

## CHEMICAL VARIATION IN LEAF OILS OF *Pistacia chinensis* FROM FIVE LOCATIONS IN CHINA

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*Hydrodistilled leaf oils of Pistacia chinensis Bunge from five locations in China were analyzed using GC/MS. A total of 58 compounds was identified in the oils, and a relatively high variation in their contents was found. The major compounds include  $\beta$ -phellandrene (0.54~53.86%),  $\alpha$ -pinene (4.74~54.44%),  $\beta$ -pinene (0.49~42.90%), caryophyllene (5.64~20.01%), cis-ocimene (tr~43.93%), eudesmadiene (0~15.06%), and camphene (tr~20.57%). Cluster analysis classified the leaf oils into two chemotypes: one rich in  $\alpha$ -pinene and  $\beta$ -pinene, and the other rich in  $\beta$ -phellandrene.*

**Key words:** *Pistacia chinensis*, leaf oils, GC/MS analysis, chemotype.

Chinese pistache, *Pistacia chinensis* Bunge, is a perennial arbor distributed mainly in eastern and southern China. Its galls induced by aphids are a rich source of tannins and used as a Chinese folk medicine for sore-treating, cough-relieving, and hemostasis. The seed of *P. chinensis* is a source of edible or industrial oil. Because of its excellent adaptation to infertile soils and dry climate, *P. chinensis* is also considered a pioneer species for reforestation. In addition, *P. chinensis* can serve as a good rootstock for *P. vera* L. in horticultural applications.

The composition of essential oils of *P. lentiscus* L. was extensively investigated, especially intraspecific variability and seasonal variation from different areas including Corsica [1], Sardinia [2], Turkey [3], Morocco [4], and Mediterranean countries [5]. The essential oils of other *Pistacia* species, e.g., *P. terebinthus* [6], *P. eurycarpa* [7], *P. palaestina* [8], *P. vera*, *P. terebinthus*, and *P. lentiscus* [3], were analyzed. In particular, the essential oils of *P. chinensis* from Egypt along with *P. khinjuk* and *P. lentiscus* were also analyzed [9]. However, chemical variation in the leaf oils of *P. chinensis* has not been investigated yet.

Fifty-eight compounds of the leaf oils of *P. chinensis* were identified in five samples from different locations in China, accounting for 99.10~99.88% of the oils. The oils of the samples from Beijing, Kunming, Shanghai, Nanjing, and Wuhan showed the presence of 50, 45, 52, 50, and 50 identified compounds accounting for 99.21, 99.34, 99.74, 99.88, and 99.10% of the whole oils, respectively. The major compounds in the oils from Beijing were camphene (20.57%),  $\alpha$ -pinene (17.75%),  $\beta$ -pinene, (15.96%), eudesmadiene (7.04%), caryophyllene (5.64%),  $\gamma$ -elemene (5.24%), tricyclene (5.03%), and  $\beta$ -elemene (3.43%); in the oils from Kunming were  $\alpha$ -pinene (54.44%), caryophyllene (20.01%), and  $\beta$ -pinene (11.20%); in the oils from Shanghai were cis-ocimene (43.93%),  $\beta$ -phellandrene (32.27%), caryophyllene (7.38%) and  $\alpha$ -pinene (7.35%); in the oils from Nanjing were  $\beta$ -phellandrene (53.86%), eudesmadiene (15.06%), caryophyllene (10.49%), and  $\alpha$ -pinene (7.90%); and in the oils from Wuhan were  $\beta$ -pinene (42.90%),  $\beta$ -phellandrene (37.49%), caryophyllene (7.15%), and  $\alpha$ -pinene (4.74%).

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TABLE 1. Composition of Leaf Oils of *P. chinensis* from Five Locations in China

Compound	RI	Beijing	Kunming	Shanghai	Nanjing	Wuhan
		%				
<i>trans</i> -3-Hexenol	862	0.31	Tr.	Tr.	0.16	1.20
<i>cis</i> -3-Hexenol	871	0.10	Tr.	0.46	-	-
Hexanol	874	-	-	Tr.	Tr.	0.26
Tricyclene	928	5.03	0.62	-	-	-
$\alpha$ -Pinene	941	17.75	54.44	7.35	7.90	4.74
Camphene	959	20.57	2.33	Tr.	0.10	Tr.
Sabinene	976	0.65	Tr.	0.13	1.63	0.30
$\beta$ -Pinene	984	15.96	11.20	0.49	2.07	42.90
Myrcene	988	0.49	0.92	-	-	-
$\alpha$ -Phellandrene	1006	0.53	0.15	Tr.	0.18	Tr.
$\delta$ -3-Carene	1018	0.26	0.13	Tr.	-	-
Limonene	1032	1.86	1.11	-	-	-
$\beta$ -Phellandrene	1034	0.54	0.95	32.27	53.86	37.49
<i>cis</i> -Ocimene	1046	0.19	Tr.	43.93	0.15	0.14
$\gamma$ -Terpinene	1059	0.43	0.25	Tr.	0.24	0.11
Ocimene	1072	Tr.	Tr.	0.51	Tr.	Tr.
<i>trans</i> -Linalool oxide	1074	-	Tr.	Tr.	Tr.	Tr.
<i>p</i> -Mentha-1,4(8)-diene	1085	0.25	0.27	Tr.	0.28	0.13
Linalool	1096	Tr.	Tr.	Tr.	Tr.	Tr.
<i>n</i> -Nonaldehyde	1100	Tr.	Tr.	Tr.	Tr.	Tr.
Terpineol	1144	Tr.	Tr.	Tr.	Tr.	Tr.
Camphor	1152	Tr.	Tr.	Tr.	Tr.	Tr.
Borneol	1175	0.20	0.11	Tr.	Tr.	Tr.
<i>p</i> -Menth-1-en-4-ol	1182	0.88	0.25	0.15	0.39	0.14
<i>p</i> -Menth-1-en-8-ol	1196	0.96	1.04	0.35	1.00	0.29
<i>p</i> -Menth-1-en-3-ol	1208	Tr.	Tr.	0.11	Tr.	Tr.
Bornyl acetate	1283	1.13	0.56	Tr.	Tr.	Tr.
$\delta$ -Elemene	1330	Tr.	Tr.	Tr.	Tr.	Tr.
$\alpha$ -Cubebene	1345	0.85	Tr.	Tr.	0.20	-
Copaene	1375	0.26	Tr.	Tr.	0.11	Tr.
$\beta$ -Elemene	1388	3.43	-	0.30	0.83	0.17
Caryophyllene	1424	5.64	20.01	7.38	10.49	7.15
Guaiadiene	1434	0.39	Tr.	0.19	Tr.	Tr.
Humulene	-	-	Tr.	0.18	0.79	Tr.
<i>E</i> -Farnesene	-	-	-	Tr.	0.12	Tr.
$\beta$ -Caryophyllene	1458	0.44	0.95	0.49	0.66	0.40
$\alpha$ -Humulene	1463	0.19	0.18	Tr.	Tr.	Tr.
$\gamma$ -Muurolene	1473	0.44	0.46	0.21	0.59	0.13
Germacrene D	1483	2.81	Tr.	Tr.	Tr.	Tr.
Eudesmadiene	1494	7.04	-	2.68	15.06	1.38
$\gamma$ -Elemene	1499	5.24	-	0.40	Tr.	Tr.
Cadinadiene	1516	0.42	-	0.17	Tr.	Tr.
Panasinsen	1522	0.29	-	Tr.	0.11	Tr.
$\alpha$ -Muurolene	1532	Tr.	-	-	-	-
<i>trans</i> -Nerolidol	-	Tr.	Tr.	Tr.	Tr.	Tr.
( <i>Z</i> )-3-Hexenyl benzoate	1574	0.10	Tr.	Tr.	Tr.	Tr.
Spathulenol	1581	0.51	0.45	Tr.	0.22	Tr.
Caryophyllene oxide	1586	0.13	0.94	-	-	-
Globulol	1590	0.85	Tr.	0.13	0.44	Tr.

TABLE 1. (continued)

Compound	RI	Beijing	Kunming	Shanghai	Nanjing	Wuhan
		%				
Tetramethyl octahydro naphthalenemethanol	-	-	-	0.11	0.23	Tr.
Eudesmenol	1621	0.37	0.49	0.16	0.44	Tr.
$\delta$ -Cadinol	1658	0.60	Tr.	Tr.	Tr.	Tr.
$\alpha$ -Cadinol	1664	0.50	0.11	0.14	0.51	0.10
Farnesol	-	0.13	0.21	0.13	0.17	0.16
9,12,15-Octadecatrienol	1883	0.10	-	-	-	0.56
Phytol	-	-	-	0.15	Tr.	0.21
<i>n</i> -Pentacosane	-	-	-	Tr.	Tr.	Tr.
<i>n</i> -Heptacosane	-	-	-	Tr.	Tr.	Tr.

RI: retention index; Tr.: trace quantities (<0.1% detected).

In order to determine the variations of leaf oils of *P. chinensis* from different locations in China, the composition data were analyzed using cluster analysis. The result shows that the difference in leaf oils between the two locations from Beijing and Kunming was relatively small. These two locations were well clustered into a group, and separated from the rest of the three locations, suggesting that the five locations be grouped into two chemotypes: the samples from Beijing and Kunming featured rich in  $\alpha$ -pinene (17.75%~54.44%) and  $\beta$ -pinene (11.20%~15.96%), whereas the samples from the other three locations rich in  $\beta$ -phellandrene (32.27%~53.86%).

Our study shows the variability of the chemical constituents of leaf oils of *P. chinensis* in different regions of China. This variability reveals the wide adaptation of *P. chinensis* to different environments. Further investigation on the chemical composition of *P. chinensis*, especially expanding to other regions of different ecological conditions, is still needed.

## EXPERIMENTAL

Samples of *P. chinensis* were collected from five locations in China from May 26 to June 2, 2004: Tianmashan in Shanghai (N: 31.1°, E: 121.1°, 60 ATH), Moshan in Wuhan (N: 30.3°, 114.2°, 25 ATH), Zhongshan in Nanjing (N: 32.1°, E: 118.5°, 35 ATH), Helongtang in Kunming (N: 25.0°, E: 102.4°, 1990 ATH), and Xiangshan in Beijing (N: 39.5°, E: 116.3°, 65 ATH) (Table 1). No obvious morphological variation was found among these samples. They were identified and voucher specimens were deposited in the MOE Laboratory for Biodiversity Science and Ecological Engineering, Fudan University.

The leaves of the five samples, *i.e.*, Shanghai (365 g), Wuhan (350 g), Nanjing (323 g), Kunming (402 g), and Beijing (340 g), were dried and cut into fragments and then subjected to hydrodistillation for 3 h in a Clevenger-type apparatus. The oils were collected and then stored with anhydrous sodium sulfate in Eppendorf tubes at 4°C until analyzed and tested.

The leaf oils of *P. chinensis* were analyzed based on previously described protocols [10]. GC analyses were carried out on a HP-6890 gas chromatograph equipped with a FID and a HP-5 capillary column (30 m length, 0.25 mm diameter, 0.25  $\mu$ m film thickness) using N<sub>2</sub> as carrier gas (1 mL/min). A 1  $\mu$ L aliquot of oil was injected into the column using a 10:1 split injection, with temperature set at 250°C. The GC program was initiated by a column temperature set at 60°C for 2 min, increased to 250°C at a rate of 10°C/min, and held for 10 min.

GC/MS analyses were performed on a combined GC/MS instrument (Finnigan Voyager, San Jose, CA, USA) with a HP-5 fused silica capillary column (30 m length, 0.25 mm diameter, 0.25  $\mu$ m film thickness) using He as carrier gas (1  $\mu$ L/min). The mass spectrometer was operated in the 70 eV EI mode with scanning from 41 to 450 amu at 0.5 s, and the mass source was set at 200°C. The identifications of the volatile constituents were based on GC retention indices (relative to *n*-alkanes, from C8 to C20) and computer matching of their mass spectral fragmentation patterns with those stored in the spectrometer database using the National Institute of Standards and Technology Mass Spectral database (NIST-MS, 1998). Relative percentage amounts of the identified components were calculated from the total ion chromatograms by a computerized integrator.

An average Euclidean distance matrix based on the composition percentages of leaf oils from the five locations of *P. chinensis* was calculated. Cluster analysis based on the distance matrix was performed with the UPGMA method [11] using the NTSYSpc version 2.02 [12].

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## REFERENCES

1. V. Castola, A. Bighelli, and J. Casanova, *Biochem. Syst. Ecol.*, **28**, 79 (2000).
2. R. Congiu, D. Falconieri, B. Marongiu, A. Piras, and S. Porcedda, *Flavour Frag. J.*, **17**, 239 (2002).
3. M. E. Duru, A. Cakir, S. Kordali, H. Zengin, M. Harmandar, S. Izumi, and T. Hirata, *Fitoterapia*, **74**, 170 (2003).
4. S. Zrira, A. Elamrani, and B. Benjlali, *Flavour Frag. J.*, **18**, 475 (2003).
5. O. Barazani, N. Dudai, and A. Golan-Goldhirsh, *J. Chem. Ecol.*, **29**, 1939 (2003).
6. M. Couladis, M. Ozcan, O. Tzakou, and A. Akgul, *J. Sci. Food Agr.*, **83**, 136 (2003).
7. F. Demirci, K. H. C. Baser, I. Calis, and E. Gokhan, *Chem. Nat. Compd.*, **37**, 332 (2001).
8. G. Flamini, A. Bader, P. L. Cioni, A. Katbeh-Bader, and I. Morelli, *J. Sci. Food Agr.*, **52**, 572 (2004).
9. H. L. De Pooter, N. M. Schamp, E. A. Aboutabl, S. F. El Tohamy, and S. L. Doss, *Flavour Frag. J.*, **6**, 229 (1991).
10. P. Nan, S. L. Peng, S. H. Shi, H. Ren, J. Yang, and Y. Zhong, *Z. Naturforsch.*, **58 c**, 57 (2003).
11. P. H. A. Sneath and R. R. Sokal, *Numerical Taxonomy*, Freeman, San Francisco, CA (1973).
12. F. J. Rohlf, *NTSYSpc 2.02*. Exeter Software, Setauket, NY, USA (1998).