	–	0.11
8	3,7-dimethyl-(Z)-2,6-octadienal	$C_{10}H_{16}O$	152	1.77	0.34	5.41
9	3,7-dimethyl-(E)-2,6-octadienal	$C_{10}H_{16}O$	152	–	0.17	7.77
10	Eucalyptol	$C_{10}H_{18}O$	154	–	–	–
11	3,7-dimethyl-1,6-octadien-3-ol	$C_{10}H_{18}O$	154	–	0.03	0.09
12	Dehydroelsholtzia ketone	$C_{10}H_{12}O_2$	164	2.77	5.47	10.28
13	Elsholtzia ketone	$C_{10}H_{14}O_2$	166	1.39	1.68	3.22
14	Geranic acid	$C_{10}H_{16}O_2$	168	–	1.36	–
15	2,6-octadienoic acid,3,7-dimethyl-methyl ester	$C_{11}H_{18}O_2$	182	10.16	63.30	31.76
16	2,6-octadien-1-ol,3,7-dimethyl-acetate, (Z)-	$C_{12}H_{20}O_2$	196	–	–	0.14
17	Caryophyllene	$C_{15}H_{24}$	204	55.02	4.48	5.86
18	trans-α-bergamotene	$C_{15}H_{24}$	204	–	0.16	–
19	7,11-dimethyl-3-methylene-1,6,10-dodecatriene, (Z)-	$C_{15}H_{24}$	204	6.48	7.86	5.91
20	7,11-dimethyl-3-methylene-1,6,10-dodecatriene, (E)-	$C_{15}H_{24}$	204	10.56	–	–
21	1-methyl-5-methylene-8-(1-methylethyl)-1,6-cyclodecadiene [s-(E,E)]-	$C_{15}H_{24}$	204	0.19	0.08	–
22	2,6-dimethyl-6-(4-methyl-3-pentenyl)-bicyclo[3.1.1]hept-2-ene	$C_{15}H_{24}$	204	–	–	0.09
23	α-farnesene	$C_{15}H_{24}$	204	–	–	0.03
24	2,6,10-trimethyl-dodecane	$C_{15}H_{32}$	212	–	–	0.03
25	Caryophyllene oxide	$C_{15}H_{24}O$	220	4.15	0.94	8.21
26	1,5,5,8-tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene	$C_{15}H_{24}O$	220	0.38	0.08	0.36
27	Farnesene epoxide, E-	$C_{15}H_{24}O$	220	–	–	0.11
28	4,4-dimethyl-tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol	$C_{15}H_{24}O$	220	–	–	0.10
29	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.36	–	–
30	n-hexadecanoic acid	$C_{16}H_{32}O_2$	256	0.52	–	–
31	6,10,14-trimethyl-2-pentadecanone	$C_{18}H_{36}O$	268	0.73	–	0.31
32	2,6,11,15-tetramethyl-hexadeca-2,6,8,10,14-pentane	$C_{20}H_{32}$	272	0.32	–	0.16
33	2,3-dimethyl-5-(2,6,10-trimethylundecyl) furan	$C_{20}H_{36}O$	292	–	–	0.10
34	9,12,15-octadecatrienoic acid,2-phenyl-1,3-dioxan-5-yl ester	$C_{28}H_{40}O_4$	440	0.15	–	
Total				95.32	86.66	80.18

* WFE: variation of *E. argyi* from Sanmen Pb/Zn mined area; PFE1: *E. argyi* from Sanmen Pb/Zn mined area; PFE2: *E. argyi* from Jiuxi uncontaminated agricultural area

Among the common components in the essential oils, 10 compounds out of the total oils in PFE1 and WFE, while 12 compounds of the total oils in PFE1 and PFE2, were observed. PFE1 and WFE contained many more of the components such as 3,7-dimethyl-2,6-octadienal (Z)-, caryophyllene, dehydroelsholtzia ketone, elsholtzia ketone, and caryophyllene oxide. All the three Elsholtzia plants contain Elsholtzia ketone, which is the bacterium and epiphyte resisting characteristic of Elsholtzia plants. While WFE contain much more caryophyllene than other *E. argyi* plants. Caryophyllene is the perfume ingredient in plants, and can be exploited for versatile uses as fragrances.

Phytoremediation has been considered as a promising approach to remove metals from contaminated soils. Disposal of remedial plant materials after harvest is an unsolved problem (Sas-Nowosielska *et al.*, 2004). Several methods such as composting, compaction, incineration, ashing, pyrolysis, direct disposal and liquid extraction are used for the disposal of the contaminated plant materials after phytoextraction process (Kumar *et al.*, 1995; Salt *et al.*, 1995, 1998; Raskin *et al.*, 1997; Blaylock *et al.*, 1997; Garbisu and Alkorta, 2001; Mulligan *et al.*, 2001). But these disposal treatments are based on the idea that the contaminated plant materials are dealt with as castoff rather than better utilized as resources. *Thlaspi* can be incinerated to obtain Zn in its ash after harvest from Zn contaminated soil, which is called plant mining. The selected *Elsholtzia argyi* plants exhibited difference in their volatile components, but the perfume ingredient and antibacterial and antiseptic compounds in the essential oils of the three Elsholtzia plants can be fully utilized for the manufacture of perfumery products and antimicrobial and antiseptic agents.

CONCLUSION

Mined ecotypes of *Elsholtzia argyi* are Pb-accumulating plant species native to China, and have been suggested to be used for phytoremediation of metal contaminated soils, due to its great biomass and wide adaptation. The disposal of remedial materials after removal of their heavy metals from contaminated soil has become a key problem. The results from this study indicate that the flowers of the selected *Elsholtzia argyi* plants contain many perfume components, antibacterial and antiseptic ingredients, which can be utilized for flavor and antiseptics manufacture. The full utilization of phytoremediated plant materials is particularly important for implementation of innovative environmental biotechnology.

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