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Essential oil of *Actinidia macrosperma*, a catnip response kiwi endemic to China*

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Abstract: Objective: To identify compounds that may be responsible for catnip response of *Actinidia macrosperma*, and compare chemical compositions in the wild and in vitro regenerated plants. Methods: GC-MS and relative retention indices with *n*-alkanes as reference points were used for compound identification, and component relative percentage was calculated based on GC peak areas without using correction factors. Results: There are 28 compounds (92.72%) and 15 compounds (93.88%) identified in the essential oils from the wild and regenerated plants, respectively. Dihydronepetalactone, iridomyrmecin, and dihydroactinidiolide, which are believed to be attractive to felines, are present in both wild and regenerated plants. Actinine was not detected, and beta-pheylethyl alcohol was only present in wild plant. In addition, short-chain enol derivatives, messengers in chemical communication, are commonly present in wild plant of *A. macrosperma*, but absent in regenerated one. Conclusion: Dihydronepetalactone, iridomyrmecin, and dihydroactinidiolide are responsible for the catnip response of *A. macrosperma*.

Key words: *Actinidia macrosperma*, GC-MS, Dihydronepetalactone, Iridomyrmecin, Dihydroactinidiolide, Catnip response
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INTRODUCTION

Actinidia macrosperma is a deciduous scandent shrub with white flowers and orange fruits. It is endemic to China, and grows in thickets, mixed forest, forest margins on low mountains, and moist places in Zhejiang, Jiangxi, Hubei and Jiangsu Provinces (Liang, 1984).

A. macrosperma was named "ginseng of cat" by a herbalist of Zhejiang in the 1960's because it was observed that cats preferred to eat its fresh leaves and twigs to excite themselves and cure wounds. Its root and stem have been used to treat lung cancer, esophageal cancer, and leprosy in the folks of China (Jiangsu New Medicine College, 1984; Yao and Wang, 1989). It has been enrolled in TCM (traditional Chinese medicine) standards of Zhejiang Province (Health Bureau of Zhejiang Province, 1994). Because of the enormous demand, the wild resource of this species has decreased rapidly, even become exhausted, based on our field and market investigation and folk inquiry. Great attention should be paid to its effective protection and reasonable development. Therefore, in recent years we implemented a series of research projects focusing on the chemistry and tissue culture (Jiang and Li, 2003; Feng *et al*., 2004; Lu *et al*., 2004; Zhao *et al*., 2006). The objectives of this study were to identify constituents of the essential oil of *A. macrosperma*, provide clues for the attraction of the plant to cats, and to determine whether there are differences of essential oils in wild and regenerated plants.

MATERIALS AND METHODS

Fresh leaves of the wild plant were collected in April 2005 from Fuyang County, Zhejiang Province.

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The site was in mixed forest of piedmont at 200 m above sea level. Fresh leaves of the 2 years old regenerated plant from tissue culture were sampled in a cultivation base of *A. macrosperma* in Fuyang at the same time. A voucher specimen (No. A2005006) is deposited in the Herbarium of Zhejiang University (HZU). The fresh materials of 100 g were separately distilled with *n*-hexane in a Clevenger-type apparatus for 2 h. The yield was 0.l0% and 0.08% (w/w) for wild plant and regenerated one respectively.

GC analyses were conducted using an HP 6890 gas chromatograph equipped with an FID and HP-5 capillary column (30 m×0.25 mm, 0.25 μm film thickness) working with the following temperature program: 50 °C for 3 min, rising at 10 °C/min to 260 °C, and finally isothermal at 260 °C; injector and detector temperatures 260 °C; carrier gas, helium (1 ml/min); detector, dual FID; split ratio, 1:10. Component relative percentage was calculated based on GC peak areas without using correction factors. *n*-alkanes were used as reference points in the calculation of relative retention indices.

GC-MS analyses were conducted under the same conditions with GC using a chromatograph HP 6890 interfaced to an HP 5973 mass spectrometer (ionization voltage 70 eV) and equipped with an HP-5 capillary column (30 m×0.25 mm, 0.25 μm film thickness).

Identification of the constituents was based on comparison of their relative retention indices and mass spectra with those of NIST 2003 library data of the GC-MS system and literature data (Adams, 2001).

RESULTS

The composition of the essential oil of *A. macrosperma* is listed in Table 1. A total of 28 and 15 compounds were identified, amounting to 92.72% and 93.88% of the whole oil, from wild and regenerated plants, respectively.

The main constituents of the essential oil were straight-chain fatty acid derivatives, among which α-Linolenic acid constituted the most (63.97% from wild plants and 81.71% from regenerated plants), followed by triterpenes. Short-chain enol derivatives may act as messengers in chemical communication (Du, 2001). Interestingly, while absent in regenerated ones, these compounds were common in wild plants of *A. macrosperma*. We speculate that artificial habitat and immature growth stage might lead to absence of these enol derivatives. However, the differences of behavior in chemical communication between wild and regenerated plants need further observation.

DISCUSSION

A. macrosperma is named "ginseng of cat" in China, because cats prefer its fresh leaves and twigs to cure wounds and excite themselves. During our cultivation of tissue-cultured plantlets, cats were frequently observed to tear the plantlets in green house into pieces and become frantic. Similar behavior was first observed in catnip (*Nepeta cataria*), later named catnip response. Todd (1962) described the response of the domestic cat to catnip as sniffing, licking and chewing with head shaking, chin and cheek rubbing, and head-over rolling and body rubbing. Many other species have been reported to be intriguing to cats and other feline, including *Nepeta* spp., *Actinidia* spp., *Valeriana* spp., *Teucrium marum*, *Boschniakia rossica*, *Menyanthes trifoliata*, *Nemophila menziesii*, *Origanum dictamnus*, *Lippia javanica*, *Viburnum opulus* (Tucker and Tucker, 1988).

Fourteen compounds have been shown to elicit catnip response, including nepetalactone, epinepetalactone, dihydronepetalactone, isodihydronepetalactone, neonepetalactone, iridomyrmecin, boschnialactone, onikulactone, actinidine, boschniakine, actinidiolide and dihydroactinidiolide (Tucker and Tucker, 1988). We have detected three of them in the essential oil of both wild plant and regenerated one from tissue culture of *A. macrosperma*, including dihydronepetalactone, iridomyrmecin, dihydroactinidiolide. The three compounds have been identified in essential oils of *Nepeta cataria*, *N. nepetella*, *A. polygama*, algae (*Cladophora vagabunda*) (Elenkov *et al*., 1995) and defensive secretions of some ants, e.g., *Iridomyrmex pruinosus*, *I. purpureus*, and rove beetle (*Creophilus maxilosus*). Their effect on the physiological activities of cats has been confirmed (Tucker and Tucker, 1988). Actinidine is another stimulant to feline and was firstly isolated from *A. polygama*. Later, it was found in *A. arguta*, *Valeriana officinalis*, *Tecoma stans*, as well as rove beetles of

Table 1 Composition of the essential oil from leaves of *Actinidia macrosperma*

No.	RI*	Composition	Relative (%)	
			Wild	Regenerated
1	850	2-hexenal	0.01	−
2	856	3-hexenol	0.26	−
3	862	2-hexenol	0.06	−
4	1007	3-hexen-1-ol, acetate, (Z)-	0.03	−
5	1010	3-methyl-3-heptanol	0.05	−
6	1037	Benzyl alcohol	0.31	−
7	1098	β-Linalool	0.93	−
8	1101	3-Decyn-2-ol	0.02	−
9	1110	β-phenylethyl alcohol	0.04	−
10	1200	Dodecane	0.24	−
11	1209	*cis*-α-terpineol	0.18	−
12	1220	(*R*)-citronellol	0.13	−
13	1255	(*E*)-geraniol	0.52	−
14	1292	2-methyl bicyclo[3,2,1]octane	−	0.62
15	1301	1,2-dimethyl-indoline	0.23	1.12
16	1318	Dodeca-1,6-dien-12-ol-6,10-dimethyl	7.81	1.60
17	1346	3-hexadecyne	1.41	−
18	1400	Tetradecane	0.16	−
19	1404	Limonene-1,2-epoxide	−	0.19
20	1430	Dihydronepetalactone	1.97	1.23
21	1463	Iridomyrmecin	0.40	0.15
22	1588	Dihydroactinidiolide	0.18	0.15
23	1600	Hexadecane	0.08	−
24	1843	3,7,11,15-tetramethyl-2-hexadecenol	0.24	−
25	1846	2-pentadecanone-6,10,14-trimethyl	0.04	−
26	1953	(*Z*)-11-hexadecenoic acid	−	0.19
27	1972	*n*-hexadecanoic acid	4.46	4.08
28	2101	Linolenic acid, methylester	0.31	0.63
29	2114	Phytol	1.64	0.80
30	2178	α-Linolenic acid	64.97	81.71
31	2198	Linolenic acid ethylester	−	0.17
32	2660	Squalene	6.04	0.42
33	2702	Linolenic acid, phenylmethyl ester	−	0.82

* RI: linear retention index relative to *n*-alkanes on HP-5 column

the genera of *Cafius*, *Creophilus*, *Gabrius*, *Hesperus*, and *Philonthus*, family Staphylinidae. These beetles produce actinidine in their defensive secretions (Tucker and Tucker, 1988; Blake, 2004). However, we did not detect any actinidine in *A. macrosperma*. Beta-pheylethyl alcohol also induces cat salivation, although it is present in wild plant of *A. macrosperma*, but absent in the regenerated plant. It is likely that the presence of dihydronepetalactone, iridomyrmecin and dihydroactinidiolide is responsible for the attraction of *A. macrosperma* to cats.

The current intrageneric subdivisions within *Actinidia* included four sections and four series, *Leicarpae* (*Lamellatae* and *Solidae*), *Maculatae*, *Strigosae*, and *Stellatae* (*Perfectae* and *Imperfectae*) (Liang, 1984). Although the other three traditional sections are controversial, *Leiocarpae* is well supported as a monophyletic group by different metrics, such as general morphology, leaf flavonoid content, cpDNA, and RAPDs (Fig.1) (Huang *et al*., 2002). Interestingly, the *Actinidia* species (*A. polygama*, *A. kolomikta*, *A. arguta*, *A. macrosperma*) reported to

elicit catnip response all belong to Sect. *Leiocarpae*. No species of the other sections were found to have such characteristic to date. Whether the reaction in cats or its responsible compounds are common in Sect. *Leiocarpae* species or widespread in the genus needs further test. The differences of cat attracting substances among different *Actinidia* species may also have chemotaxonomic implications.

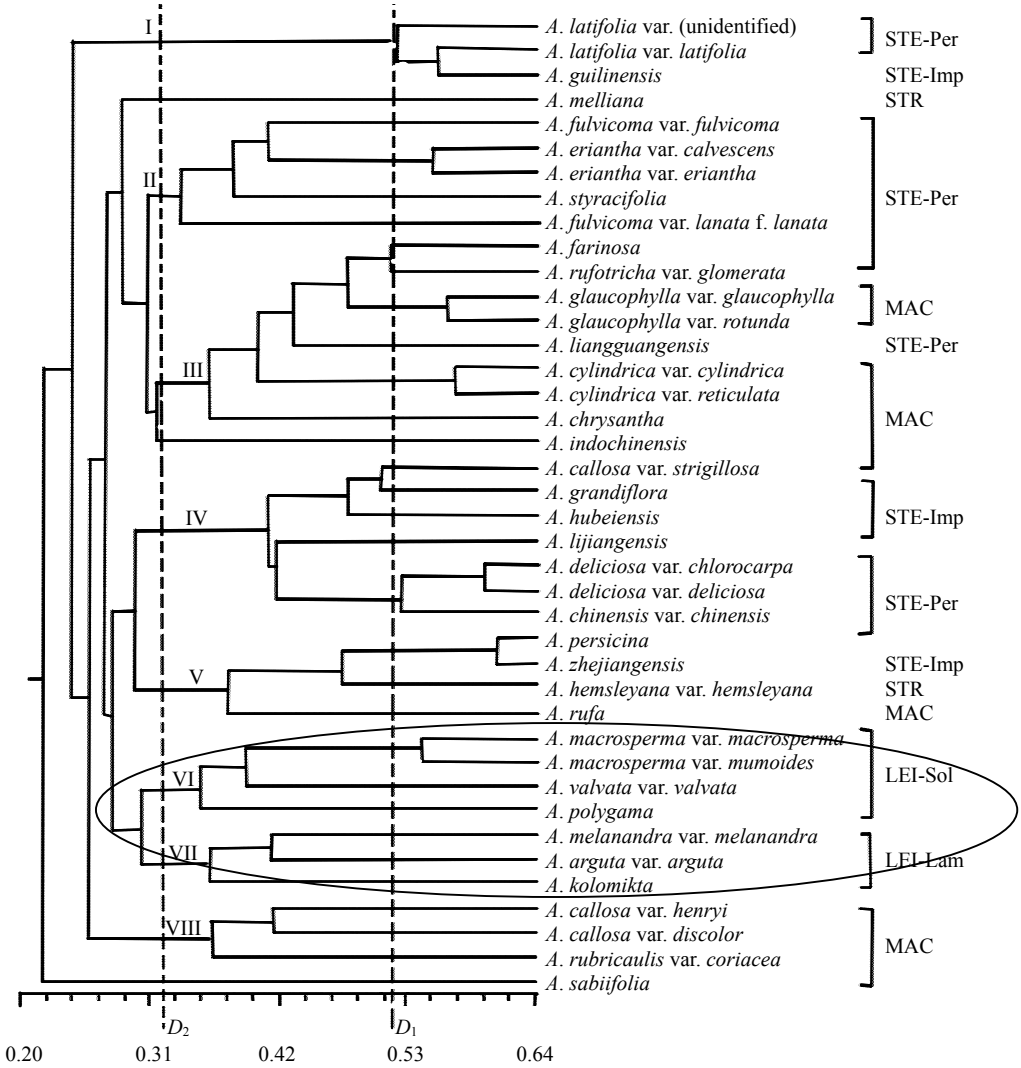

Fig.1 UPGMA (unweighted pair group method using arithmetic average) phenogram based on the similarity (Jaccard's coefficient) matrix calculated from RAPD (random amplified polymorphic DNA) data of 40 *Actinidia* taxa

The dotted lines indicate the two cut-off points, D_1=0.52 and D_2=0.32. Numbers on the phenogram label the seven major clusters. Cophenetic correlation coefficient is 0.76. LEI: Sect. *Leiocarpae*; Lam: Ser. *Lamellatae*; Sol: Ser. *Solidae*; MAC: Sect. *Maculatae*; STR: Sect. *Strigosae*; STE: Sect. *Stellatae*; Per: Ser. *Perfectae*; Imp: Ser. *Imperfectae* (Huang *et al*., 2002)

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