Pak. J. Bot., 42(6): 4379-4386, 2010.

IDENTIFICATION OF ESSENTIAL OILS FROM THE LEAVES OF 11 SPECIES OF *ERIOBOTRYA*

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Abstract

Essential oils are regarded as the major bioactive compounds and exhibit antibacterial, antifungal, anti-viral and antioxidant activities. The contents and components of essential oils present in plant tissues depend largely on germplasm resource. In this study, essential oils in leaves of 11 species of *Eriobotrya* were extracted with ethyl ether and were then analyzed by gas chromatography-mass spectrometry. A total of 66 components were identified and the major components were benzoic acid methyl ester, heptacosane, squalene, neophytadiene and hexacosane. The species 'Obovata leaf' exhibited higher contents of squalene and benzoic acid methyl ester compared to other species, suggesting a higher utilization value. The clustering analysis on the basis of the essential oils indicated that the 11 species of *Eriobotrya* can be classified into 3 groups, i.e. the first group 'Oak leaf', 'Taiwan', 'Bengal', 'Obovata leaf', 'Hengchun', 'Guangxi' and 'Big flower' loquats, the second group the common loquat, and 'Tibet', 'Zaozhong 6' and 'Daduhe' loquats, and the third group 'Fragrant' loquat. The differences in contents of phytol, squalene, neophytadiene and lup-20(29)-en-28-oic acid 3-hydroxyl-methyl ester among the three groups indicated the oil profiles of the 11 species of *Eriobotrya*. The study can help to increase utilization of *Eriobotrya* species.

Introduction

Loquat (*Eriobotrya japonica* Lindl.) has become an important fruit in most of the Asian countries while fruit production has increased tremendously in the last few decades (Lin, 2007). China exhibits a rich wild loquat resource with about 20 species. More attention has been paid to the wild species of *Eriobotrya* besides the commercial loquat species (Lee *et al.*, 2001; Hong *et al.*, 2008b). Current study concerns largely phenolics and triterpenes present in leaf of *Eriobotrya* (Ito *et al.*, 2000; Taniguchia *et al.*, 2002; Ju *et al.*, 2003; Hong *et al.*, 2007; Hong *et al.*, 2008a).

Essential oils are among the secondary metabolites in higher plants. In modern pharmacology, essential oils are regarded as the major bioactive compounds, exhibiting antibacterial, antifungal, anti-viral and antioxidant activities (Govinden-Soulange *et al.*, 2004; Salehi *et al.*, 2005; Mevy *et al.*, 2007; Saïdana *et al.*, 2008). Shaw and Wilson (Shaw *et al.*, 1982) reported 18 volatile compounds from loquat fruit, in which the major components consisted of phenylethyl alcohol, 3-hydroxy-2-butanone, phenylacetadehyde and hexen-l-ols , and the minor components concluded ethyl acetate, methyl cirmamate and β-ionone, contributing greatly to the fruit flavor. Furthermore, Tai *et al.* (Tai *et al.*, 2008) found that 77 compounds of the essential oils from *Eriobotrya japonica* leaf were identified and the principal constituents included n-hexadecanoic acid, (E)-nerolidol, (Z,Z,Z)-9,12,15-qctadecatrien-1-ol, (+)-carvone, 2-hexanoylfuran, elemicin, dihydroactinidiolide, farnesyl acetate, farnesol and alpha-bisabolol.

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Essential oils display various chemical profiles from different species or different regions of plants (Lu *et al.*, 2006; Hu *et al.*, 2006). The amount of the essential oils in plants depends largely on germplasm resource and thus can be used to determine the genetic relationship among different genus (Pedro *et al.*, 2001; Böszörményi *et al.*, 2009). Hierarchical clustering analysis is usually performed to differentiate or classify different species, samples from different regions or natural and cultured samples, based on the extent of compounds(Hong *et al.*, 2007; Hu *et al.*, 2006; Yang *et al.*, 2009; Guo *et al.*, 2009). Thus, the clustering analysis can be used for chemotaxonomic characterization of various plant species.

This objective of the study was to investigate the essential oils from leaves of 11 species of *Eriobotrya*. A comparative analysis of the wild species with the commercial species of loquat was conducted in relation to their genetic relationships using the clustering analysis. Based on the profile and amount of the essential oils, the study can be helpful in better utilization of the wild *Eriobotrya* species.

Materials and methods

Materials and reagents: The mature leaves were obtained in April 2005 from 11 species of *Eriobotrya* trees grown in the experimental orchard in South China Agriculture University. These leaves were collected, dried 3–4 days at about 40°C, and then crushed into powder (80 mesh). The ground powder was stored at about -20°C prior to analysis. The 12 samples of 11 species used in this study are listed in Table 1.

Sample preparation: The dried powder (0.5 g) of each sample was suspended in 20 mL of ethyl ether, and then exposed twice to ultrasonic treatment with each for 20 min at an interval of 10 min. After the suspension was placed overnight and then recovered to the original volume by the additional ethyl ether, the supernatant was collected and filtered though a 0.22 μm membrane. The filtered solution was stored at -20°C before gas chromatography-mass spectrometry (GC-MS) analysis.

GC-MS analysis: The analysis was performed by a trace 2000 GC/MS (Finnigan, USA). The injector temperature was kept at 250°C, with a helium carrier flow of 1 mL/min in a splitless mode. The filtered solution (0.5 μL) was injected into a DB-1 capillary column (30 m in length and 0.25 mm in diameter). The column was maintained for 1 min at 45 °C after injection, programmed at 3°C/min from 45 to 60°C for 10 min and then increased at 10 °C/min from 60 to 250°C for 10 min. The mass spectrometer was operated in the electron-impact (EI) mode. The ionization energy was 70 eV while the scan range was 35~335 m/z. All compounds were identified by the similarity match using NIST-library spectra while their relative contents were calculated by the area normalization method of the total ion chromatogram (TIC).

Data handling: The data was analyzed by the Computer Aided Similarity Evaluation System of the Central South University and SPSS10.0. The Computer Aided Similarity Evaluation System was mainly applied in the chromatographic and spectral pattern (Meng *et al.*, 2005). In this study, the software coded in MATLAB 6.5 for Windows was employed to synchronize the chromatographic peaks and then to calculate the correlation coefficients between the entire chromatographic profile. Based on the signal intensity of sample, all samples were clustered into different groups using SPSS 10.0.

Table 1. Sample codes of the materials used.

Species	Sample code	English name
E. japonica cv. Zaozhong No. 6	S1	Common loquat cv. Zaozhong 6
E. japonica Lindl.	S2	Common loquat (wild tree)
E. fragrans Champ	S3	Fragrant loquat
E. elliptica Lindl.	S4	Tibet loquat
E. prinoides var. *dadunensis*	S5	Daduhe loquat
E. prinoides Rehd & Wils	S6	Oak leaf loquat
E. deflexa Nakai	S7	Taiwan loquat
E. deflexa var. *koshunensis*	S8	Hengchun loquat
E. kwangsiensis Chun	S9	Guangxi loquat
E. bengalensis Hook. f.	S10	Bengal loquat
E. obovata W. W. Smith	S11	Obovata leaf loquat
E. cavaleriei Rehd	S12	Big flower loquat

Result and discussion

Components of the different species and their relative contents: The components from leaves of *Eriobotrya* species were analyzed by GC-MS. Compared with the NIST library, 66 components were identified, including 23 alkanes, 12 esters and some alcohol, olefin and acids (Table 2). There were 16 identified terpenoids included terpineol, α-ionone, neryl acetone, farnesan, farnesene, d-nerolidol, trans-nerolidol, α-eudesmol, (−)-loliolide, β-eudesmol, neophytadiene, phytol, 3-hydroxy-urs-12-en-28-oic acid-methyl ester(3α), methyl-3α-hydroxyolean-12-en-28-oate, squalene and 3-hydroxyl lup-20(29)-en- 28-oic acid-methyl ester. Furthermore, Table 3 presents the 8 major compounds of all samples. In 'Fragrant' loquat, the highest component was lup-20(29)-en-28-oic acid 3-hydroxyl-methyl ester (No. 66) while squalene (No. 65) was the highest in common loquat, 'Oak leaf', 'Taiwan', 'Obovata leaf', 'Hengchun' and 'Big flower' loquats. In 'Tibet', 'Daduhe' and 'Zaozhong 6' loquats, the highest compound was identified as heptacosane (No. 62). However, the compound with a retention time (RT) of 36.92 min exhibiting the highest content in 'Guangxi' and 'Bengal' loquats requires further identification.

Most of *Eriobotrya* exhibited a high content of benzoic acid methyl ester. The compound showed good antioxidant and antimicrobial activities (Chipley *et al.*, 1980; Choudhary *et al.*, 2008). In addition, the existence of squalene could indicate the cytoprotective activity against chemotherapy-induced toxicity and anticancer activity (Das *et al.*, 2008; Waterman *et al.*, 2007). In this study, 'Fragrant' loquat showed the highest content of benzoic acid methyl ester while 'Big flower' loquat had the highest squalene. Besides 'Fragrant' and 'Big flower'loquats, 'Obovata leaf' loquat exhibited relatively higher contents of squalene and benzoic acid methyl ester than other species. Considering high levels of total phenolics and flavonids (Hong *et al.*, 2008a), and strong antioxidant activity of benzoic acid methyl ester, 'Obovata leaf' loquat appeared to exhibit a high utilization value.

Terpenoid is an important bioactive compound. Among these 11 species, 'Fragrant', 'Obovata leaf', 'Big flower', 'Hengchun', 'Taiwan' and 'Bengal' loquats exhibited a higher content of terpenoid than the common loquat, suggesting a better utilization potential.

Table 2. Components of the ethyl ether extract from 11 *Eriobotrya* species.

No.	Retention time	Component	Molecular formula
1.	12.08	Hexanoic acid	$C_6H_{12}O_2$
2.	17.75	Benzoicacid methyl ester	$C_8H_8O_2$
3.	21.57	Terpineol	$C_{10}H_{18}O$
4.	21.76	Hexylbutyrate	$C_{10}H_{20}O_2$
5.	21.84	Benzothiazole	C_7H_5NS
6.	22.3	Dodecane	$C_{12}H_{26}$
7.	23.75	2,3,7-Trimethyl octan	$C_{11}H_{24}$
8.	24.16	Tridecan	$C_{13}H_{28}$
9.	25.16	4-Methyl-tridecan	$C_{14}H_{30}$
10.	25.38	Tetradecane, 2,6,10-trimethyl-	$C_{15}H_{32}$
11.	25.68	Tetradecane	$C_{14}H_{30}$
12.	25.75	α-Ionone	$C_{13}H_{20}O$
13.	25.98	Decahydro-4,4,8,9,1-pentamethyl	$C_{15}H_{28}$
14.	26.07	Neryl acetone	$C_{13}H_{22}O$
15.	26.47	1-Tridecanol	$C_{13}H_{26}O$
16.	26.55	Farnesan	$C_{12}H_{20}O_2$
17.	26.82	Dihydroactinidiolide	$C_{11}H_{16}O_2$
18.	26.92	Methyl 4,4,7-trimethyl-4,7-dihydroindan-6-carboxylate	$C_{15}H_{24}O$
19.	26.96	Farnesene	$C_{15}H_{24}$
20.	27.02	Pentadecane	$C_{15}H_{32}$
21.	27.09	D-nerolidol	$C_{15}H_{26}O$
22.	27.61	trans-Nerolidol	$C_{15}H_{26}O$
23.	27.75	2-Ethyl-didecanol	$C_{14}H_{30}O$
24.	28.24	Hexadecane	$C_{16}H_{34}$
25.	28.67	α-Eudesmol	$C_{15}H_{24}O_4$
26.	28.92	1-Hexadecanol, 2-methyl-	$C_{17}H_{36}O$
27.	29.07	Hexadecanol, 3-methyl-	$C_{17}H_{36}$
28.	29.16	2,4',5-Trimethyl diphenylmethane	$C_{16}H_{18}$
29.	29.38	Tetradecane, 2,6,10-trimethyl-	$C_{17}H_{36}$
30.	29.5	Pentadecan,2,6,10,14-tetramethyl	$C_{19}H_{40}$
31.	29.56	(−)-Loliolide	$C_{11}H_{16}O_3$
32.	30.35	β-Eudesmol	$C_{15}H_{26}O$
33.	30.47	Heptdecane	$C_{17}H_{36}$
34.	30.75	Phthalic acid,diisobutyl ester	$C_{16}H_{22}O_4$
35.	30.78	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$
36.	30.85	Neophytadiene	$C_{20}H_{38}$
37.	31.28	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$
38.	31.49	Octadecane	$C_{18}H_{38}$
39.	31.57	Methyl palmitate	$C_{17}H_{34}O_2$
40.	31.67	Dibutyl phthalate	$C_{16}H_{22}O_4$
41.	31.89	Hexadecane	$C_{16}H_{32}O_2$
42.	32.46	Nonadecane	$C_{19}H_{40}$
43.	33.14	Linoleic acid, methyl ester	$C_{19}H_{34}O_2$
44.	33.18	Linoleic acid,ethyl ester	$C_{20}H_{36}O_2$
45.	33.23	Oleic acid,methyl ester	$C_{19}H_{36}O_2$
46.	33.39	Phytol	$C_{20}H_{40}O$
47.	33.49	1-mono Linoleinin	$C_{21}H_{36}O_4$
48.	33.52	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	$C_{19}H_{34}O_2$

Table 2. (Cont'd.).

No.	Retention time	Component	Molecular formula
49.	33.6	N,N-Diethyl-3-methyl-10-(trimethylsilyl) bicycle [4.4.1] undeca-4,6,8,10-tetraen-2-carboxamid	$C_{20}H_{31}NOSi$
50.	33.86	Isochiapin B	$C_{19}H_{22}O_6$
51.	34.22	Octadecan acetate	$C_{20}H_{40}O_2$
52.	34.29	Eicosan	$C_{20}H_{42}$
53.	35.15	Heneicosan	$C_{21}H_{44}$
54.	35.36	Urs-12-en-28-oic acid,3-hydroxy-methyl ester(3α)	$C_{31}H_{50}O_3$
55.	35.8	p-Cresol,2,2'-methylene-bis[6-tert-butyl-]	$C_{23}H_{32}O_2$
56.	36.24	Dihydrochrysin	$C_{15}H_{12}O_4$
57.	36.59	Docosane	$C_{22}H_{46}$
58.	37.24	Tetracosane	$C_{24}H_{50}$
59.	38.65	Pentacosane	$C_{25}H_{52}$
60.	38.74	Chrysin	$C_{15}H_{10}O_4$
61.	40.41	Hexacosane	$C_{26}H_{54}$
62.	41.4	Heptacosane	$C_{27}H_{56}$
63.	42.48	Methyl-3α-hydroxyolean-12-en-28-oate	$C_{31}H_{50}O_3$
64.	42.66	Octacosane	$C_{28}H_{58}$
65.	42.89	Squalene	$C_{30}H_{50}$
66.	44.37	Lup-20(29)-en-28-oic acid, 3-hydroxyl-methyl ester	$C_{31}H_{50}O_3$

Table 3. Percent contents of the major component peaks.

Component number	Retention time	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
2	17.75	3.20	3.72	3.07	2.23	3.23	3.78	3.31	3.73	3.93	5.06	4.55	
34	30.75	4.67							2.91				2.62
35	30.78											1.78	
36	30.85	3.08	2.32		2.81	3.24		2.82		2.94	2.48	1.95	
-	31.08	4.94			4.59								3.19
41	31.89						3.94						
44	33.18		2.31							3.05			2.95
46	33.39								4.77	3.33		2.16	
47	33.49									3.61			
53	35.15						4.35						
54	35.36		2.04			4.09							
55	35.8					3.63							
56	36.24			1.90				2.60					
57	36.59					5.49		2.38				2.64	
-	36.92	5.46	7.92	1.80	4.41	3.96	6.91	8.40	5.65	11.48	7.28	4.80	2.51
-	37.99				4.14								3.09
-	38.56							2.65					
-	40.29		3.10			3.50	6.19						2.60
61	40.41			2.69	5.21		2.89		3.08		3.29		
-	40.86	3.34											
62	41.4	11.9		2.55	15.2	5.79	4.58	3.91	8.49		4.18	3.38	7.30
-	42.02									2.49			
63	42.48			2.02									
64	42.66		2.11	3.69					3.52		3.38		
65	42.89	8.76	10.50		7.27		8.09	15.90	15.41	2.40	3.31	31.50	24.25
66	44.37			44.50							6.61		
Total percentage		**34.0**	**36.2**	**45.9**	**62.2**	**32.9**	**40.7**	**42.0**	**33.2**	**47.6**	**35.6**	**52.8**	**48.5**

The sample codes indicated the different materials mentioned in Table 1 while the component number was provided in Table 2

"-": Not identified

In this study, 66 compounds were identified, which was less than those reported by Tai *et al.* (Tai *et al.*, 2008) using simultaneous distillation and solvent extraction, and among them only 8 compounds were the same, which may be due to the different extraction methods used (Galhiane *et al.*, 2006; Yamini *et al.*, 2008; Chen *et al.*, 2009). Currently, ultrasonic-assisted extraction with ethyl ether is widely used to extract the essential oil from plant tissue. The advantage of this method is that it needs a little material and a short extraction time. Thus, a number of samples can be used for a comparative analysis of 11 species of *Eriobotrya* in this study.

Similarity analysis of GC chromatogram and initiative clustering: Similar analysis over the whole chromatograms was conducted using the Computer Aided Similarity Evaluation System of Central South University as a selection of RT 5–43.5 min, the coefficients of 11 *Eriobotrya* species were from 0.7883 to 0.9949. SPSS10.0 software was further used to conduct cluttering analysis to the original chromatogram data. The clustering analysis on the basis of these essential oils indicated that the 11 species of *Eriobotrya* can be classified into 3 groups, i.e., the first group 'Oak leaf', 'Taiwan', 'Bengal', 'Obovata leaf', 'Hengchun', 'Guangxi' and 'Big flower' loquats, the second group the common loquat, and 'Tibet', 'Zaozhong 6' and 'Daduhe' loquats, and the third group 'Fragrant' loquat. A similar result was also reported by Yang *et al.* (Yang *et al.*, 2007), who suggested that 'Hengchun' loquat, 'Guangxi' loquat, the common loquat and 'Tibet' loquat belonged to the same group using the molecular marker. In addition, 'Daduhe' loquat and the common loquat including the cultivated and wild species fell into one group while 'Oak leaf', 'Obovata leaf', 'Guangxi' and 'Big flower' loquats became other group based on the terpenoid profile by high performance liquid chromatography (Hong *et al.*, 2007). Thus, it suggested that the GC-MS chromatograph could indicate the genetic variance of the 11 *Eriobotrya* species.

Among these three groups, 'Obovata leaf', 'Hengchun' and 'Guangxi' loquats, and 'Obovata leaf' and 'Big flower' loquats exhibited higher contents of phytol and squalene in the first group compared with other species respectively, while 'Zaozhong 6' and 'Daduhe' loquats in the second group had a high neophytadiene content. In the third group, 'Fragrant' loquat had the highest content of lup-20(29)-en-28-oic acid 3-hydroxyl-methyl ester. These results suggested that these wild species could be utilized better for the essential oils.

Acknowledgements

We thank Dr. Y.M. Jiang of South China Botanical Garden, Chinese Academy of Sciences for providing some useful suggestions. We also thank Ms. Y.F. Chen of South China Agricultural University for GC-MS analyses. This work was supported by Guangdong Provincial Natural Science Foundation (Grant No. 07118121) and Fujian Provincial Youth Project (Grant No. 2008F3080).

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(Received for publication 22 February 2010)