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Composition of the essential oil and the hydrosol of the roots of *Ligusticum porteri*

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Abstract
Monoterpenes and monoterpenes derivatives constitute the main part of the essential oil of the roots of *Ligusticum porteri*. Sabinene (9.0-15.5%) and *p*-cymene (6.5-9.7%) are the main compounds of monoterpene hydrocarbons along with a rather unusual compound: viridene (19.4%). *trans*-Sabinyl acetate (22.6%) is by far the most important oxygenated monoterpene. Several compounds are observed among the sesquiterpene group with percentage generally lower than 1%. The most important compound in one sample is thapsadiene (0.6%) and in the second sample is α-selinene (0.7%). Finally, several recently described oxygenated sesquiterpenoids are among the minor compounds. The most important are: α-prethapsenol (1.7-3.3%), α-preisothapsenol (0.8-1.6%) and β-isoligustigrenol (0.6-1.3%) along with (*Z*)-ligustilide (0.7%). The hydrosol does not show the presence of hydrocarbon compounds. However, oxygenated compounds such as terpinen-4-ol (16.5 mg/L), including some compounds not observed in the oil such as hexanal (2.5 mg/L), furfural (2.0 mg/L) and *p*-cymen-8ol (2.5 mg/L), are observed in the hydrosol.

Keywords: Essential oil, *Ligusticum porteri*, *trans*-sabinyl acetate, viridene, sabinene, α-prethapsenol.

1. Introduction
Ligusticum porteri J. M. Coult. & Rose (Apiaceae) –syn.: Osha, Bear Root, Chuchupate, Indian parsley- belongs to the Apiaceae family (Umbelliferae). It is a perennial herb that can be found on the East side of the Rocky Mountains from Montana to Chihuahua, Mexico [1]. In 2005, 350 and 1642 pounds of dried cultivated and wild roots were harvested in the US [2]. This plant has many uses in Native American medicine [3] and exhibits several pharmacological activities [3, 4]. Several studies deal with the composition of natural products extracted with organic solvents. For example, Delgado *et al*. published the composition of the hexane extract of the roots. The main observed products are (*E*)- and (*Z*)-ligustilide (ca. 24 and 18% , respectively) as well as α-phellandrene (12.6%) [5]. A study of the acetone extract from the fresh rhizomes showed the presence of dimeric phthalides [6]. An oil obtained through hydrodistillation contained an undetermined isomer of sabinyl acetate (56.6%) and (*Z*)-ligustilide (39.1%) as its main compounds [7]. Recently, another report indicated that the chemical composition of an essential oil obtained by hydrodistillation was (3*Z*)-butylidenephthalide (24.4%) and (*Z*)-ligustilide (20.2%) for the main products [8]. In the same study, several monoterpenes and sesquiterpenes were also identified in the 0-5 % range. These are thuj-2,4(10-diene (4.2%), β-phellandrene (3.6%), γ-gurjunene (4.5%), α-acoradiene (3.5%) and eudesma-2,4(15),11-triene (3.2%). Thus, there are large variations in observed compositions of the volatile extracts of Osha roots. The diversity of phthalides from the Apiaceae and their bioactivities is also available [9]. We take advantage of trials to introduce new commercial formulations to analyze these essential oils further.

2. Materials and methods
2.1 Plant Material
Several kg of wild dried material of Osha roots were collected in August and purchased from Mountain Rose Herbs, Eugene, Oregon, Lot 7999. This material was sliced in thin pieces and manually grinded.

2.2 Oil Isolation

1.2 kg of this material was steam distillated during 2 h 45 min and produced 30 ml of oil and 1.1 L of hydrosol. For solvent extraction, 50 g were macerated during 30 days with 250 mL of either anhydrous ethanol or acetone at room temperature. Samples were kept at -5 °C before injection on GC-FID or GC/MS.
For hydrosol, a 100.0 mL sample was extracted with 3 × 25 mL dichloromethane. The organic layers were combined and dried over $MgSO_4$. A tetradecane internal standard was added (1.6 mL), and the extract was gently concentrated under reduced pressure at room temperature. The residue was resuspended in dichloromethane and kept at -5 °C before injection.

2.3 GC and GC/MS

Essential oils, hydrosols and organic extracts were analyzed in duplicate by GC on an Agilent 6890N gas chromatograph with an automatic injector and FID detector equipped with two columns: a polar Solgel-Wax and a non-polar DB-5 fused silica capillary column (30 m × 0.25 mm × 0.25 µm). Detector and injection temperatures were 220 and 260 °C, respectively. The oils were also analyzed by GC/MS on an Agilent 5975C InertXL EI/CI triple axis detector mass spectrometer at 70 eV coupled to an Agilent 7890A GC equipped with the same columns as above. The temperature program for both GC/FID and GC/MS was from 40 °C (2 min) to 210 °C (33 min) at a rate of 2 °C/min. The carrier gas was He at a flow of 1.4 mL/min; inlet pressure: 104.5 kPa. Injection volume and split ratio: 3 µL and (50:1). Operating conditions for the MS: the carrier gas was He at a flow of 1.0 mL/min; inlet pressure 48.5 kPa; temperature of the source 280 °C and scan speed, 0.6 s between 40 and 450 amu. Identification of the components was done by comparison of their retention indices (RI) with normal hydrocarbons ranging from C8 to C30 and by comparison of their mass spectra with literature data [10-13] and with our own data bases. Quantitative data were obtained electronically from GC-FID area percentages.

3. Results & Discussion

3.1 The essential oil

The composition of the essential oil of two samples of the roots of Osha appears in Table 1. *trans*-Sabinyl acetate (22.6%) is the main observable compound in agreement with one of the above reported paper [6]. However, the percentages of ligustilide as well as those of phthalide compounds are below 1% [3, 6-7]. Finally, sabinene is the main observed monoterpene hydrocarbon (9-15%) and α-phellandrene (0.2%) is well below the 12.6% reported above. Thus, as far as the percentages of the main compounds are concerned, there are large differences with literature values.
Several monoterpene and sesquiterpene molecules are observed in other studies, see table 1 [7, 8]. *p*-Cymene and γ-terpinene are in concentrations ten times larger than previously published values [7, 8]. Viridene, a compound not reported until now in essential oils of *L. porteri*, constitutes an obvious great difference with a percentage of almost 20%. The same observation applies to norviridene and ar-norviridene previously observed as minor products in the oil of *Ligusticum grayi* roots [14]. Similar conclusions can be drawn for oxygenated mono- and sesquiterpenoid molecules although their concentration lies in the 0-1% range.
Finally, the presence of a series of rather rare compounds has to be mentioned. These are thapsenol and ligustigrenol derivatives with α-prethapsenol as the main compound (1.7-3.3%). Thapsadiene (0.6%) and isothapsadiene (0.2%) observed in sample a must be added to this family of compounds. To our knowledge, these compounds have never been mentioned in *L. porteri* extracts. Yet, they were observed in hexane extract of *Ligusticum grayi* roots [15]. Viridene is also the main compound observed in this last oil with a percentage similar to that observed in the Osha essential oil (Table 1). On the other hand, sabinyl acetate was not reported in the hexane extract of *L. grayi*.

Table 1: Chemical constituents of the root essential oil of *Ligusticum porteri.*

S. No.	Compounds *	RI a	RI b	% Composition (FID)				Identification c
				Essential oil		Extracts		
				A	B	alcohol	ketone	
1	hexanal *	798	1092	t				a, b
2	unidentified **I** *	897		0.1				
3	α-thujene	911	1022	0.2	0.2	t	0.1	a, b
4	α-pinene	917	1015	0.4	0.5	0.3	0.1	a, b
5	camphene*	934	1057	0.1	0.1	t		a, b
6	sabinene	964	1115	15.5	9.1	4.4	4.4	a, b
7	β-pinene	967	1099	1.0	0.9	0.3	0.1	a, b
8	myrcene	988	1161	2.8	1.9	0.6	0.5	a, b
9	Δ^2-carene *	998	1125	0.4	0.3	t	0.1	a, b
10	4-methyl-3-hexanol acetate *	1000	1203	0.1	0.4			c
11	α-phellandrene	1001	1153	0.2	0.2	t		a, b
12	4-methyl-3-hexanol acetate isomer*	1006	1215	0.1				
13	α-terpinene	1013	1170	0.2	0.4	t	t	a, b

14	*p*-cymene	1020	1264	9.7	6.5	1.6	1.9	a, b
15	limonene	1027	1186	0.8	0.6	0.2	0.2	a, b
16	β-phellandrene	1027	1193	0.8	0.9	0.1	0.1	a, b
17	1,8-cineol *	1027	1197		t			a, b
18	γ-terpinene	1048	1238	5.8	4.4	1.5	1.8	a
19	*cis*-sabinene hydrate *	1054	1455	0.1	0.1	t		a
20	norviridene *	1071	1312	0.4	0.4	0.1	0.1	d
21	ar-norviridene *	1076	1393	0.1	0.1			d
22	terpinolene *	1069	1277	0.1	0.2	0.1		a, b
23	*trans*-sabinene hydrate *	1092	1548	t	t	t		a
24	linalool *	1099	1548	t	0.1			a, b
25	α-thujone	1101	1395	0.1	0.1	0.1	t	a, b
26	β-thujone *	1113	1416	0.1	0.1	t	t	a, b
27	*cis*-*p*-menth-2-en-1-ol *	1117	1550	0.2	0.6			a, b
28	*trans*-sabinol	1135	1674	0.3		t	0.3	a, b
29	*trans*-*p*-menth-2-en-1-ol *	1135	1571	0.2	1.0			a, b
30	dictyotene *	1155	1326		t			e
31	viridene	1159	1380	19.3	19.5	5.6	4.3	a, b, d
32	1-pentenylbenzene	1165	1477	2.5	2.0	0.4	0.3	f
33	terpin-1-en-4-ol	1173	1580	0.2	0.6	0.1	0.1	a, b
34	α-terpineol	1187	1674	t	0.1			a, b
35	*cis*-piperitol *	1198	1711	t	0.1			a, b, e
36	*trans*-piperitol	1203		0.1	0.2			a, b, e
37	isothymol methyl ether	1228	1567	0.1	0.1	0.1	t	
38	thymol methyl ether	1231	1588	0.1	0.1			a, b
39	carvacrol methyl ether	1242	1586	1.2	1.2	0.4	0.2	a, b
40	piperitone	1247	1705		0.1			a
41	linalyl acetate *	1256	1551	0.2	0.4	0.1	0.1	a, b
42	3-isothujanyl acetate	1265		0.1	0.1	0.1		a
43	bornyl acetate	1283	1560	0.7	0.8	0.2	0.2	a, b
44	*trans*-sabinyl acetate	1292	1632	22.6	22.6	11.1	8.4	a, b
45	*p*-vinylguaiacol	1309	2167	0.2	1.0	4.4	5.3	a, b
46	*cis*-piperitol acetate *	1331	1658	t	t			a
47	*trans*-piperitol acetate *	1338	1691	1.4	0.8	0.9		a, b
48	α-terpinyl acetate	1347	1684	0.4	0.4	0.2	0.2	a, b
49	*cis*-carvyl acetate *	1360		0.1		0.2		a, b
50	2-*epi*-α-funebrene *	1374		t	0.1			a
51	β-elemene *	1387	1571	t	t			a, b, e
52	methyl eugenol	1401	1997	t	0.5			a, b
53	α-barbatene	1408	1566	0.1	0.1	t	0.1	a, b
54	β-funebrene	1408		t	0.1			a, b
55	2,5-dimethoxy-*p*-cymene	1420	1842	0.1	0.1	0.1	0.1	a, b
56	*cis*-thujopsene (widdrene)	1423	1625	0.1	0.2			a, b
57	isobazzanene *	1430	1576	0.2	0.6	0.2	0.1	a, b
58	isothapsadiene *	1430	1632	0.2		0.2		f
59	thapsadiene *	1433		0.6				f
60	β-barbatene	1433	1577	0.2				a, b

61	α-humulene *	1446	1641	t	0.1		0.1	a, b
62	(*E*)-β-farnesene	1457	1651	0.2	0.3	0.1	0.2	a, b
63	β-acoradiene *	1462	1659	0.2	0.3	0.1	0.1	a, b
64	4,5-di-*epi*-aristolochene ?	1471		0.1	0.2			a, b
65	γ-curcumene *	1478	1677	0.1	0.1		0.1	a, b
66	β-selinene *	1483	1689	0.2		0.1	0.1	a, b, e
67	α-gorgonene ?	1487		0.1	0.1			e
68	α-selinene *	1493	1696	0.3	0.7	0.2	0.2	a, b, e
69	β-dihydroagarofuran *	1494	1732	0.1	0.3	0.1	0.1	a, b, e
70	β-himachalene *	1495	1737	0.2	0.1		0.2	a, b, e
71	α-chamigrene	1500	1741	0.1	t		0.1	a, b, e
72	cuparene *	1507	1792		t			a, b, e
73	β-bisabolene	1509	1708	0.1				a, b, e
74	cyclobazzanene * + other compound	1509	1674	0.4				e
75	myristicin	1517	2203	t	0.1		t	a, b
76	γ-cuprenene *	1520	1674	0.1	0.1			a, e
77	elemicin	1549	2218	t	t	0.1	0.1	a, b
78	unidentified **II** *	1556		0.1	0.4	0.2	0.1	
79	10-*epi*-γ-eudesmol *	1614	2067	t	0.1	0.1	0.1	a, b, e
80	3-propylidene-4,5-dihydrophthalide	1622		t		t	0.1	a
81	*trans*-sesquilavandulol *	1630	2148	0.4	0.8	2.0	1.8	a, b
82	α-isothapsenol *	1642		0.5	0.8	2.7	2.5	f
83	β-eudesmol *	1648	2158	0.1	t	0.1	0.2	a, b, e
84	α-cadinol *	1653	2185	0.1	0.2	0.3	0.5	a, b, e
85	α-preisothapsenol *	1658	2248	0.8	1.6	6.4	6.8	f
86	(*Z*)-3-butylidenephthalide	1665	2492	t	t		t	a, b, g
87	α-prethapsenol *	1665	2309	1.7	3.3	12.5	12.0	d
88	β-prethapsenol *	1679	2339	0.3	0.6	2.4	2.4	f
89	unidentified **III** *	1715		0.2	0.5	1.6	1.8	
90	α-isoligustrenol *	1717		0.2	0.5	2.3	1.9	f
91	senkyunolide A *	1719				1.1	1.0	b, g
92	(*Z*)-ligustilide	1728	2520	0.7	0.6	3.8	3.7	a, b, e
93	α-ligustigrenol *	1731	2351	-	0.2		6.0	f
94	(*E*)-sesquilavandulyl acetate *	1746	2098			0.8	1.0	a, b
95	β-isoligustigrenol ?*	1741	2410	0.6	1.3	2.3	1.7	f
96	β-*epi*-thapsenol *	1753		0.1		0.1	0.2	f
97	β-ligustigrenol *	1759	2496	0.1	0.3	0.4	1.2	f
98	(*E*)-ligustilide	1785				t	0.1	a, b, e
99	ferulic acid *	1870				0.2	0.2	c, g
100	rimuene *	1887	2103	t				a, e
101	beyerene *	1913	2142	0.1				a, e
102	hexadecanoic acid *	1960				0.2	0.1	a, b, e
103	4-hydroxy-3-butylidenephthalide *	2084				0.1	0.1	g
104	linoleic acid *	2134				0.3	0.3	a, b
105	ethyl linoleate *	2139				0.2	0.2	c
Monoterpene hydrocarbons (viridene compounds included)				57.9	45.4	14.8	13.7	
Oxygenated monoterpenes				28.4	31.0	18.5	15.3	

Sesquiterpene hydrocarbons	3.5	3.0	1.0	1.3	
Oxygenated sesquiterpenes	5.9	11.6	38.2	45.6	
Total	98.5	94.2	73.3	76.7	

*: Compounds not reported in previous hydrodistillation studies [6, 7]; [a]: Retention index on a-polar DB-5 column; [b]: Retention index on polar Solgel-wax column; [c]: Identification, a: GC/MS and retention index (see Material and methods: GC and GC/MS); b: ref. 11; c: MS, ref. [13]; d: MS, ref. [14]; e: MS, ref. [12]; f: MS, ref. [15]; g: MS, ref. [16]; t: trace <0.05%. MS of unidentified compounds, m/z (relative intensity):

I: 43(100), 101(24), 69(12), 115(10)…;
II: 163(100), 121(70), 107(40), 220(33), 205(32)…;
III: 137(100), 109(50), 121(42), 136(30), 135(28), 91(24), 220(22)…

The compositions of organic extracts are roughly similar either qualitatively or quantitatively to that of the essential oil (Table 1). As such, they do not need more comments except for the presence of new heavier compounds such as ferulic, hexadecanoic and linoleic acids. Senkyunolide A, 4-hydroxy-3-butylidenephthalide, hexadecanoic acid and ethyl linoleate have been reported in extracts of *Ligusticum chuanxiong* Hort [16].

3.2 The hydrosol

The analysis of the hydrosol appears in Table 2. It is well known that hydrocarbon compounds have a very low solubility in water. Indeed, their solubility is lower than 10 mg/L [17]. Here, if they are present in the hydrosol, they have concentration below their detection limit as only oxygenated compounds are observed. By contrast, alcohols, aldehydes and organic acids have water solubility above 1 g/L. Thus, compounds such as furfural, hexanol, 1-octen-3-ol, linalool oxides with a low concentration in the plant, are not observed in the essential oil but in the hydrosol. In between, oxygenated compounds with a high concentration in the plant are observable both in the essential oil and in the hydrosol [18]. This is the case for terpinen-4-ol and thapsenol derivatives.

Table 2: Chemical constituents of the hydrosol of *Ligusticum porteri.*

No	Compounds [a]	RI [b]	RI [c]	Quantity mg/L	Water solubility mg/L [17]
1	2-penten-1-ol		765	0.9	
2	3-methyl-2-butenal	~784	778	0.2	
3	hexanal	~797	801	2.5	5.82×10^3
4	isovaleric acid ?	817	827	t	6.0×10^4
5	furfural	821	828	2.0	7.41×10^4
6	2-furanmethanol	842	851 [11]	t	1.0×10^6
7	hexanol	853	863	0.1	5.9×10^3
8	2-acetylfuran	893	909	0.2	
9	2-cyclohexen-1-one	912	914 [19]	0.2	
10	benzaldehyde	944	952	0.2	6.95×10^3
11	5-methylfurfural	950	957	0.1	
12	1-octen-3-ol	972	974	t	
13	unidentified **I***	992		1.5	
14	(*E*,*E*)-2,4-heptadienal	1007	1005	t	
15	1,4-cineole	1011	1012	0.3	153
16	1,8-cineole	1025	1026	0.1	3.5×10^3
17	benzyl alcohol	1028	1026	t	42.9×10^3
18	salicylaldehyde	1034	1039	0.1	1.7×10^4
19	benzeneacetaldehyde	1036	1036	0.6	3.03×10^3
20	*cis*-linalool oxide (fur.)	1068	1067	0.7	
21	*trans*-linalool oxide (fur.)	1083	1084	0.7	
22	*o*-guaiacol	1087	1087	t	1.87×10^4
23	linalool	1097	1095	0.6	1.59×10^3
24	α-thujone	1100	1101	0.3	
25	β-phenylethyl alcohol	1107	1106	t	

26	β-thujone	1110	1112	0.1	
27	*cis*-*p*-menth-2,8-dien-1-ol	1130	1133	0.2	
28	3-isothujanol	1133	1134	0.1	
29	camphor	1135	1141	0.4	1.6×10^3
30	sabina ketone	1149	1154	0.3	
31	borneol	1166	1165	0.6	738
32	terpinen-4-ol	1172	1174	16.5	~2200 ppm [20]
33	*p*-methylacetophenone	1176	1179	0.4	372
34	*m*-cymen-8-ol	1178	1176	0.2	
35	*p*-cymen-8-ol	1181	1179	2.5	
36	α-terpineol	1186	1186	0.2	710
37	*cis*-piperitol	1198	1195	0.2	
38	2α-hydroxy-1,8-cineole	1219	1218 [13]	0.2	
39	2,3-dimethoxytoluene	1237	1247 [13]	0.4	
40	unidentified **II**	1248		1.2	
41	piperitone	1249	1249	0.3	
42	*p*-cymen-7-ol	1285	1289	0.1	
43	*trans*-sabinyl acetate	1292	1289	2.2	
44	thymol	1294	1289	0.2	900
45	carvacrol	1304	1298	0.2	1250
46	*p*-vinylguaiacol	1310	1309	6.6	
47	exo-2-hydroxycineole acetate	1339	1344 [13]	0.2	
48	eugenol	1353	1356	0.3	2460
49	unidentified **III**	1377		0.3	
50	vanillin	1387	1393	1.7	1.1×10^4
51	unidentified **IV**	1390		2.5	
52	unidentified **V**	1456		1.1	
53	unidentified **VI**	1466		4.9	
54	unidentified	1554		0.4	
55	elemicin	1555	1555	0.7	
56	*cis*-3-propylidenephthalide	1619		t	
57	*trans*-sesquilavandulol	1628	1607	t	
58	α-isothapsenol	1650		0.4	
59	(*E*)-asarone	1655	1675	t	
60	α-preisothapsenol	1657		0.4	
61	*cis*-3-butylidenephthalide	1661	1671	0.3	
62	α-prethapsenol	1666		1.6	
63	β-prethapsenol	1680		0.2	
64	(*Z*)-ligustilide	1726	1734	1.0	
65	β-isoligustigrenol	1768		0.1	
66	unidentified	2245		0.8	
67	4,4'-dihydroxy-3,3'-dimethoxy-1,1'-biphenyl ??	2463		0.6	
68	unidentified **VII**	2684		2.2	
69	Total of reported compounds (mg/L)			65.4	
70	Total of observed peaks (mg/L)			100.6	

a: Identification by GC/MS and retention index (see Material and methods: GC and GC/MS); b: Retention index on a-polar DB-5 column; c: Retention index from ref. [10] otherwise indicated; t: trace <0.05 mg/L. ? : needs to be ascertained;
MS of unidentified compounds, m/z (relative intensity):
I: 91(100), 95(67), 92 ~ 110(41), 94(34), 77(33), 79(32), 65(28), 51(24) ...;
II: 119(100), 91(92), 117(60), 134(48), 132(44), 115(33), 109(40), 77(30) …;
III: 119(100), 91(42), 81(38), 71(30), 84(28), 109(24), … 152(10), 169(1);
IV: 119(100), 109(62), 91(44), 81(36), 84(38), 134(24), 71(20) …;
V: 93(100), 153(92), 135(82), 77(77), 150(76), 95(72), 168(66), 67(61) …;
VI: 150(100), 135(96), 153(82), 93(80), 77(70), 107(56), 168(56), 65(40) …;
VII: 300(100), 285(75), 161(58), 253(30), 137 ~ 253 ~ 301(18)...

4. Conclusions

The analyzed essential oil seems to be a kind of "combination" of the Osha essential oil and that of *L. grayi*, and in any case offers a profile that is quite different from other reports. As mentioned by a reviewer this observation could be due to a mixture of plant material from the commercial supplier. Thus, it is too early to make any hypothesis. Obviously, more research is needed to shed light on this "combination" and these disparities. Oxygenated compounds present in low concentration in the plant, not observed in the essential oil, are seen in the hydrosol.

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