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Comparative analysis of essential oil components of two *Cryptomeria* species from China

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ABSTRACT

The essential oils obtained by hydrodistillation from leaves of Chinese native *Cryptomeria japonica* and *Cryptomeria fortunei* were analyzed by GC and GC–MS. Fifty-seven compounds were identified in the essential oils of *C. japonica* with α-elemol (20.12%), kaur-16-ene (14.84%), β-phellandrene (5.97%), β-elemene (5.87%), α-eudesmol (5.62%) and β-eudesmol (5.03%) as main constituents. Forty components were identified in the oil of *C. fortunei* with kaurene (34.04%), α-elemol (13.34%), γ-eudesmol (10.80%), β-eudesmol (10.16%), α-pinene (2.75%) and γ-cadinene (1.92%) as the most abundant components. This study demonstrated the occurrence of α-elemol chemotype in *C. japonica* and kaurene chemotype in *C. fortunei* from China. The essential oil compositions of two *Cryptomeria* samples were shown that they can be used for green plant protection, pharmaceutical, perfume and food industries.

1. Introduction

Essential oils were largely employed for their properties had already been observed in nature. At present, approximately 3000 essential oils are known, 300 of which are commercially important especially in the pharmaceutical, agronomic, food, sanitary, cosmetic and perfume industries (Bakkali et al., 2008). In Japan and Taiwan, many studies had already reported the genus *Cryptomeria* notably the *Cryptomeria japonica* species. According to recent studies, the extracts and the essential oils from *C. japonica* have been shown to possess antitermite (Yatagai et al., 1991; Sogabe et al., 2000; Cheng et al., 2007), antimosquito (Cheng et al., 2003, 2008, 2009; Gu et al., 2009a,b), antisilverfish (Wang et al., 2006), antimite (Morita et al., 1991; Ando, 1993; Morita and Yatagai, 1994), antimicrobial (Matsushita et al., 2006; Cha et al., 2007; Oh et al., 2007; Lee et al., 2009), antibacterial (Li et al., 2008; Yoon et al., 2009), antifungal (Cheng et al., 2005), antiinflammatory (Shyur et al., 2008; Yoon et al., 2009), antiulcer (Matsunaga et al., 2000), anticancer (Chen et al., 2010), antiallergic (Morimoto et al., 2003), hepatoprotective (Shyur et al., 2008), angiotensin (Tsutsumi et al., 1998), anxiolytic and analgesic (Cheng et al., 2009) activities. These activities were attributed to a plethora of components, notably flavonoids, terpene (monoterpenes, sesquiterpenes and diterpene) and terpenols (Ohmoto and Yoshida, 1983; Cheng et al., 2009). The volatile constituents that contributed to a part of these activities were also documented (Chen et al., 2001; Kashiwagi et al., 2007; Wu et al., 2008). In the past, some papers have been reported on the bioactive compositions in the essential oil of *C. japonica*, but they didn't give homogeneous results. These may be due to the genetic, environmental factors, ontogeny, season, plant part analyzed and analytical methods (Appleyon et al., 1968; Cheng et al., 2005, 2009; Li et al., 2008).

The genus *Cryptomeria* belongs to Taxodiaceae family, including only 2 species. China has the both two species, and *C. fortunei* Hooibrenk is the most abundant one and unique to China (Xia et al., 2004). In contrast, the distribution areas of *C. japonica* was restricted to the humid forest of the East Asia. The chemical composition of the *C. japonica* essential oils in Japan, Korea and Taiwan had been studied (Shieh et al., 1981; Lee and Lin, 1986; Cheng et al., 2005; Wang et al., 2006; Oh et al., 2007; Cheng et al., 2009a,b; Lee et al., 2009; Ho et al., 2010). As far as we were concerned, the chemical composition of *C. fortunei* essential oils was reported only one time in China (Wu, 2006). Despite the great scientific interest, the high potential exploitation value and economic value of *C. japonica* that is native in China are unfortunately neglected. In order to exploit those species resource rationally, comprehensive researches on their phytochemical characteristic seems to be necessary. Thus, this paper dealt with the chemical composition of the essential oils isolated from wild-growing *C. japonica* and *C. fortunei*, and compared with the results reported in the previous studies.

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2. Materials and methods

2.1. Plant material

The fresh leaves of *C. japonica* and *C. fortunei* were collected from two wild population located respectively in Hefei (latitude 31°52′(N); longitude 117°17′(E); altitude 20 m) and Mount. Lu (latitude 29°56′(N); longitude 115°96′(E); altitude 1200 m) during May 2009. The plant material was botanically identified by Dr. Wei xing (College of Life Sciences & Technology, Huazhong Agricultural University) and according to the morphological description presented in China flora (Zheng and Fu, 1978).

2.2. Isolation of essential oils

The essential oils were isolated from the fresh plant material (100 g) by conventional hydrodistillation for 4 h. The hydrodistillation was performed by a simple laboratory apparatus which consisted of a 1000 ml steam generator flask, a distillation flask, a condenser and a receiving vessel. The obtained distillate was extracted twice with petroleum ether, and the solvent was concentrated in a rotary evaporator under vacuum. The obtained essential oil was dried with anhydrous Na_2SO_4 and stored in a sealed vial at 4 °C for further analysis.

2.3. Gas-chromatography (GC) analysis

GC analysis was performed using an Agilent 7890 series gas chromatograph equipped with a HP-5 capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). Samples (1 μl) were injected with a split ratio of 1:50, and a continuous flow rate of chromatographic grade nitrogen was 1 ml/min. The oven temperature was held at 50 °C for 10 min, and then ramped at 10 °C/min up to 250 °C. Injector and FID detector temperature were held at 250 °C.

2.4. Gas chromatography–mass spectrometry (GC–MS) analysis

The GC–MS analysis was performed on a gas chromatograph Agilent 6890A interfaced with an Agilent 5975C mass spectrometer. A HP-5 MS (cross-linked 5% phenyl methyl silox) capillary column (30 m × 0.25 mm, 0.25 μm film thickness) was used. The column temperature was programmed to rise from 50 to 250 °C at a rate of 10 °C/min. The carrier gas was helium with a flow rate of 1 ml/min. MS were taken at 70 eV and a mass range of 15–500. The oils components were identified by matching their recorded mass spectra with the data bank mass spectra (NISt08.L libraries) and by comparing their retention indices relative to a series of n-alkanes (C9–C22) with literature values (Van den Dool and Kratz, 1963). The quantity of compounds was obtained by integrating the peak area of the spectrograms.

3. Results and discussion

3.1. Extraction and yield of essential oils

The leaves of *C. japonica* and *C. fortunei* yielded 1.15% and 0.82% (w/w) of pale yellowish or deep yellow oils by hydrodistillation, respectively (Table 1). Compared with the results of previous studies, the oil yield we obtained was similar to that some observed in *C. japonica* from Taiwan (Cheng et al., 2009a,b), meanwhile, lower than some results that observed in *C. japonica* from Taiwan (Cheng et al., 2005, 2009; Wang et al., 2006; Ho et al., 2010), but higher than that of Korea (Oh et al., 2007; Cha et al., 2007; Lee et al., 2009; Yoon et al., 2009). In conclusion, genetic difference, different envi-

Table 1
Composition of the essential oils of *C. fortunei* and *C. japonica*.

No.	Constituents	RI[a]	*C. fortunei* (%)[b]	*C. japonica* (%)[b]
1	Tricyclene	927	0.05	0.12
2	α-Thujene	930	0.12	0.47
3	α-Pinene	939	2.75	8.00
4	Camphene	954	0.39	0.81
5	Sabinene	975	1.07	–
6	β-Pinene	979	0.23	0.66
7	2-Carene	1001	–	0.36
8	α-Phellandrene	1003	–	0.07
9	3-Carene	1011	0.30	0.53
10	α-Terpinene	1018	0.16	0.05
11	p-Cymene	1025	–	0.12
12	β-Phellandrene	1030	–	5.97
13	Limonene	1031	0.72	1.63
14	γ-Terpinene	1060	0.29	0.63
15	Terpinolene	1091	–	0.28
16	Borneol	1169	0.08	0.03
17	Terpinen-4-ol	1180	0.40	0.69
18	Anethol	1262	–	0.01
19	Borneol acetate	1288	0.36	0.51
20	β-Patchoulene	1374	–	0.07
21	Isoledene	1375	–	0.19
22	Copaene	1376	–	0.03
23	β-Maaliene	1380	0.71	–
24	β-Elemene	1392	0.15	5.87
25	Longifolene	1410	0.10	0.46
26	α-Gurjunene	1411	–	0.05
27	β-Caryophyllene	1414	–	0.25
28	γ-Elemene	1423	–	0.22
29	Calarene	1432	0.09	–
30	Aromadendrene	1437	0.25	0.04
31	α-Farnesene	1438	0.42	–
32	α-Caryophyllene	1455	0.06	–
33	α-Humulene	1458	–	0.12
34	C15H24	1459	–	0.08
35	γ-Gurjunene	1470	–	0.15
36	Aristolene	1472	–	0.11
37	γ-Muurolene	1477	0.19	0.18
38	Valencene	1479	–	0.19
39	γ-Selinene	1480	–	0.26
40	β-Selinene	1481	0.26	–
41	Germacrene D	1483	0.45	0.87
42	α-Selinene	1485	–	0.04
43	β-Ionone	1488	–	0.70
44	Alloromadendrene	1496	0.10	–
45	Eremophilene	1502	0.13	–
46	α-Muurolene	1504	0.46	0.33
47	γ-Cadinene	1519	1.92	1.47
48	δ-Cadinene	1525	1.45	1.73
49	α-Amorphene	1530	0.41	0.41
50	Selina-3,7(11)-diene	1547	–	0.04
51	Elemol	1550	–	0.54
52	Germacrene D-4-ol	1576	0.30	1.15
53	Viridiflorol	1590	–	0.29
54	α-Elemol	1625	13.34	20.12
55	γ-Eudesmol	1634	10.80	4.12
56	β-Eudesmol	1654	10.16	5.03
57	α-Eudesmol	1657	–	5.62
58	Farnesol	1700	–	0.02
59	Nerolidol	1715	–	0.30
60	Liriodenine	1780	–	2.72
61	Sandaracopimaradiene	1960	0.44	–
62	Epimanoyl oxide	2012	0.06	–
63	Kaurene	2025	34.04	1.47
64	Kaur-16-ene	2054	0.05	14.84
65	Abietatriene	2059	0.33	0.37
66	Pimaral	2187	0.22	–
67	Cupressene	2200	–	1.16
68	Ferruginol	2334	0.06	0.04
	Identified compound (%)		83.87	92.59
	Montoterpene hydrocarbons		6.08	19.7
	Oxygenated monoterpenes		0.84	1.94
	Sesquiterpene hydrocarbons		7.15	13.16
	Oxygenated sesquiterpenes		34.6	37.19
	Diterpene hydrocarbons		34.86	17.84
	Oxygenated diterpenes		0.34	0.04
	Others		–	2.72
	Oil yield (%, w/w)		0.82	1.15

[a] Kovats index on a HP-5 column in reference to n-alkanes (Van den Dool and Kratz, 1963).
[b] Relative percentage of the identified volatiles based on GC-FID.