Composition of the Leaf Oils of *Prunus phaeosticta* var. *phaeosticta* From Taiwan[1]

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Abstract

The leaf essential oils of *Prunus phaeosticta* var. *phaeosticta* were isolated and analyzed using hydrodistillation and headspace-GC methods to determine their composition and yield. Seventy-six compounds were identified in the hydrodistilled leaf oil, and 58 compounds were identified by the headspace, respectively, using GC and GC/MS. The main components of the oils were benzaldehyde (73.3%), 1,8-cineole (5.4%), and α-terpinyl acetate (4.4%).

Key Word Index

Prunus phaeosticta var. *phaeosticta*, Rosaceae, essential oil composition, benzaldehyde, headspace volatiles.

Introduction

The dark-spotted cherry, *Prunus phaeosticta* var. *phaeosticta*, is an evergreen tree in the *Prunus* genus of the Rosaceae family. It is mainly distributed in Taiwan and southeastern China (1). Although the essential oil composition of a number of *Prunus* species have been published (2–5), there appears to be no report on the oil composition of *P. phaeosticta* var. *phaeosticta*. Therefore, we used hydrodistillation and headspace-GC (HS-GC) methods to collect its leaf oils and GC-FID and GC/MS to analyze the composition of the oils and headspace volatiles. To determine the essential oil yields, a multiple headspace extraction (MHE) method was employed. The purpose of this study was to establish a chemical basis for the effective multipurpose utilization of the species.

Experimental

Plant materials: The fresh leaves of *P. phaeosticta* var. *phaeosticta* were collected from Taiwan Forestry Research Institute Lienhuachih Research Center in central Taiwan, where a specimen has been deposited in the Herbarium, in May 2006. Leaves of the species were collected for subsequent oil isolation and analysis.

Isolation of leaf oils and determination of composition and yield: Hydrodistillation extraction: One kg of the leaves of *P. phaeosticta* var. *phaeosticta* was placed in a round-bottom flask and 3 L of distilled water poured in. It was hydrodistillated for 8 h and the oil removed from the partitioned water layer. Anhydrous sodium sulfate was added to dewater. The yield of oil was determined. All test data are the average of triplicate analyses.

GC and GC/MS analyses: A Hewlett-Packard HP6890 gas chromatograph equipped with a DB-5 fused silica capillary column (30 m × 0.25 mm, 0.25 µm film thickness, J&W Scientific) and a FID detector was used for the quantitative determination of oil components. Oven temperature was programmed as follows: 50°C for 2 min, rising to 250°C at 5°C/min. Injector temperature was 270°C. Carrier gas was He with a flow rate of 1 mL/min. Detector temperature was 250°C, split ratio: 1:10. One µL sample was injected. Identification of the oil components was based on their retention indices and mass spectra, obtained from GC/MS analysis on a Hewlett-Packard HP6890/HP5973 equipped with a DB-5 fused silica capillary column (30 m × 0.25 mm, 0.25 µm film thickness, J&W Scientific). The GC analysis parameters were the ones listed above and the MS was operating (full scan mode: scan time: 0.3 s, mass range was *m/z* 30–500) in the EI mode at 70 eV. All test data are the average of triplicate analyses.

[1] Contract/grant sponsor: Council of Agriculture, Executive Yuan, Taipei, Taiwan. Contract/grant number: 96AS-11.4.1-FI-G2

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Received: August 2007
Revised: November 2007
Accepted: January 2008

1041-2905/09/0004-0345$14.00/0

Oil yield: The total amount of oil in each sample was determined by HS-GC. Calibration curves were made with different quantities (0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 µL) of leaf oil previously obtained by hydrodistillation. A special quantitative method, MHE, was used. According to Kolb (6), the matrix effect can be eliminated by using the MHE method. The total area of each oil volume was calculated according to the following equation:

$$\Sigma A = A_1^2/(A_1 - A_2) \quad \text{(a)}$$

where: ΣA is the total area; A_1 is the first area value; and A_2 is the second area volume from two successive chromatograms.

The HS-GC analyses were accomplished using a Hewlett-Packard HP6890 GC equipped with a FID detector and combined with a Perkin Elmer Headspace Turbomatrix 40. The GC analysis programs used were as described in the above section. Conditions of the headspace sampler were as follows: the sample size was 0.1 µL oil and 20 mg plant material (dried leaves). In MHE analyses of the oil, the vial oven and transfer line temperature were both 100°C; the needle temperature was 110°C; treatment time in the oven with shaking was 50 min; pressurization time was 3.0 min; and thermostat time was 50 min.

Component identification: Identification of the leaf oil constituents was based on comparisons of the peaks Retention indices (RI) (7), their retention times (RT), and mass spectra with those obtained from authentic standards and/or the NIST and Wiley libraries spectra and literature (8–9).

Results and Discussion

Leaf oil yields: The leaf oil yields by hydrodistillation of leaves of *P. phaeosticta* var. *phaeosticta* was 0.90 ± 0.03 (mL/100 g), respectively.

Leaf volatiles determination by the HS-GC method: The value of the total area corresponding to each volume of leaf essential oil submitted to the MHE of the headspace-GC were calculated by means of a previously described equation (a) in experimental section. The leaf volatile calibration curve obtained from the value corresponded to the regression equation $y = a + bx$, where value for the leaf volatile was $a = 38.769$ and $b = 3782.5$, $r^2 = 0.9984$ (Table I).

Table II shows the area values corresponding to different quantities of plant material (leaves) submitted to the multiple consecutive extraction of the headspace-GC unit. By using the MHE method and extrapolating the area values of the leaf volatiles calibration curves, we obtained respective yield values of 0.92 ± 0.01 (mL/100 g). The value was very close to the hydrodistillation yields, and the result suggest that the HS-GC method can be used to determine the essential oil yield (Table III) for *P. phaeosticta* var. *phaeosticta*.

Comparison of leaf oil compositions: From the *P. phaeosticta* var. *phaeosticta* leaf oil obtained by hydrodistillation, 76 compounds were identified with the main components being benzaldehyde (73.3%), 1,8-cineole (5.4%), α-terpinyl acetate (4.4%), methyl salicylate (3.3%), isopimara-9(11),15-diene (1.5%), α-terpineol (1.4%) and phytol (1.4%). The constituents were divided into monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, diterpenes and non-terpenoids. When these groups were tallied, the non-terpenoids had the highest area percentage of 79.0%, including benzaldehyde, etc. Oxygenated monoterpenes accounted for 13.5%, monoterpene hydrocarbons for 1.4%, oxygenated sesquiterpenes for 2.0%, sesquiterpene hydrocarbons for 1.2%, and diterpenes for 3.0%. In the HS-GC analysis, 58 compounds were identified, again with benzaldehyde as the main component, accounting for 73.4% of the total. It was followed by 1,8-cineole (5.4%), α-terpinyl acetate (4.5%), methyl salicylate (3.2%), isopimara-9(11),15-diene (1.5%), α-terpineol (1.5%), phytol (1.4%), etc. The non-terpenoid group (78.9%) also accounted for the highest fraction among the identified compounds.

Table I. The values of the total area corresponding to each quantity of *Prunus phaeosticta* var. *phaeosticta* oil subjected to MHE on HS-GC

P. phaeosticta var. *phaeosticta* (µL)	Area
0.1	423.21 ± 8.61
0.2	803.76 ± 9.81
0.3	1216.24 ± 12.33
0.4	1588.28 ± 13.16
0.5	1896.36 ± 15.62
0.6	2286.68 ± 17.21

Table II. Area values corresponding to different quantity of plant material subjected to MHE on HS-GC

Plant material (mg)	Area
10	385.91 ± 10.16
20	721.34 ± 12.38
30	1093.63 ± 11.68
40	1421.58 ± 13.85

The above yield values and compositions indicate that hydrodistillation and the HS-GC methods gave comparable leaf oil yields. When the composition of the oil was compared, however, the minor components obtained by hydrodistillation (content < 0.1%) could not be detected by HS-GC. The major reason was probably due to the small size of the specimens used, as the former needed ca. 1 kg of sample, while HS-GC only took 20 mg. Overall, the HS-GC yielded main components and compound groups similar to those of the hydrodistillation results. The methodology proved that HS-GC can be an effective method for an essential oil compositional analysis; furthermore, it requires only a minute amount of specimen and a long period of distillation is not needed.

The predominant compound in the leaf oil was benzaldehyde, making up ca. 73% of all volatile fractions. Natural benzaldehyde is one of the main materials for making food flavoring agents and for preparing industrial dyestuffs and spices. The present supply of benzaldehyde is obtained by artificial synthetic chemical reactions from bitter almond oil-containing fruit kernels or natural cinnamon oil. The conversion process often produces harmful byproducts (10). Thus, the presence of this compound in the leaf oil may serve as a source of benzaldehyde directly from nature. Its harvest and isolation from this species may be an ideal means of obtaining the needed chemical without significantly harming the plants. There appears to be no information in the literature pertaining to the species we studied. Thus, this paper represents the first study of the leaf essential oil of *P. phaeosticta* var. *phaeosticta*.

Table III. Chemical composition of the leaf oils and headspace volatiles of *Prunus phaeosticta* var. *phaeosticta*

RI[a]	Compound ID	Concentration(%)		Identification[d]
		HD[b]	HS[c]	
854	(E)-3-hexenol	t[e]	-[f]	MS, RI, ST
855	(E)-2-hexenal	t	-	MS, RI, ST
859	(Z)-3-hexenol	0.2	0.3	MS, RI, ST
888	ethyl 4-pentenoate	0.3	0.3	MS, RI
930	α-thujene	0.1	0.1	MS, RI, ST
939	α-pinene	0.1	0.1	MS, RI, ST
954	camphene	t	t	MS, RI, ST
960	benzaldehyde	73.3	73.4	MS, RI, ST
986	6-methyl-5-hepten-2-one	0.1	0.2	MS, RI, ST
1003	α-phellandrene	t	-	MS, RI, ST
1008	dehydroxy-*cis*-linalool oxide	0.1	t	MS, RI
1025	p-cymene	0.5	0.6	MS, RI, ST
1029	limonene	0.5	0.6	MS, RI, ST
1031	1,8-cineole	5.4	5.4	MS, RI, ST
1032	benzyl alcohol	0.2	0.2	MS, RI, ST
1045	salicylaldehyde	0.1	0.1	MS, RI, ST
1060	γ-terpinene	0.1	0.1	MS, RI, ST
1068	octanol	0.1	0.1	MS, RI, ST
1073	*trans*-linalool oxide (furanoid)	0.1	0.2	MS, RI, ST
1087	*cis*-linalool oxide (furanoid)	0.1	0.2	MS, RI, ST
1089	terpinolene	t	-	MS, RI, ST
1091	methyl benzoate	0.1	0.1	MS, RI, ST
1097	linalool	0.4	0.4	MS, RI, ST
1101	nonanal	0.1	0.1	MS, RI, ST
1122	*cis*-p-menth-2-en-1-ol	0.1	0.1	MS, RI, ST
1141	*trans*-p-menth-2-en-1-ol	0.1	t	MS, RI, ST
1142	benzyl nitrile	0.1	0.2	MS, RI, ST
1153	citronellal	0.1	0.1	MS, RI, ST
1159	sabina ketone	t	-	MS, RI, ST
1162	benzyl acetate	t	-	MS, RI, ST
1177	terpinen-4-ol	0.3	0.3	MS, RI, ST
1189	α-terpineol	1.4	1.5	MS, RI, ST
1192	methyl salicylate	3.3	3.2	MS, RI, ST
1193	hexyl butyrate	t	-	MS, RI, ST
1196	*cis*-piperitol	t	-	MS, RI, ST
1200	*trans*-dihydrocarvone	0.1	0.1	MS, RI
1205	verbenone	0.2	0.1	MS, RI, ST
1215	*trans*-pulegol	0.1	0.2	MS, RI, ST
1226	citronellol	0.2	0.2	MS, RI, ST
1237	pulegone	t	-	MS, RI, ST
1253	geraniol	0.1	t	MS, RI, ST
1264	(E)-2-decenal	t	-	MS, RI, ST
1270	ethyl salicylate	0.1	0.1	MS, RI, ST
1276	*trans*-carvone oxide	t	-	MS, RI, ST
1289	bornyl acetate	0.1	t	MS, RI, ST
1291	p-cymen-7-ol	t	0.1	MS, RI
1303	*o*-vanillin	t	-	MS, RI, ST
1318	*cis*-dihydro-α-terpinyl acetate	0.3	0.4	MS, RI
1341	5-indanol[g]	0.3	0.3	MS, RI
1349	α-terpinyl acetate	4.4	4.5	MS, RI, ST
1359	eugenol	0.2	0.3	MS, RI, ST
1377	α-copaene	t	-	MS, RI, ST
1385	(E)-β-damascenone	0.1	0.2	MS, RI, ST
1391	β-elemene	0.1	0.2	MS, RI, ST
1394	vanillin	0.1	0.1	MS, RI, ST
1409	β-caryophyllene	0.1	0.1	MS, RI, ST
1409	dodecanal	0.1	0.1	MS, RI, ST
1430	(E)-α-ionone	t	-	MS, RI, ST
1480	γ-muurolene	0.1	0.1	MS, RI, ST
1489	(E)-β-ionone	0.1	0.1	MS, RI, ST
1493	δ-selinene	t	-	MS, RI, ST
1494	zingiberene	t	-	MS, RI, ST
1497	viridiflorene	t	-	MS, RI, ST
1506	(E,E)-α-farnesene	0.1	0.2	MS, RI, ST
1514	γ-cadinene	0.3	0.3	MS, RI, ST
1523	δ-cadinene	0.1	0.1	MS, RI, ST
1529	*trans*-calamenene	0.2	0.2	MS, RI, ST
1550	elemol	0.1	0.1	MS, RI, ST
1563	(E)-nerolidol	0.2	0.2	MS, RI, ST
1566	carvotacetone acetate[g]	t	-	MS, RI
1567	(Z)-3-hexenyl benzoate	0.1	0.1	MS, RI
1583	caryophyllene oxide	0.8	0.8	MS, RI, ST
1646	α-muurolol	0.6	0.6	MS, RI, ST
1654	α-cadinol	0.1	0.1	MS, RI, ST
1906	isopimara-9(11),15-diene	1.5	1.5	MS, RI
1943	phytol	1.4	1.4	MS, RI
	Monoterpene hydrocarbons (%)	1.4	1.4	
	Oxygenated monoterpenes (%)	13.5	13.6	
	Sesquiterpene hydrocarbons (%)	1.2	1.3	
	Oxygenated sesquiterpenes (%)	2.0	1.9	
	Diterpenes (%)	3.0	2.9	
	Others (%)	79.0	78.9	
	Oil Yield (mL/100 g)	0.90±0.03	0.92±0.01	

[a] Retention index on a DB-5 column in reference to n-alkanes (7). [b] HD, Hydrodistillation extraction. [c] HS, Headspace-GC extraction. [d] MS, NIST and Wiley libraries spectra and the literature; RI, Retention index; ST, authentic standard compounds. [e] trace < 0.1%. [f] Not detected. [g] tentative identification. 5-indanol: 134[M]$^+$(73), 133(100), 105(15), 77(11), 51(8), 117(7), 135(7), 79(7), 115(6), 107(57); carvotacetone acetate: 196[M]$^+$(2), 150(28), 135(10), 108(40), 95(16), 91(10), 82(20), 59(24), 54(12), 43(100).

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