

# Analysis of leaf essential oils from the indigenous five conifers of Taiwan

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**ABSTRACT:** The chemical composition of leaf essential oils of five conifers in Taiwan were examined. In *Chamaecyparis formosensis* leaf oil 60 compounds were identified, with  $\alpha$ -pinene (71.6%),  $\delta$ -2-carene (4.6%),  $\beta$ -myrcene (4.1%),  $\gamma$ -muurolene (3.1%),  $\beta$ -pinene (2.7%) and  $\alpha$ -caryophyllene (2.0%) predominating. Forty-four compounds were identified in the leaf oil of *C. obtusa* var. *formosana* with  $\alpha$ -pinene (76.7%),  $\beta$ -myrcene (5.7%),  $\beta$ -pinene (3.2%),  $\gamma$ -muurolene (2.8%),  $\delta$ -2-carene (2.1%) and  $\beta$ -phellandrene (2.1%) the major components. In leaf oil of *Calocedrus formosana*, we identified 81 compounds, with  $\alpha$ -pinene (69.8%),  $\beta$ -caryophyllene (4.7%),  $\beta$ -myrcene (4.3%) and limonene (2.0%) predominating. For the leaf oil of *Taiwania cryptomerioides*, 56 compounds were found, with limonene (44.5%),  $\alpha$ -pinene (14.4%), caryophyllene oxide (8.5%),  $\delta$ -2-carene (3.9%),  $\alpha$ -terpineol acetate (3.6%) and  $\beta$ -myrcene (2.6%) the main components. In leaf oil of *Cunninghamia lanceolata* var. *konishii*, there were 68 identified compounds, with  $\alpha$ -pinene (36.4%),  $\alpha$ -thujene (11.4%),  $\alpha$ -eudesmol (8.1%), elemol (5.8%),  $\beta$ -elemene (3.5%),  $\gamma$ -eudesmol (2.8%) and  $\gamma$ -himachalene (2.7%) the major constituents. All five species had monoterpene hydrocarbons as their main components. Except for *Taiwania*, in which limonene predominated, in all other species  $\alpha$ -pinene was the predominant component. Copyright © 2006 John Wiley & Sons, Ltd.

**KEY WORDS:** leaf essential oils; *Chamaecyparis formosensis*; *Chamaecyparis obtusa* var. *formosana*; *Calocedrus formosana*; *Taiwania cryptomerioides*; *Cunninghamia lanceolata* var. *konishii*

## Introduction

Taiwan is an island off the southeastern Asian mainland, which has floral compositions extending from tropical to alpine climates in vertical distribution. The diverse floral resources represent a veritable treasure house for the study of natural chemical products. The term 'five conifers of Taiwan' originated in the Meiji era during the Japanese occupation of the island (1895–1945), and referred to certain valuable timber species endemic to the island. In the first year of Daisho (1912), an English scholar, Elwis, studied the flora of Taiwan, and found that the Taiwan red false-cypress (*Chamaecyparis formosensis* Matsum.), Taiwan hinoki false-cypress [*C. obtusa* (Sieb. & Zucc) Endl. var. *formosana* (Hayata) Rehder], Taiwan incense-cedar (*Calocedrus formosana* Florin), Taiwan fir (*Taiwania cryptomerioides* Hayata) and Konishi China-fir [*Cunninghamia lanceolata* (Lamb.) Hook. var. *konishii* (Hayata) Fujita] were very special trees, and in addition to possessing high economic value, they have special significance in terms of botany, geological history, ecology and natural products. Hence the five species of conifers were designated the five most-

valuable conifers of Taiwan, or simply the five precious conifers or five conifers of Taiwan.

The first three species on the list belong to the family Cupressaceae; with the first two in the genus *Chamaecyparis*, and the third species belonging to the genus *Calocedrus*. The other two species are in the family Taxodiaceae and respectively belong to the *Taiwania* and *Cunninghamia* genera. *Chamaecyparis formosensis* has thin bark and pale-pinkish-colored wood, and is distributed in mid-elevation montane forests from 1500 to 2150 m above sea level in Taiwan. *C. obtusa* var. *formosana*, on the other hand, has thicker bark and pale-yellowish wood, and is distributed in Taiwan's northern and central montane forests between the elevations of 1300 and 2800 m. The entire *Chamaecyparis* genus comprises six species and one variety. In addition to the above two species, there are *C. obtusa* and *C. pisifera* of Japan and *C. lawsoniana*, *C. nootkensis* and *C. thyoides* of North America. *Calocedrus* is a relict species left over from a past geological period. There are a total of three species in the genus, distributed in North America, China and Taiwan, respectively. Taiwan incense-cedar is endemic to Taiwan and is distributed in the northern and central montane forests between 500 and 900 m elevation.<sup>1</sup> Taiwan fir, an evergreen tree, is also endemic to Taiwan, and was the only species named after the island. The species greatly resembles Japanese cedar (*Cryptomeria japonica*), hence the species name

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'*cryptomerioides*.' The tree, together with the ginkgo (*Ginkgo biloba*), *Sequoiadendron giganteum* and *Metasequoia glyptostroboides* are relict species of the last Ice Age, and are globally rare and precious species.<sup>1</sup> It is distributed between 1300 and 2600 m. Konishi China-fir is also an indigenous species of Taiwan. It is an ever-green tree whose wood has a strong fragrance, hence it is called fragrant China-fir in the local market. The species is distributed in northern and central forests at 1300–2000 m. All five species are class I conifers in Taiwan, which means that they possess excellent wood quality and wide applications, such as construction, furniture, coffins, vehicles, carving, etc. The leaf and wood essential oils have multiple biological activities such as antibacterial,<sup>2,3</sup> antifungal,<sup>4,5</sup> anti-mite,<sup>6</sup> anti-termite,<sup>5,7</sup> etc. However, we found few reports dealing with the compositions of the essential oils or the active components of the essential oils. Thus, in this report, we present the results of separating hydrodistilled leaf essential oils from the five species, identifying individual components and determining their contents using GC and GC-MS analyses and evaluations. As for the evaluation of the active ingredients from the essential oils, we shall report those results in another paper.

## Experimental

### Plant materials

In the study, we obtained the leaves of five conifers: *Chamaecyparis formosensis* Matsum., *Chamaecyparis obtusa* (Sieb. & Zucc) Endl. var. *formosana* (Hayata) Rehder, *Calocedrus formosana* Florin, *Taiwania cryptomerioides* Hayata, and *Cunninghamia lanceolata* (Lamb.) Hook. var. *konishii* (Hayata) Fujita from various locales. The *C. formosensis* leaves were collected from Chilan Mt in northeast Taiwan; *C. obtusa* var. *formosana* and *T. cryptomerioides* leaves were collected from the Hsin-sheng Nursery near Taipei, while *C. formosana* and *C. lanceolata* var. *konishii* leaves were from the Lienhuachih Research Center of the Taiwan Forestry Research Institute (TFRI). All trees sampled were aged about 22 years.

Fresh leaves shipped to the Taipei headquarters of the TFRI were hydrodistilled to extract the leaf essential oils. The procedure entailed placing 1 kg of fresh leaves of each species in a large round-bottom flask, adding 3 l of distilled water, then flash heating this for 8 h to obtain the essential oil fraction on top of the recondensed water. Anhydrous sodium sulfate was then added to the oils to remove traces of water. The essential oils thus obtained were placed in specimen vials for yield determination. All test data are the average of triplicate analyses.

### GC and GC-MS analysis

A Hewlett-Packard HP6890 gas chromatograph equipped with a DB-5 fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness, J&W Scientific) and an FID detector

was used for the percentage determination of oil composition. Oven temperature was programmed as follows: 50 °C for 2 min, rising to 250 °C at 5 °C/min; injector temperature 270 °C; and carrier gas He with a flow rate of 1 ml/min. The detector temperature was 250 °C. The split ratio was 1:10; and 1 µl of sample was injected. Identification of the oil components was based on their retention indices and mass spectra, obtained from the GC-MS analysis on a Hewlett-Packard HP6890/HP5973 equipped with a DB-5 fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness, J&W Scientific). The GC analysis parameters are the same as the ones listed above, and the MS was operated (full-scan mode) in the EI mode at 70 eV. All test data are the average of triplicate analyses.

### Component identification

The identification of the chemical constituents was based on comparisons of their Kovats indices (KI),<sup>8</sup> their retention times (RT) and mass spectra with those obtained from authentic standards and/or the NIST and Wiley libraries spectra and literature.<sup>9,10</sup>

## Results and discussion

### Yields of leaf essential oils

The leaf essential oil yields after hydrodistillation for *C. formosensis*, *C. obtusa* var. *formosana*, *C. formosana*, *T. cryptomerioides* and *C. lanceolata* var. *konishii* were respectively 0.69 ± 0.06, 0.43 ± 0.07, 0.63 ± 0.03, 0.06 ± 0.01 and 0.46 ± 0.05 ml/100 g o.d. weight of the leaves. Among the species, *T. cryptomerioides* had the lowest leaf essential oil yield, while *C. formosensis* had the highest.

### Compositions and contents of leaf essential oils

The compositions and contents of individual components of the leaf essential oils of the five conifers are shown in Table 1.

#### *Composition and contents of leaf essential oil of Chamaecyparis formosensis*

From the leaf essential oil of *C. formosensis* we separated 60 compounds, with  $\alpha$ -pinene predominant and accounting for 71.6% of the total. Then sequentially, there were  $\delta$ -2-carene,  $\beta$ -myrcene,  $\gamma$ -muurolene,  $\beta$ -pinene,  $\alpha$ -caryophyllene,  $\beta$ -phellandrene,  $\delta$ -cadinene, kaur-16-ene, etc. All identified components were separated into the five categories of monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and diterpenes. Upon integrating the areas of the individual categories, we found that monoterpene hydrocarbons accounted for the largest fraction at 86.2%,

**Table 1.** Chemical composition of the essential oils of the five most valuable conifers in Taiwan

Peak no.	Constituent	KI <sup>a</sup>	Concentration (%)					Identification <sup>b</sup>
			I	II	III	IV	V	
1	Tricyclene	927	0.1	0.1	0.2	0.1	0.1	MS, KI, ST
2	$\alpha$ -Thujene	930	t <sup>c</sup>	t	0.3	0.1	11.4	MS, KI, ST
3	$\alpha$ -Pinene	939	71.6	76.7	69.8	14.4	36.4	MS, KI, ST
4	Camphene	954	0.5	0.5	0.5	0.4	0.4	MS, KI, ST
5	Verbenene	968	— <sup>d</sup>	—	t	0.1	t	MS, KI
6	Sabinene	975	—	t	1.1	0.2	1.0	MS, KI, ST
7	1-Octen-3-ol	979	0.1	0.1	t	0.3	t	MS, KI
8	$\beta$ -Pinene	979	2.7	3.2	2.0	0.4	1.2	MS, KI, ST
9	$\beta$ -Myrcene	991	4.1	5.7	4.3	2.6	2.4	MS, KI, ST
10	$\delta$ -2-Carene	1002	4.6	2.1	0.4	3.9	1.6	MS, KI, ST
11	$\alpha$ -Terpinene	1017	t	—	0.2	t	0.2	MS, KI, ST
12	<i>p</i> -Cymene	1025	0.1	0.1	0.4	0.4	1.5	MS, KI, ST
13	Limonene	1029	—	—	2.0	44.5	—	MS, KI, ST
14	$\beta$ -Phellandrene	1030	1.8	2.1	—	—	1.8	MS, KI, ST
15	1,8-Cineole	1031	t	—	t	0.4	—	MS, KI, ST
16	<i>trans</i> - $\beta$ -Ocimene	1050	t	t	t	—	t	MS, KI, ST
17	$\gamma$ -Terpinene	1060	t	—	0.3	0.2	0.5	MS, KI, ST
18	<i>iso</i> -Terpinolene	1088	t	—	—	—	—	MS, KI
19	Terpinolene	1089	0.8	0.4	0.8	0.1	0.6	MS, KI, ST
20	<i>p</i> -Cymenene	1091	—	—	0.1	0.1	0.1	MS, KI
21	Linalool	1097	—	—	0.1	1.4	t	MS, KI, ST
22	Perillene	1101	—	—	0.1	—	0.1	MS, KI
23	endo-Fenchol	1117	—	—	t	—	—	MS, KI, ST
24	$\alpha$ -Campholenal	1126	—	—	t	—	0.1	MS, KI
25	1-Terpineol	1134	—	—	—	—	t	MS, KI
26	<i>trans</i> -Pinocarveol	1139	—	—	t	—	0.1	MS, KI, ST
27	Camphor	1146	0.2	0.2	0.1	0.6	t	MS, KI, ST
28	Citronellal	1153	—	—	—	0.2	—	MS, KI, ST
29	$\alpha$ -Phellandren-8-ol	1170	—	—	0.1	0.1	—	MS, KI
30	4-Terpineol	1177	t	t	1.1	0.3	2.2	MS, KI, ST
31	<i>p</i> -Cymen-8-ol	1183	—	t	0.1	0.1	0.2	MS, KI, ST
32	$\alpha$ -Terpineol	1189	0.3	0.2	0.6	0.5	0.7	MS, KI, ST
33	n-Decanal	1202	—	—	0.1	—	—	MS, KI
34	Verbenone	1205	—	—	t	—	0.1	MS, KI
35	Endo-fenchyl acetate	1220	—	—	0.5	—	—	MS, KI
36	Carvacrol, methyl ether	1245	—	—	t	0.1	t	MS, KI, ST
37	Methyl citronellate	1261	—	—	t	0.1	—	MS, KI
38	Methyl nerolate	1283	—	—	0.1	0.3	t	MS, KI
39	Bornyl acetate	1289	0.4	0.3	0.9	0.3	0.1	MS, KI, ST
40	<i>trans</i> -Sabinyl acetate	1291	—	—	t	—	—	MS, KI
41	Terpinen-4-ol acetate	1300	—	—	0.1	0.1	t	MS, KI
42	Methyl geranate	1325	—	—	0.1	0.1	—	MS, KI, ST
43	$\delta$ -Elemene	1338	0.1	0.1	t	—	0.4	MS, KI, ST
44	$\alpha$ -Terpinyl acetate	1349	0.4	0.3	—	3.6	0.3	MS, KI, ST
45	$\alpha$ -Cubebene	1351	—	—	0.3	—	—	MS, KI, ST
46	Neryl acetate	1362	t	—	—	—	t	MS, KI, ST
47	$\alpha$ -Ylangene	1375	t	—	—	—	t	MS, KI, ST
48	$\alpha$ -Copaene	1377	0.1	t	0.1	0.2	0.1	MS, KI, ST
49	Geranyl acetate	1381	t	—	—	—	—	MS, KI, ST
50	$\beta$ -Bourbonene	1388	—	—	t	—	—	MS, KI
51	$\beta$ -Elemene	1391	0.1	0.1	0.4	0.1	3.5	MS, KI, ST
52	$\beta$ -Longipinene	1401	—	—	—	0.8	—	MS, KI
53	Methyl eugenol	1404	—	—	t	—	—	MS, KI, ST
54	$\alpha$ -Cedrene	1412	0.1	0.2	0.1	—	0.2	MS, KI, ST
55	$\beta$ -Caryophyllene	1419	0.5	0.3	4.7	1.3	1.2	MS, KI, ST
56	$\beta$ -Cedrene	1421	0.1	0.1	0.1	—	0.1	MS, KI, ST
57	$\beta$ -Copaene	1432	0.1	0.1	0.1	—	0.5	MS, KI, ST
58	<i>cis</i> - $\alpha$ -Ambrinol	1441	0.1	0.1	t	—	t	MS, KI
59	Bakerol	1446	0.2	—	—	—	0.1	MS, KI
60	<i>cis</i> -Murola-3,5-diene	1450	t	0.2	t	—	—	MS, KI
61	$\alpha$ -Caryophyllene	1455	2.0	0.3	0.4	0.3	0.5	MS, KI, ST
62	9-epi-( $\beta$ )-Caryophyllene	1466	0.2	0.1	0.1	—	—	MS, KI, ST
63	$\beta$ -Acoradiene	1471	—	—	0.2	—	—	MS, KI
64	<i>trans</i> -Cadina-1(6),4-diene	1477	0.1	—	0.1	—	—	MS, KI
65	$\gamma$ -Muurolene	1480	3.1	2.8	1.9	0.1	0.8	MS, KI, ST
66	$\gamma$ -Himachalene	1483	0.1	t	0.1	—	2.7	MS, KI
67	Germacrene D	1485	0.2	0.2	0.2	0.4	—	MS, KI, ST

Table 1. (Continued)

Peak no.	Constituent	Kl <sup>a</sup>	Concentration (%)					Identification <sup>b</sup>
			I	II	III	IV	V	
68	<i>cis</i> - $\beta$ -Guaiene	1493	0.1	0.1	0.2	—	2.2	MS, KI
69	$\alpha$ -Muurolene	1500	0.3	0.2	0.3	—	—	MS, KI, ST
70	<i>trans</i> - $\beta$ -Guaiene	1503	0.1	—	—	—	—	MS, KI, ST
71	$\beta$ -Himachalene	1505	—	—	—	—	0.1	MS, KI
72	$\beta$ -Bisabolene	1506	—	—	0.1	—	0.2	MS, KI, ST
73	$\gamma$ -Cadinene	1514	0.4	0.3	0.2	0.2	0.2	MS, KI, ST
74	$\delta$ -Cadinene	1523	1.1	0.8	0.7	2.6	0.5	MS, KI, ST
75	<i>trans</i> -Calamenene	1529	0.1	—	0.1	0.5	—	MS, KI, ST
76	<i>trans</i> -Cadina-1(2),4-diene	1535	t	—	t	0.2	0.1	MS, KI
77	$\alpha$ -Cadinene	1539	t	t	t	—	0.1	MS, KI, ST
78	$\alpha$ -Calacorene	1546	t	—	—	0.2	0.1	MS, KI, ST
79	Elemol	1550	t	—	0.2	0.2	5.8	MS, KI, ST
80	Germacrene B	1561	—	—	0.1	—	1.8	MS, KI
81	( <i>E</i> )-Nerolidol	1563	—	t	0.1	0.1	—	MS, KI, ST
82	Spathulenol	1578	—	—	t	0.1	—	MS, KI, ST
83	Caryophyllene oxide	1583	—	—	0.5	8.5	0.3	MS, KI, ST
84	Globulol	1585	—	—	t	—	t	MS, KI, ST
85	Humulene epoxide ?	1608	t	—	0.1	0.8	0.1	MS, KI
86	1,10-di-epi-Cubenol	1619	t	—	t	0.1	—	MS, KI
87	10-epi- $\gamma$ -Eudesmol	1624	—	—	t	—	0.2	MS, KI
88	1-epi-Cubenol	1629	t	—	0.1	2.3	—	MS, KI
89	$\gamma$ -Eudesmol	1632	—	—	0.1	0.1	2.8	MS, KI, ST
90	$\tau$ -Cadinol	1640	0.5	0.3	0.3	1.3	0.3	MS, KI
91	$\tau$ -Muurolol	1642	t	0.3	—	—	0.1	MS, KI
92	$\delta$ -Cadinol	1646	t	—	0.1	0.2	0.2	MS, KI, ST
93	$\alpha$ -Eudesmol	1654	—	—	—	—	8.1	MS, KI, ST
94	$\alpha$ -Cadinol	1654	0.3	0.2	0.6	0.7	—	MS, KI, ST
95	Intermedeol	1667	—	—	—	0.1	0.3	MS, KI
96	14-Hydroxy-9-epi-( <i>E</i> )-caryophyllene	1670	—	—	—	0.9	—	MS, KI
97	Benzyl benzoate	1760	—	—	0.1	—	0.1	MS, KI
98	Sandaracopimara-8(14),15-diene	1969	t	—	—	—	—	MS, KI
99	Kaur-15-ene	1998	0.1	t	—	—	—	MS, KI
100	Kaurene	2043	1.0	1.0	0.2	—	t	MS, KI
<i>Grouped components</i>								
Monoterpene hydrocarbons (%)			86.2	90.8	82.2	67.2	58.9	
Oxygenated monoterpenes (%)			1.4	1.1	4.1	8.3	3.9	
Sesquiterpene hydrocarbons (%)			8.8	5.8	10.4	6.7	15.2	
Oxygenated sesquiterpenes (%)			1.2	0.8	2.1	15.3	18.3	
Diterpenes (%)			1.1	1.1	0.2	—	t	
Others (%)			0.1	0.1	0.4	0.2	0.2	
Total identified (%)			98.8	99.7	99.3	97.6	96.6	
Oil yield (ml/100 g)			0.69 $\pm$ 0.06	0.43 $\pm$ 0.07	0.63 $\pm$ 0.03	0.06 $\pm$ 0.01	0.46 $\pm$ 0.05	

I, *Chamaecyparis formosensis*; II, *Chamaecyparis obtusa* var. *formosana*; III, *Calocedrus formosana*; IV, *Taiwania cryptomerioides*; V, *Cunninghamia lanceolata* var. *konishii*.

<sup>a</sup> Kovats index on a DB-5 column in reference to n-alkanes<sup>8</sup>

<sup>b</sup> MS, NIST and Wiley libraries spectra and the literature; KI, Kovats index; ST, Authentic standard compounds.

<sup>c</sup> t, trace, <0.1%.

<sup>d</sup> —, Not detected.

followed by sesquiterpene hydrocarbons, oxygenated monoterpenes, oxygenated sesquiterpenes and diterpenes.

There are several reports on the compositions of the root, bark, wood, pericarps and leaves of *C. formosensis*.<sup>11–21</sup> There are few reports on the leaf essential oil of *C. formosensis*. In 1931, Kafuku and Ichikawa<sup>11,12</sup> were the first to extract its essential oil using hydrodistillation and to analyze its composition. They found that  $\alpha$ -pinene predominated at 85%. Other compounds they found included camphene, dipentene, cineol,  $\alpha$ -terpinene,  $\beta$ -terpinene, borneol, bornyl acetate, bornyl formate, humulene and cadinene. In 1986, Fang

*et al.*<sup>19</sup> extracted the leaf essential oils of the species and obtained 18 monoterpenes, 14 sesquiterpenes and three diterpenes. They also found  $\alpha$ -pinene as the main ingredient, making up ca. 57% of the total. Other compounds they found included 3-carene,  $\beta$ -pinene,  $\alpha$ -terpineol,  $\gamma$ -muurolene and kaurene. Comparing the results in the literature, all studies indicated that  $\alpha$ -pinene was the main ingredient. We identified far more components than were included in those two studies. However, several compounds were first discovered by us to be associated with the species, including  $\beta$ -myrcene,  $\delta$ -2-carene,  $\beta$ -phellandrene, terpinolene, camphor,  $\alpha$ -terpineol acetate,

$\delta$ -elemene,  $\alpha$ -cedrene,  $\beta$ -cedrene,  $\alpha$ -caryophyllene,  $\alpha$ -muurolene,  $\delta$ -cadinene and  $\tau$ -cadinol.

#### *Composition and contents of the leaf essential oil of Chamaecyparis obtusa var. formosana*

In total, 44 compounds were identified from the leaf essential oil of *C. obtusa* var. *formosana*. Again,  $\alpha$ -pinene was the predominant component, accounting for 76.7% of the total. It was followed by  $\beta$ -myrcene,  $\beta$ -pinene,  $\gamma$ -muurolene,  $\delta$ -2-carene,  $\beta$ -phellandrene, kaur-16-ene and  $\delta$ -cadinene. Monoterpene hydrocarbons made up 90.8% of the total, followed by sesquiterpene hydrocarbons, oxygenated monoterpenes, diterpenes and oxygenated sesquiterpenes.

There are a few reports on the chemical composition of this species.<sup>22–24</sup> Kafuku *et al.*<sup>22</sup> and Kafuku and Nozone<sup>23</sup> studied the leaf essential oil of this species. In their reports, they mentioned that the acidic fraction had a main component of C<sub>10</sub>H<sub>16</sub>O<sub>2</sub> and hinokic acid, while the remaining components were caproic acid and phenolic compounds. As for monoterpenes, they found D-sabinene, chamene, etc. Other components they identified included D- $\alpha$ -pinene, *p*-cymene,  $\alpha$ -terpinene,  $\gamma$ -terpinene,  $\alpha$ -thujene, and a trace of dipentene. The fraction of terpene alcohols included D-4-terpineol, L-linalool, borneol, etc. The sesquiterpenes contained D-cadinene, and the sesquiterpene alcohol fraction had D-cadinol. Compared with our results, their main components differed. Many of the compounds identified in our study were not previously known to exist in the leaf essential oil of this species, such as  $\beta$ -myrcene,  $\delta$ -2-carene,  $\beta$ -phellandrene,  $\gamma$ -muurolene and kaur-16-ene.

#### *Composition and contents of the leaf essential oil of Calocedrus formosana*

For the leaf essential oil of *C. formosana*, we identified 81 compounds, with  $\alpha$ -pinene predominant at 69.8%. It was followed by  $\beta$ -caryophyllene,  $\beta$ -myrcene, limonene,  $\gamma$ -muurolene, sabinene, 4-terpineol, bornyl acetate,  $\delta$ -cadinene,  $\alpha$ -cadinol and caryophyllene oxide. Among the compounds, monoterpene hydrocarbons accounted for 82.2% of the total, followed by sesquiterpene hydrocarbons, oxygenated monoterpenes, oxygenated sesquiterpenes and diterpenes.

There are a limited number of previous studies on the chemical composition of the species.<sup>25–29</sup> Lin<sup>26</sup> extracted the leaf essential oil and identified  $\beta$ -caryophyllene as having the highest content (25.5%), followed by limonene (21.2%),  $\alpha$ -pinene (6.3%) and 22 other compounds. Cheng *et al.*<sup>5</sup>, however, identified 19 terpenoids from the leaf essential oil of the species with  $\alpha$ -pinene the highest at 44.2%, followed by limonene (21.6%),  $\beta$ -myrcene (8.9%) and  $\beta$ -caryophyllene (8.2%). Compared with our results, the compositions differed based on dif-

ferent studies. The differences could have arisen from differences in the chemotype, location and site factors. Overall, we found more compounds than the other studies, such as sabinene, endo-fenchyl acetate, bornyl acetate,  $\alpha$ -cubebene,  $\beta$ -elemene and kaur-16-ene.

#### *Composition and contents of the leaf essential oil of Taiwania cryptomerioides*

In total, 56 compounds were identified from the leaf essential oil of *T. cryptomerioides*, with limonene predominant, which made up 44.5% of the total. Other compounds in the order of abundance were  $\alpha$ -pinene, caryophyllene oxide,  $\delta$ -2-carene,  $\alpha$ -terpineol acetate,  $\beta$ -myrcene,  $\delta$ -cadinene, 1-epi-cubenol,  $\tau$ -cadinol and  $\beta$ -caryophyllene. Monoterpene hydrocarbons had the highest proportion at 67.2%, followed by oxygenated sesquiterpenes, oxygenated monoterpenes and sesquiterpene hydrocarbons, with no trace of diterpenes.

Prior studies on the chemical composition of *T. cryptomerioides* included several reports.<sup>3,4,30–37</sup> Among these, Kato<sup>32,33</sup> was the first to study the leaf essential oil of the species, and found  $\alpha$ -pinene, camphene, limonene, cadinane type and  $\tau$ -cadinol compounds in the leaf essential oil. Chang *et al.*<sup>3,4</sup> studied the cadinane-type compounds, i.e.  $\tau$ -cadinol,  $\tau$ -muurolol, and  $\alpha$ -cadinol, from the essential oil. None of the other compounds had previously appeared in the literature. Thus our results represent the first report on the detailed composition of the leaf essential oil for this species.

#### *Composition and contents of the leaf essential oil of Cunninghamia lanceolata var. konishii*

From the leaf essential oil of *C. lanceolata* var. *konishii*, we identified 68 compounds. The main components in the order of abundance were  $\alpha$ -pinene,  $\alpha$ -thujene,  $\alpha$ -eudesmol, elemol,  $\beta$ -elemene,  $\gamma$ -eudesmol,  $\gamma$ -himachalene,  $\beta$ -myrcene, *cis*- $\beta$ -guaiene, 4-terpineol,  $\delta$ -2-carene, *p*-cymene,  $\beta$ -caryophyllene and  $\beta$ -pinene. Monoterpene hydrocarbons had the highest fraction at 58.9%, followed by oxygenated sesquiterpenes, sesquiterpene hydrocarbons, oxygenated monoterpenes and diterpenes. We failed to find any literature reference to the leaf essential oil of this species, hence the report represents the first publication on the leaf essential oil composition of this species.

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