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Comparative Analysis of Chemical Composition of Three *Elsholtzia* Volatile Oils

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Abstract

Objective: Volatiles oils from three *Elsholtzia* plants (*E. capituligera* C.Y.Wu, *E. fruticosa* (D.Don) Rehd. and *E. bodinieri* Vaniot) were extracted by simultaneous distillation extraction device (SDE). Their volatile compounds were isolated and characterized by GC-MS, in order to provide a theoretical basis for its further development and utilization.

Methods: Under the same conditions, the main volatile compounds were identified by WILEY and NIST and the relative percentage of volatile oil were determined by using the normalization method.

Results: The main volatile compounds for each species were Eucalyptol (Relative content: 31.55%), (-)-Verbenone (Relative content: 25.36%) and 2, 5-dimethyl-2-Isopropenyl-1-cyclohexanone (Relative content:12.26%) in *Elsholtzia capituligera* C.Y.Wu; 2, 5-dimethyl-2-Isopropenyl-1-cyclohexanone (Relative content: 60.78%) and (-)-Verbenone (Relative content: 19.78%) in *Elsholtzia Fruticosa* (D.Don) Rehd; Eucalyptol (Relative content: 55.08%) and Terpinyl acetate (Relative content: 5.64%) in *Elsholtzia bodinieri* Vaniot.

Conclusion: Comparing of volatile compounds of these three samples showed that the volatiles oil of these genus plants have some differences, the reason might be determined by genotype. This study not only provides a lot of valuable experimental data, but also greatly promoted the volatiles oil research for other researchers.

Keywords*: *Elsholtzia capituligera C.Y.Wu; Elsholtzia fruticosa (D.Don) Rehd.; Elsholtzia bodinieri vaniot; Gas chromatography-mass spectrometry (GC-MS); Volatile oil

Introduction

The genus *Elsholtzia willd* (Laminaceae) is mainly distributed in eastern Asia, of which three species distributed in Ethiopia in Africa, and only one was found in extension to Europe and North America. It is reported that this genus has about 40

species, of which 33 species, 15 varietals and 5 forma are distributed in China [1]. In china 26 species and 11 varietals could be found in Yunnan Province [2]. Many species of this genus have been used as Traditional Chinese Medicine for a long time. The volatile oil of Elsholtzia exhibit some beneficial pharmacological effects, such as analgesia, sedation, antispasmodic, antibacterial, anti-inflammatory and antioxidant [3-7]; and could be used in natural air fresheners, food preservation, food additives, rare wild vegetable oil and other fields [8,9].

E. capituligera C.Y.Wu, mainly distributed in the northwest of Yunnan Province and southwest of Sichuan Province, grows in the weathered gravel with dry and abundant sunshine at elevations of 2000 meters to 3000 meters [10]. In the Tibetan medicine system, the growth of a year of foliage and inflorescence medicine has been used to treat anal, fetal, skin, and gastrointestinal diseases and topical anti-mosquito bites.

E. fruticosa (D.Don) Rehd., mainly distributed in the south of Gansu Province (Bailongjiang River Basin), western of Hubei Province, Sichuan Province, Tibet Autonomous Region, Yunnan Province, Guizhou Province and Guangxi Province, and Bhutan, Nepal, Sikkim, Northern India, grows in the valley side, bottom, roadside, hillside and grass at elevations of 1200-3200 meters. It has been used to the treatment of rash and detoxification [11].

E. bodinieri Vaniot, mainly distributed in the southwest, western, central and southern of Yunnan province, grows in the slopes and in a drier environment at elevations of 1200 meters to 3000 meters. It has been used to treat the headache fever, toothache, sore throat, diarrhea, indigestion, eye pain, urinary and hepatitis [12]. To the best of our knowledge, *Elsholtiza bodinieri* Vaniot is the only edible plant in this genus [13].

Materials and Methods

Herbs

The whole plant of *E. capituligera* C.Y.Wu was collected from the Lancang River Basin RUMEI power station in Mangkang County, Tibet Autonomous Region, May, 2016 (Specimen number: YNNI16-05-11). *E. fruticosa* (D.Don) Rehd. was collected from the Lancang River Valley 318 line (La Wu village) in Mangkang County, Tibet Autonomous Region, May, 2016 (Specimen number: YNNI16-05-12). *E. bodinieri* Vaniot was collected from Yuxi City Xinping County, July, 2016 (Specimen number: YNNI16-07-18). The plant samples were identified by Dr. Yang Lipan from Yunnan College of traditional Chinese medicine. The samples were stored in the Key Laboratory of Chemistry in Ethnic Medicinal Resouces, State Ethnic Affairs Commission and Ministry of Education, Yunnan Minzu University. All these herbs are air-dried.

Reagents

All reagents were analytical grade. Dichloromethane, sodium chloride and anhydrous sodium sulfate were obtained from the Tianjin Fengchuan Chemical Reagents Technology Co., Ltd. Distilled water was purchased from Watsons.

Instruments and equipment

Simultaneous Distillation Extraction Device was homemade; PE Clarus 600 Gas Chromatography Mass Spectrometer with Electron Impact Ion Source (EI) and WILEY, NIST Mass Spectrometry Database were from PerkinElmer, USA; DB-5MS capillary column (30 m × 0.25 mm × 0.25 μm) was produced by the United States Agilent; one thousandth of the electronic

analytical balance was obtained from the Austrian Hauser Instruments Shanghai Co., Ltd.; KDM-type adjustable temperature heating sets 250 mL, 2000 mL was produced by Shandong Juancheng Hualu Electric Instrument Co., Ltd. Rotary Evaporator was purchased from Shanghai Ailang Instrument Co., Ltd.

Test conditions

Gas chromatographic conditions: Column: DB-5MS capillary column (30 m × 0.25 mm × 0.25 μm), inlet temperature of 250°C. Temperature program: the initial temperature of 50°C and keep 5 min, then to 2°C/min the heating rate of up to 80°C, then to 3°C/min the heating rate of up to 230°C and keep the temperature of 16 min, then to 3°C/min the heating rate of up to 250°C and keep the temperature of 20 min. The final set helium flow rate of 0.7 mL/min, injection volume of 2.0 μL and adjust the gas flow rate of 30: 1.

Mass spectrometry conditions: Ion source is electron bombardment (EI) at 180°C, and the ionization energy is 70 eV. Transmission line temperature is 260°C. The monitoring mode is full ion scanning, and the range of mass scan is m/z35 ~ 400. The solvent delay time is 5 min.

Experimental Content

Pretreatment of herbs

Dried herbs were cut into small pieces of 2 ~ 3 cm, spare.

Simultaneous distillation extraction of volatile components from herbs

The pre-processed herbs (10.0 g) were treated by simultaneous distillation extraction method for 4 hours with 300 mL of distilled water (dissolved in 30.0 g sodium chloride, the purpose is to reduce the volatile oil components dissolved in water) and 50 mL of dichloromethane. Then the samples were cooled to room temperature, followed by extracting with methylene chloride and dichloromethane together. The anhydrous sodium sulfate was used to remove water from the extract. Finally, the yellow oil was collected after filtration and distillation, which was stored in sealed vials in a refrigerator at 0°C before analysis.

The relative content of volatile oil were determined

The volatile oil is a complex mixture, and its boiling point is generally between 70~300°C. Therefore, the relative content of volatile oil was determined by GC-MS with peak area normalization method.

Calculation formula : $$\omega\% = \frac{A_i}{A_1 + A_2 + \cdots + A_n} \times 100$$

Note: "A_i" means Peak area. "$\omega\%$" means relative content.

Ingredient identification

The total ion chromatogram of the volatile components was analyzed as following (FIG. 1 to FIG. 3) under the conditions of the gas chromatographic mass spectrometry (GC-MS) given above.

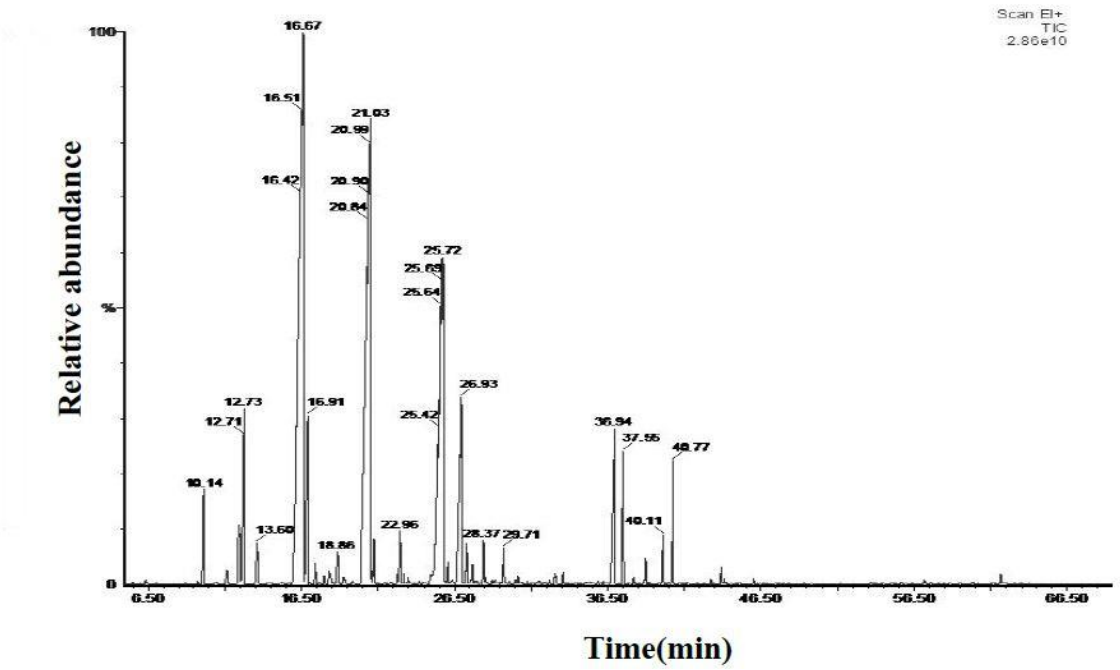

FIG. 1. **The total ion chromatogram of chemical constiuents in essential oil from *Elsholtzia capituligera* C.Y.Wu.**

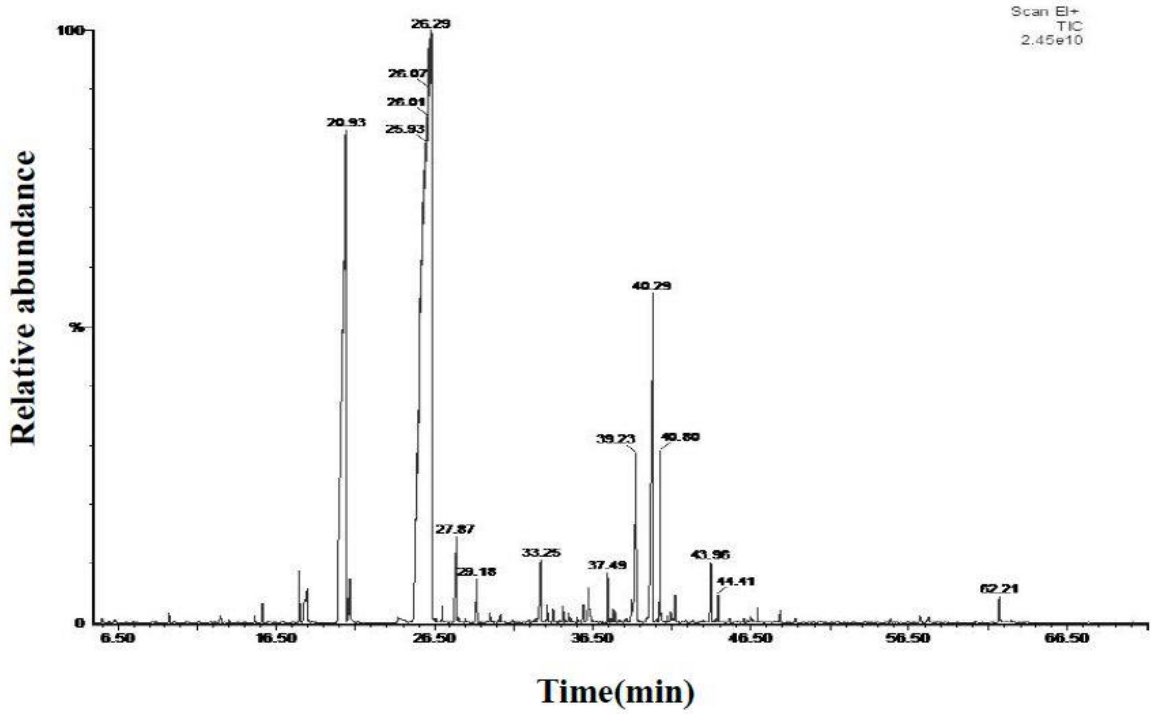

FIG. 2. **The total ion chromatogram of chemical constituents in essential oil from *Elsholtzia fruticosa* (D.Don) Rehd.**

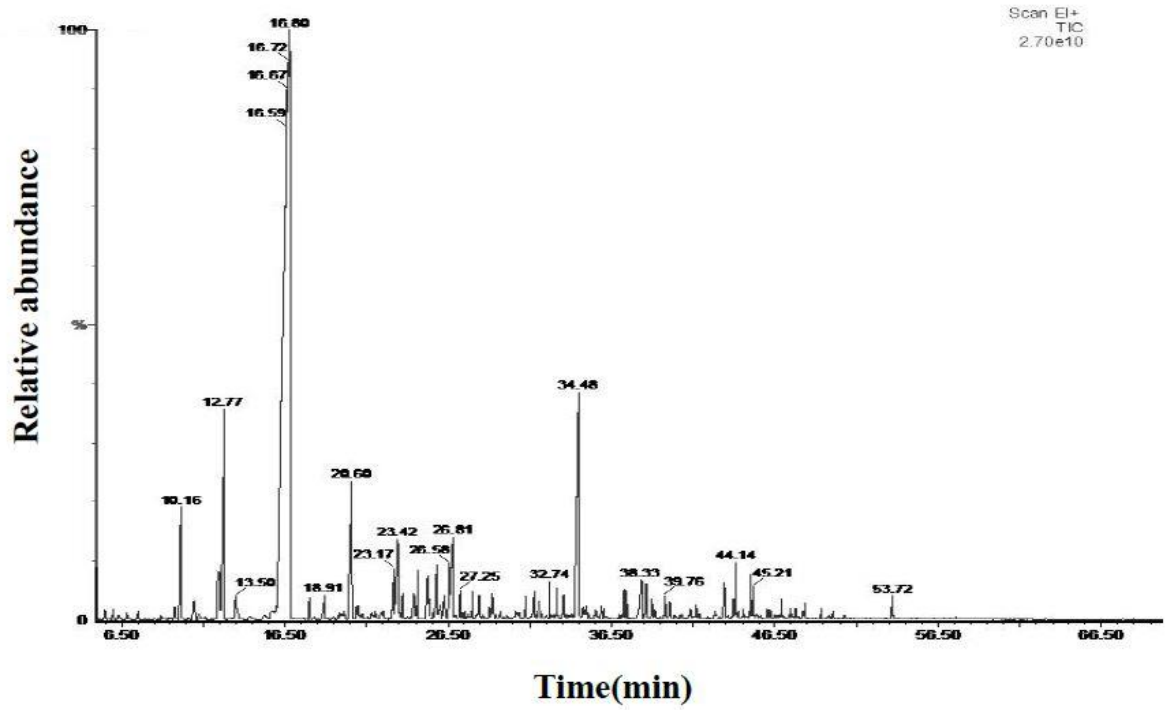

FIG. 3. **The total ion chromatogram of chemical constituents in essential oil from *Elsholtiza bodinieri* Vaniot.**

According to the WILEY and NIST Mass Spectrometry Database, the main chemical constituents of these three volatile oil were retrieved (TABLE 1**)** and the relative percentage of each compound was calculated by initial quantification.

TABLE 1. **Chemical constituents of volatile oils.**

Serial No.	**Name**	**CAS no.**	**Formula**	***E. capituligera* C.Y.Wu**		***E. fruticosa* (D.Don) Rehd.**		***E. bodinieri* Vaniot**	
				RT (minutes)	**RC (%)**	**RT (minutes)**	**RC (%)**	**RT (minutes)**	**RC (%)**
1	Furfural	98-01-1	$C_5H_4O_2$	5.5	0.01	5.49	0.04	5.49	0.13
2	2-Hexenal	505-57-7	$C_6H_{10}O$	6.32	0.03	-	-	-	-
3	(*E*)-4-Hexen-1-ol	928-92-7	$C_6H_{12}O$	6.42	0.01	-	-	-	-
4	5-methyl-2-Furancarboxaldehyde	620-02-0	$C_6H_6O_2$	8.92	0.01	8.92	0.02	-	-

5	4-methyl-1-(1-methylethyl)-Bicyclo[3.1. 0] hexane didehydro deriv.	58037-87-9	$C_{10}H_{16}$	9.75	0.03	9.75	0.08	9.76	0.21
6	(1*R*)-(+)-α-pinene	7785-70-8	$C_{10}H_{16}$	10.14	1.19	-	-	10.16	1.87
7	4, 4-Dimethyl-2-buten-4-olide	20019-64-1	$C_6H_8O_2$	10.87	0.01	10.84	0.03	10.94	0.37
8	Camphene	79-92-5	$C_{10}H_{16}$	10.99	0.03	-	-	11	0.26
9	Benzaldehyde	100-52-7	C_7H_6O	11.64	0.22	11.62	0.02	-	-
10	4-methylene-1-(1-methylethyl)-Bicyclo [3.1.0] hexane	3387-41-5	$C_{10}H_{16}$	12.46	1.12	12.39	0.02	12.48	1.33
11	Myrcene	123-35-3	$C_{10}H_{16}$	13.61	0.67	13.54	0.02	-	-
12	Pseudolimonene	499-97-8	$C_{10}H_{16}$	14.34	0.01	-	-	-	-
13	3-Methylene-1, 5, 5-trimethylcyclohexene	16609-28-2	$C_{10}H_{16}$	15.24	0.04	-	-	-	-
14	Eucalyptol	470-82-6	$C_{10}H_{18}O$	16.67	31.55	-	-	16.8	55.08
15	(*E*)-3,7-Dimethyl-1,3,6-octatriene	3779-61-1	$C_{10}H_{16}$	16.91	2.57	-	-	-	-
16	Benzeneacetaldehyde	122-78-1	C_8H_8O	17.04	0.02	16.93	0.03	-	-
17	3, 7-dimethyl-1, 3, 6-Octatriene	13877-91-3	$C_{10}H_{16}$	17.41	0.21	-	-	-	-
18	γ-Terpinene	99-85-4	$C_{10}H_{16}$	18.03	0.1	18	0.67	18.06	0.32
19	Acetophenone	98-86-2	C_8H_8O	18.31	0.28	18.43	0.81	-	-
20	cis-4-Thujanol	15537-55-0	$C_{10}H_{18}O$	18.86	0.64	18.86	0.03	20.93	0.17
21	α-Pinene epoxide	1686-14-2	$C_{10}H_{16}O$	19.26	0.13	-	-	-	-
22	(+)-4-Carene	29050-33-7	$C_{10}H_{16}$	19.83	0.05	-	-	-	-
23	(-)-Verbenone	1196-01-6	$C_{10}H_{14}O$	21.03	25.36	20.93	19.78	27.24	0.4

24	Perillene	539-52-6	$C_{10}H_{14}O$	21.14	0.07	21.05	0.11	-	-
25	Linalool	78-70-6	$C_{10}H_{18}O$	21.23	0.4	21.15	0.34	21.02	0.18
26	2-Ethenyl-1, 1-dimethyl-3-methylenecyclohexane	95452-08-7	$C_{11}H_{18}$	21.97	0.01	-	-	-	-
27	trans-p-Mentha-2,8-dienol	-	$C_{10}H_{16}O$	22.27	0.03	-	-	-	-
28	cis-1-methyl-4-(1-methylethyl)-2-Cyclohexen-1-ol	29803-82-5	$C_{10}H_{18}O$	22.41	0.02	-	-	-	-
29	(4*E*,6*Z*)-2, 6-Dimethyl-2, 4, 6-octatriene	7216-56-0	$C_{10}H_{16}$	22.81	0.08	-	-	-	-
30	1, 4-dimethyl-Bicyclo[2.1.0]pentane	17065-18-8	C_7H_{12}	22.96	0.56	-	-	-	-
31	Limonene 1, 2-epoxide	1195-92-2	$C_{10}H_{16}O$	23.21	0.11	-	-	-	-
32	Pinocarveol	5947-36-4	$C_{10}H_{16}O$	23.33	0.01	-	-	-	-
33	3,7 -dimethyl-1,7-octadien-3-ol	598-07-2	$C_{10}H_{18}O$	23.49	0.06	-	-	-	-
34	2-(2-Propenyl) bicyclo[2.2.1]heptane	2633-80-9	$C_{10}H_{16}$	24.17	0.06	24.18	0.29	-	-
35	1-(2-furanyl)-3-methyl-2-Butanone	20907-04-4	$C_9H_{12}O_2$	24.48	0.04	-	-	-	-
36	2, 6-Dimethyl-1-nonen-3-yn-5-ol	-	$C_{11}H_{18}O$	25.43	2.38	-	-	-	-
37	2, 5-dimethyl-2-Isopropenyl-1-cyclohexanone	6711-26-8	$C_{11}H_{18}O$	25.72	12.26	25.29	60.78	-	-
38	(-)-4-Terpineol	20126-76-5	$C_{10}H_{18}O$	26.04	0.15	-	-	-	-

39	(*E*)-2, 6-Dimethyl-3,7-octadiene-2,6-diol	13741-21-4	$C_{10}H_{18}O_2$	26.37	0.07	-	-	-	-
40	α-Terpineol	98-55-5	$C_{10}H_{18}O$	26.93	4.06	26.94	0.02	-	-
41	2, 5-Dimethylfuran	625-86-5	C_6H_8O	27.7	0.22	27.87	1.11	-	-
42	2-Cyclopentene-1-carboxylic acid, 1, 2-dimethyl-, ethyl este	5809-03-0	$C_{10}H_{16}O_2$	27.83	0.02	-	-	-	-
43	7-methyl-3-methylene-6-octen-1-ol	13066-51-8	$C_{10}H_{18}O$	27.95	0.04	-	-	-	-
44	1, 3, 3-trimethyl-2-Oxabicyclo[2.2.2]octan-6-ol	18679-48-6	$C_{10}H_{18}O_2$	28.37	0.54	-	-	28.39	0.45
45	Citronellol	106-22-9	$C_{10}H_{20}O$	28.53	0.06	-	-	-	-
46	(*Z*)-3, 7-dimethylocta-2, 6-dienal	106-26-3	$C_{10}H_{16}O$	28.98	0.03	-	-	-	-
47	(1α, 2β, 5α)-2-Methyl-5-(1-methylvinyl) cyclohexan-1-ol	38049-26-2	$C_{10}H_{18}O$	29.16	0.03	-	-	-	-
48	2, 6-Dimethyl-1,7-octadien-3-ol	22460-59-9	$C_{10}H_{18}O$	29.52	0.02	-	-	-	-
49	*p*-menth-1-en-3-one	89-81-6	$C_{10}H_{16}O$	29.71	0.41	-	-	-	-
50	2-(2-methylpropylidene)-Cyclohexanone	43108-69-6	$C_{10}H_{16}O$	30.09	0.02	-	-	-	-
51	(*E*)-3, 7-dimethyl-2,6-Octadienal	141-27-5	$C_{10}H_{16}O$	30.51	0.03	-	-	-	-

52	3-(1-Methylethenyl)-2, 5-dimethyl-3,4-hexadien-2-ol	15448-75-6	$C_{11}H_{18}O$	30.65	0.07	30.68	0.07	-	-
53	2-Oxabicyclo[2.2.2]octan-6-ol, 1, 3, 3-trimethyl-, acetate	57709-95-2	$C_{12}H_{20}O_3$	31.26	0.02	-	-	-	-
54	Dehydroelsholtzione	6138-88-1	$C_{10}H_{12}O_2$	33.02	0.03	-	-	-	-
55	Elixene	8/8/3242	$C_{15}H_{24}$	33.56	0.11	33.6	0.15	-	-
56	8-(1-Methylethylidene)bicyclo [5.1.0]octane	54166-47-1	$C_{11}H_{18}$	35.36	0.02	-	-	-	-
57	β-Bourbonene	5208-59-3	$C_{15}H_{24}$	35.88	0.03	35.91	0.21	-	-
58	β-Elemene	515-13-9	$C_{15}H_{24}$	36.21	0.02	36.24	0.39	-	-
59	Methyl eugenol	93-15-2	$C_{11}H_{14}O_2$	36.94	3.05	-	-	-	-
60	β-Caryophyllene	87-44-5	$C_{15}H_{24}$	37.55	1.63	37.49	0.54	37.48	0.38
61	1-Cyclopropyl-1-propanone	6704-19-4	$C_6H_{10}O$	38.19	0.06	-	-	38.33	0.97
62	1, 1, 7-Trimethyl-4-methylenedecahydro-1H-cyclopropa[e]azulene	25246-27-9	$C_{15}H_{24}$	38.54	0.01	-	-	39.16	0.12
63	α-Caryophyllene	6753-98-6	$C_{15}H_{24}$	39.01	0.26	-	-	39	0.26
64	(+)-Aromadendrene	489-39-4	$C_{15}H_{24}$	39.17	0.01	-	-	-	-
65	Germacrene D	23986-74-5	$C_{15}H_{24}$	40.11	0.52	40.29	6.1	-	0.23
66	(+)-Ledene	21747-46-6	$C_{15}H_{24}$	40.51	0.01	-	-	-	-
67	α-Farnesene	502-61-4	$C_{15}H_{24}$	41.2	0.01	41.22	0.07	-	-
68	Thymoquinone	490-91-5	$C_{10}H_{12}O_2$	42.81	0.01	-	0.03	-	-

69	Geranyl isobutyrate	2345-26-8	$C_{14}H_{24}O_2$	43.26	0.04	-	-	-	-
70	Spathulenol	77171-55-2	$C_{15}H_{24}O$	43.91	0.17	46.06	0.04	43.96	0.26
71	Caryophyllene oxide	1139-30-6	$C_{15}H_{24}O$	44.09	0.06	-	-	44.14	0.81
72	Globulol	51371-47-2	$C_{15}H_{26}O$	44.24	0.01	44.26	0.02	44.28	0.08
73	n-Hexadecanoic acid	57-10-3	$C_{16}H_{32}O_2$	57.6	0.01	57.72	0.08	-	-
74	Phytol	150-86-7	$C_{20}H_{40}O$	62.19	0.09	62.21	0.24	-	-
75	Diisooctyl phthalate	27554-26-3	$C_{24}H_{38}O_4$	74.54	0.02	74.56	0.02	-	-
76	1-Acetyl-2-methylcyclopentene	3168-90-9	$C_8H_{12}O$	-	-	5.98	0.02	-	-
77	Furfuryl alcohol	98-00-0	$C_5H_6O_2$	-	-	6.31	0.04	-	-
78	Mushroom alcohol	3391-86-4	$C_8H_{16}O$	-	-	13	0.09	-	-
79	Terpinolene	586-62-9	$C_{10}H_{16}$	-	-	15.17	0.09	-	-
80	1-isopropyl-2-methylbenzene	527-84-4	$C_{10}H_{14}$	-	-	15.68	0.21	15.71	0.37
81	Dipentene	138-86-3	$C_{10}H_{16}$	-	-	15.98	0.02	-	-
82	2-Methylbutyl 2-methylbutyrate	2445-78-5	$C_{10}H_{20}O_2$	-	-	21.34	0.02	-	-
83	Octen-1-ol acetate	32717-31-0	$C_{10}H_{18}O_2$	-	-	21.67	0.02	39.76	0.28
84	2-(4-Methylphenyl)propan-2-ol	1197-01-9	$C_{10}H_{14}O$	-	-	26.62	0.02	26.29	0.51
85	Cuminaldehyde	122-03-2	$C_{10}H_{12}O$	-	-	27.03	0.13	29.06	0.11
86	Benzylacetone	2550-26-7	$C_{10}H_{12}O$	-	-	29.18	0.46	-	-
87	Benzalacetone	122-57-6	$C_{10}H_{10}O$	-	-	29.85	0.02	-	-
88	4-Phenyl-2-butanol	2344-70-9	$C_{10}H_{14}O$	-	-	30.02	0.08	-	-
89	Lavandulol, acetate	-	$C_{12}H_{20}O_2$	-	-	31.46	0.03	-	-
90	(*E*)-Benzalacetone	1896-62-4	$C_{10}H_{10}O$	-	-	34.67	0.17	-	-

91	nerol acetate	141-12-8	$C_{12}H_{20}O_2$	-	-	34.96	0.07	35.87	0.21
92	α-Copaene	3856-25-5	$C_{15}H_{24}$	-	-	35.56	0.06	35.58	0.17
93	α-Gurjunene	489-40-7	$C_{15}H_{24}$	-	-	36.95	0.02	-	-
94	β-Cubebene	13744-15-5	$C_{15}H_{24}$	-	-	37.92	0.08	-	-
95	2-methylene-5-(1-methylvinyl)-8-methyl-Bicyclo[5.3.0]decane	-	$C_{15}H_{24}$	-	-	38.27	0.02	-	-
96	(*E*)-β-Farnesene	18794-84-8	$C_{15}H_{24}$	-	-	39.23	2.24	-	-
97	(-)-Isoledene	-	$C_{15}H_{24}$	-	-	40.56	0.03	-	-
98	γ-Elemene	339154-91-5	$C_{15}H_{24}$	-	-	40.8	1.91	50.06	0.09
99	γ-Cadinene	39029-41-9	$C_{15}H_{24}$	-	-	41.44	0.07	-	-
100	(-)-β-Cadinene	523-47-7	$C_{15}H_{24}$	-	-	41.68	0.22	-	-
101	Dihydroactindiolide	15356-74-8	$C_{11}H_{16}O_2$	-	-	41.81	0.02	-	-
102	1, 2, 4a, 5, 6, 8a-Hexahydro-4,7-dimethyl-1-(1-methylethyl) naphthalene	483-75-0	$C_{15}H_{24}$	-	-	42.37	0.03	-	-
103	Nerolidol	40716-66-3	$C_{15}H_{26}O$	-	-	43.46	0.02	-	-
104	1-Hydroxy-1, 7-dimethyl-4-isopropyl-2, 7-cyclodecadiene	72120-50-4	$C_{15}H_{26}O$	-	-	43.96	0.64	-	-
105	β-Eudesmol	473-15-4	$C_{15}H_{26}O$	-	-	44.41	0.23	-	-
106	Ledene oxide-(II)	-	$C_{15}H_{24}O$	-	-	46.29	0.02	-	-
107	T-Cadinol	-	$C_{15}H_{26}O$	-	-	46.44	0.06	-	-
108	α-Cadino	481-34-5	$C_{15}H_{26}O$	-	-	46.94	0.15	46.96	0.25
109	(+)-Carotol	465-28-1	$C_{15}H_{26}O$	-	-	48.32	0.13	48.33	0.22
110	Myristinaldehyde	124-25-4	$C_{14}H_{28}O$	-	-	49.29	0.04	-	-

111	Perhydrofarnesyl acetone	502-69-2	$C_{18}H_{36}O$	-	-	53.71	0.02	53.72	0.31
112	cis, cis, cis-7,10,13-Hexadecatrienal	56797-43-4	$C_{16}H_{26}O$	-	-	55.31	0.04	-	-
113	Geranyl linalool	1113-21-9	$C_{20}H_{34}O$	-	-	57.16	0.1	-	-
114	Linolenic acid	463-40-1	$C_{18}H_{30}O_2$	-	-	62.95	0.02	-	-
115	n-Octacosane	630-02-4	$C_{28}H_{58}$	-	-	73.8	0.03	-	-
116	n-Eicosane	112-95-8	$C_{20}H_{42}$	-	-	83.4	0.03	-	-
117	rans-1-Ethoxy-1-butene	-	$C_6H_{12}O$	-	-	-	-	5.99	0.14
118	Leaf aldehyde	6728-26-3	$C_6H_{10}O$	-	-	-	-	6.33	0.08
119	Acetonyl acetate	592-20-1	$C_5H_8O_3$	-	-	-	-	6.84	0.09
120	1-Nonen-4-yne	31508-12-0	C_9H_{14}	-	-	-	-	7.5	0.13
121	4-methylene-1-(1-methylethyl)-Bicyclo [3.1.0] hex-2-ene	36262-09-6	$C_{10}H_{14}$	-	-	-	-	11.24	0.1
122	β-Pinene	127-91-3	$C_{10}H_{16}$	-	-	-	-	12.77	4.79
123	Dehydrocineole	92760-25-3	$C_{10}H_{16}O$	-	-	-	-	13.5	0.59
124	(1*S*)-(1)-β-Pinene	18172-67-3	$C_{10}H_{16}$	-	-	-	-	14.36	0.12
125	α-Terpinene	99-86-5	$C_{10}H_{16}$	-	-	-	-	15.24	0.18
126	cis-5-Ethenyltetrahydro-α,α-5-trimethyl-2-furanmethanol	5989-33-3	$C_{10}H_{18}O_2$	-	-	-	-	18.91	0.57
127	(*E*)-Linalool oxide	34995-77-2	$C_{10}H_{18}O_2$	-	-	-	-	20	0.1
128	1-methyl-4-(1-methylethenyl)-benzen	1195-32-0	$C_{10}H_{12}$	-	-	-	-	20.13	0.12

129	2-Pinen-4-one	80-57-9	$C_{10}H_{14}O$	-	-	-	-	20.6	3.18
130	7, 7-Dimethyl-bicyclo[2.2.1]heptan-2-ol	26908-71-4	$C_9H_{16}O$	-	-	-	-	21.31	0.08
131	3, 3, 6-trimethyl-1, 5-Heptadien-4-ol	27644-04-8	$C_{10}H_{18}O$	-	-	-	-	21.85	0.12
132	Fenchol	1632-73-1	$C_{10}H_{18}O$	-	-	-	-	22.05	0.08
133	trans-1-methyl-4-(1-methylethyl)-2-Cyclohexen-1-ol	29803-81-4	$C_{10}H_{18}O$	-	-	-	-	22.41	0.13
134	Campholenic aldehyde	4501-58-0	$C_{10}H_{16}O$	-	-	-	-	22.51	0.12
135	(1*R*)-(+)-Nopinone	38651-65-9	$C_9H_{14}O$	-	-	-	-	23.17	0.79
136	(-)-Trans-pinocarveol	547-61-5	$C_{10}H_{16}O$	-	-	-	-	23.42	1.74
137	(*S*)-cis-Verbenol	18881-04-4	$C_{10}H_{16}O$	-	-	-	-	23.76	0.43
138	5-(1-methylethyl)-Bicyclo[3.1.0] hexan-2-one	513-20-2	$C_9H_{14}O$	-	-	-	-	24.41	0.45
139	Pinocarvone	30460-92-5	$C_{10}H_{14}O$	-	-	-	-	24.65	0.75
140	(-)-α-Terpineol	10482-56-1	$C_{10}H_{18}O$	-	-	-	-	25.27	0.87
141	2, 2, 6-Trimethyl-6-ethenyltetrahydro-2H-pyran-3-ol	14049-11-7	$C_{10}H_{18}O_2$	-	-	-	-	25.6	0.1
142	3-acetoxy-4-(1-hydroxy-1-methylethyl)-1-methyl-Cyclohexene	-	$C_{12}H_{20}O_3$	-	-	-	-	25.35	0.21
143	Terpinen-4-ol	562-74-3	$C_{10}H_{18}O$	-	-	-	-	25.82	0.89
144	Myrtenal	564-94-3	$C_{10}H_{14}O$	-	-	-	-	26.58	0.87

145	Myrtenol	515-00-4	$C_{10}H_{16}O$	-	-	-	-	26.81	2.4
146	Decanal	112-31-2	$C_{10}H_{20}O$	-	-	-	-	27.4	0.08
147	cis-2-methyl-5-(1-methylethenyl)-2-Cyclohexen-1-ol	1197-06-4	$C_{10}H_{16}O$	-	-	-	-	28	0.44
148	2-methyl-5-(1-methylethenyl)-2-Cyclohexen-1-one	99-49-0	$C_{10}H_{14}O$	-	-	-	-	29.19	0.41
149	Lilac alcohol D	33081-37-7	$C_{10}H_{18}O_2$	-	-	-	-	29.7	0.12
150	(1*R*, 2*R*, 3*S*, 5*R*)-(-)-2, 3-Pinanediol	22422-34-0	$C_{10}H_{18}O_2$	-	-	-	-	30.66	0.17
151	4-(1-methylethyl)-1-Cyclohexene-1-carboxaldehyde	21391-98-0	$C_{10}H_{16}O$	-	-	-	-	30.83	0.09
152	Decyl alcohol	112-30-1	$C_{10}H_{22}O$	-	-	-	-	30.9	0.08
153	4-(1-methylethyl)-1, 3-Cyclohexadiene-1-methanol	1413-55-4	$C_{10}H_{16}O$	-	-	-	-	31.2	0.12
154	*L*-Borneol acetat	5655-61-8	$C_{12}H_{20}O_2$	-	-	-	-	31.29	0.29
155	p-Isopropylbenzyl alcohol	536-60-7	$C_{10}H_{14}O$	-	-	-	-	31.75	0.48
156	Perilla alcohol	536-59-4	$C_{10}H_{16}O$	-	-	-	-	32.07	0.26
157	Carvacrol	499-75-2	$C_{10}H_{14}O$	-	-	-	-	32.23	0.1
158	3, 7-dimethyl-2, 6-Octadienoic acid methyl ester	2349-14-6	$C_{11}H_{18}O_2$	-	-	-	-	33.19	0.47
159	4-(1-methylethyl)-1, 4-Cyclohexadiene-1-methanol	22539-72-6	$C_{10}H_{16}O$	-	-	-	-	33.56	0.42
160	trans-3-Caren-2-ol	—	$C_{10}H_{16}O$	-	-	-	-	34.06	0.08
161	Terpinyl acetate	80-26-2	$C_{12}H_{20}O_2$	-	-	-	-	34.48	5.64

162	decanyl acetate	112-17-4	$C_{12}H_{24}O_2$	-	-	-	-	37.27	0.36
163	α-muurolene	10208-80-7	$C_{15}H_{24}$	-	-	-	-	40.86	0.08
164	δ-Cadinene	483-76-1	$C_{15}H_{24}$	-	-	-	-	41.66	0.18
165	*d*-8-Acetoxycarvotanacetone	86421-35-4	$C_{12}H_{18}O_3$	-	-	-	-	43.42	0.52
166	Ledol	577-27-5	$C_{15}H_{26}O$	-	-	-	-	44.61	0.12
167	Himbaccol	552-02-3	$C_{15}H_{26}O$	-	-	-	-	45.02	0.65
168	Humulene oxide II	19888-34-7	$C_{15}H_{24}O$	-	-	-	-	45.21	0.39
169	Isoaromadendrene epoxide	-	$C_{15}H_{24}O$	-	-	-	-	46.07	0.14
170	11-Hexadecyn-1-ol	65686-49-9	$C_{16}H_{30}O$	-	-	-	-	47.76	0.15
171	cis-*Z*-α-Bisabolene epoxide	-	$C_{15}H_{24}O$	-	-	-	-	49.32	0.15

"-" means not detected

“RT” means Retention Time (minutes)

“RC” means Relative Content (%)

Analysis of the chemical constituents of the volatile oil of *E. capituligera* C.Y.Wu showed that 76 volatile compounds were isolated and identified. *E. fruticosa* (D.Don) Rehd. was isolated and identified 71 volatile compounds, and the *E. bodinieri* Vaniot was isolated and identified 85 volatile compounds. In total, 171 chemical constituents were identified, including 11 identical volatile compounds which were characterized in these three plants at the same time (Serial number of TABLE: 1, 5, 7, 10, 18, 20, 23, 25, 60, 70, 72), which was accounted for 18.38% of total volatile compounds.

29 identical volatile compounds were discovered in *E. capituligera* C.Y.Wu and *E. fruticosa* (D.Don) Rehd. (Serial number of TABLE: 1, 4, 5, 7, 9, 10, 11, 16, 18, 19, 20, 23, 24, 25, 34, 37, 40, 41, 52, 55, 57, 58, 60, 65, 70, 72, 73, 74, 75), which was accounted for 72.09% of total volatile compounds. The main volatile compounds was 2, 5-dimethyl-2-Isopropenyl-1-cyclohexanone (Serial number of TABLE: 37) and (-)-Verbenone (Serial number of TABLE: 23).

20 identical volatile compounds were discovered in *E. capituligera* C.Y.Wu and *E. bodinieri* Vaniot (Serial number of TABLE: 1, 5, 6, 7, 8, 10, 14, 18, 20, 23, 25, 44, 60, 61, 62, 63, 65, 70, 71, 72), which was accounted for 65.80% of total volatile compounds. The main volatile compounds were Eucalyptol (Serial number of TABLE: 14).

22 identical volatile compounds were discovered in *E. fruticosa* (D.Don) Rehd and *E. bodinieri* Vaniot (Serial number of TABLE: 1, 5, 7, 10, 18, 20, 23, 25, 60, 65, 70, 72, 80, 83, 84, 85, 91, 92, 98, 108, 109, 111), which was accounted for

18.64% of total volatile compounds. Although these two plants have 22 identical volatile components, their relative content of their major volatile components varies greatly.

Results, Discussion and Conclusion

In this experiment, the volatile oils of three kinds of *Elsholtzia* genus plants were isolated and identified by GC/MS. It was the first example to identified 171 volatile compounds from the title plants. Volatile compounds mainly included ketones, sesquiterpene hydrocarbons, alcohols, esters, and aldehydes. Through our research data, the main volatile compounds for each species were Eucalyptol (Serial number of TABLE: 14, Relative content: 31.55%), (-)-Verbenone (Serial number of TABLE: 23, Relative content: 25.36%) and 2, 5-dimethyl-2-Isopropenyl-1-cyclohexanone (Serial number of TABLE: 37, Relative content: 12.26%) in *Elsholtzia capituligera* C.Y.Wu; 2,5-dimethyl-2-Isopropenyl-1-cyclohexanone (Serial number of TABLE: 37, Relative content: 60.78%) and (-)-Verbenone (Serial number of TABLE: 23, Relative content: 19.78%) in *Elsholtzia fruticosa* (D.Don) Rehd; Eucalyptol (Serial number of TABLE: 14, Relative content: 55.08%) and Terpinyl acetate (Serial number of TABLE: 161, Relative content: 5.64%) in *Elsholtzia bodinieri* Vaniot. It is showed that *E. capituligera* C.Y.Wu and *E. fruticosa* (D.Don) Rehd. have the same major volatile components were 2,5-dimethyl-2-Isopropenyl-1-cyclohexanone (Serial number of TABLE: 37) and (-)-Verbenone (Serial number of TABLE: 23). *E. capituligera* C.Y.Wu and *E. bodinieri* Vaniot have the same major volatile components was Eucalyptol (Serial number of TABLE: 14). Although *E. fruticosa* (D.Don) Rehd and *E. bodinieri* Vaniot have 22 identical volatile components, their relative content of their major volatile components varies greatly. So the volatiles oil of these genus plants have some differences, the reason might be determined by genotype. This study not only provides a lot of valuable experimental data, but also might promote the volatile oils research for other peoples.

Elsholtzia plants are widely used in traditional Chinese medicine for the anti-virus and broad-spectrum antimicrobial. At the same time, some of these plants can also be used as food materials and nectar plants [14]. Therefore, the economic utilization and academic research of these genus plants might be increased concerned by this research.

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