Chemical composition of the essential oil from Cunninghamia konishii Hayata growing wild in Vietnam.

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Abstract
The chemical composition of one oil sample of Cunninghamia konishii Hayata isolated from wood harvested in Hà Giang province (Vietnam), was investigated by chromatographic (GC with retention indices) and spectroscopic techniques (MS, 13C NMR). Forty six compounds accounting for 88.4% of the oil have been identified. C. konishii wood produced sesquiterpene-rich essential oil, whose composition was dominated by α-cedrene (18.4%), cedrol (11.2%) and γ-eudesmol (8.8%). α-Terpineol (9.6%) was the major monoterpen. 13C NMR appeared really useful for the identification of β-funebrene which co-eluted with α-cedrene and sesquithuriferol which co-eluted with cedrol.

Keywords: Cunninghamia konishii, wood oil, chemical composition, 13C NMR, Vietnam.

1. Introduction
Cunninghamia genus (Cupressaceae family) comprises two species native from East Asia (Taiwan and China): Cunninghamia lanceolata (Lamb.) and Cunninghamia konishii Hayata [syn. Cunninghamia lanceolata (Lamb.) Hook. var. konishii (Hay.) Fujita; Cunninghamia kawakamii Hayata]. Cunninghamia konishii is an endemic species to Taiwan, growing at altitudes from 1200 m to 2700 m. Recently, this species was discovered in the North of Laos. In Vietnam, specimens have been observed in HàGiang, Thanh Hóa, Sơn La and Nghê An provinces, in dense subtropical forest, mixed with Fokienia hodginsii, Dacrydium pierrei, and Quercus bambusaeformis [2]. In Vietnam, its vernacular name is Sa mọcđau. C. konishii is an abig tree, reaching 50 m height and 1.25 m in diameter with conical, dark green crown. Brown-red bark splits into thin scales. Leaves, 2-3 cm long and 0.5 cm wide, are densely concentrated at branch-tips. Male cones numerous, terminal, are enclosed by many green scales at the base. Female cones, 2-2.5 cm by 1.3 cm, globose or ovate, terminal, first green, later brown-reddish, consist of many scale-shaped carpels. Each carpel bears three winged seeds. This species differs from C. lanceolata by shorter leaves (2-3 cm), obtuse apex and smaller female cone (less than 3 cm in diameter).

C. konishii is considered as one of the five “precious” conifers in Taiwan. Its wood is used in the construction of houses. It is resistant to rot and termites. This species is considered as endangered due to over-exploitation and possible hybridization with C. lanceolata, introduced in plantations.

Phytochemicals belonging to various families of compounds have been isolated from solvent extracts of wood of C. konishii, including new compounds whose structures have been elucidated: various sesquiterpenes [3-5] such as konishiol, cadalenol, 3-cedranol, T-cadinol, cedrol, T-muurolol, and epi-cedrol among which cedrol, T-cadinol and T-muurolol may be considered as new natural antifungal agents [6]; numerous diterpenes bearing mostly the abietane or labdane skeleton [5-11]; sterols such as β-sitosterol and stigmasterol [3, 6], and various lignans [1, 3].

In comparison, only a few studies concerned the composition of essential oils. According to Cheng et al. [12], a pioneering work has been done as early as 1929 by Ikeda and Fujita who identified various monoterpenes and cedrol in wood oil of C. konishii. Recently, these
authors \[12, 13\] reported a composition dominated by cedrol (53.0%) and \(\alpha\)-pinene (25.6%). \(\alpha\)-cedrene (6.6%) and \(\alpha\)-terpineol (4.4%) being present at substantial amounts. Wood oil of \textit{C. konishii} and cedrol are effective as antiungal agents. Other results revealed that the same wood oil sample from \textit{C. konishii} and its effective constituents served as a potential larvicide against the dengue mosquitoes. \[13\] A more or less similar composition has been reported for EO isolated from heart wood: cedrol (58.3%), \(\alpha\)-cedrene (11.8%), \(\alpha\)-terpineol (4.2%) and \(\beta\)-cedrene (3.5%) \[14\]. Antimicrobial activity and cytotoxic activity on human cancer cells have been evidenced. The active compound was determined to be cedrol. In contrast, monoterpenes were the main components of leaf oils. Indeed, \(\alpha\)-pinene (36.4%) and \(\alpha\)-thujene (11.4%) dominated the composition of Taiwanese leaf oil, accompanied by sesquiterpenes, \(\alpha\)-eusdesmol (8.1%), \(\beta\)-elemol (5.8%), \(\beta\)-elemene (3.5%), \(\gamma\)-eudesmol (2.8%) and \(\gamma\)-himachalene (2.7%).\[15\]. In a second sample of Taiwanese origin, \(\alpha\)-pinene (34.9%), \(p\)-cymene (16.7%) and \(\gamma\)-maurouene (11.4%) were the main components. \[12\] This leaf oil sample exhibited antifungal activities. Finally, two studies concerned Vietnamese \textit{C. konishii} wood oil. The composition of two oil samples isolated from plants harvested in Sơn La and Nghệ An provinces, respectively, was dominated by cedrol (30.0% and 37.0%) while \(\alpha\)-cedrene accounted for 4.5% and 5.3%, respectively. The two samples contained also a major component named \(\beta\)-penchyl alcohol (synonym, \(\beta\)-fenchyl alcohol, 16.1% and 27.5%, respectively) \[16\]. The third oil sample isolated from wood of \textit{C. konishii} collected in Tây Con Linh Nature Reserve, Hà Giang province, differed drastically from the two former samples by the occurrence of \(\alpha\)-terpineol (36.6%) and \(\alpha\)-cedrol (29.8%) as major components and it was reported as a new chemotype. In contrast, \(\beta\)-fenchyl alcohol was not detected \[17\]. In continuation of our on-going work on the characterization of essential oil bearing plants from Vietnam, we report in the present study on the chemical composition of wood oil of \textit{C. konishii}. Indeed, since previous works demonstrated that wood oil from Sơn La and Nghệ An provinces exhibited similar composition which differed drastically from that of wood oil from Hà Giang province, we investigated the composition of wood oil from Thanh Hóa province, the fourth location where \textit{C. konishii} grows wild in Vietnam. In order to avoid any misidentification of individual components which exhibit insufficiently resolved mass spectra (diastereoisomers for instance) or very close retention indices, qualitative and quantitative analysis of the oil sample has been carried out by combination of chromatographic (GC with retention indices) and spectroscopic techniques (MS, \(^{13}\)C NMR).

2. Materials and methods
2.1 Plant Material and Essential Oil Production
Wood of \textit{Cunninghamia konishii} was collected in April 2012 in Xuan Lien Nature Reserve, Thanh Hóa province (Vietnam). Wood (550g) was submitted to hydrodistillation in a Clevenger-type apparatus (4 h) and yielded 2.65 mL of EO. Plant material has been authenticated by one of the authors, Dr Nguyen Thi Ha, Vietnam National University. Voucher specimen has been deposited at the herbarium of IERUB under the accession Thai N° 06 (HN).

2.2 Analytical GC
GC analysis was carried out using a Clarus 500 Perkin Elmer apparatus equipped with FID and two fused-silica capillary columns (50 m x 0.22 mm, film thickness 0.25 µm), BP-1 (polydimethylsiloxane) and BP-20 (polyethylene glycol). The oven temperature was programmed from 60 °C to 220 °C at 2 °C/min and then held isothermal at 220 °C for 20 min; injector temperature: 250 °C; detector temperature: 250 °C; carrier gas: helium (0.8 mL/min); split: 1/60. The relative proportions of the oil constituents were expressed as percentage obtained by peak area normalization, without using correction factors. Retention indices (RI) were determined relative to the retention times of a series of \(n\)-alkanes with linear interpolation (« Target Compounds » software from Perkin Elmer).

2.3 GC-MS Analysis
GC-MS analyses were carried out using a Agilent Technologie 7890A detector (quadrupole), directly coupled to a Agilent Technologie 5975C, equipped with a fused-silica capillary column (30 m x 0.25 mm i.d., film thickness 0.25 µm), HP-MS 5% phenylmethylsiloxane. Carrier gas, helium at 1 mL/min; split, 1:80; injection volume, 0.2 µL. The injection port was set at 250 °C; the oven temperature was programmed from 60 °C to 250 °C at 4 °C/min (52.5 min). Significant quadrupole MS operating parameters: Ion source temperature, 150 °C; electron impact ionization at 70 eV with scan mass range of 33-350 m/z.

2.4 \(^{13}\)C NMR Analysis
NMR spectra were recorded on a Bruker AVANCE 400 Fourier Transform spectrometer operating at 100.63 MHz for \(^{13}\)CMR, equipped with a 5 mm probe, in CDCl\(_3\), with all shifts referred to internal TMS. \(^{13}\)CMR spectra of the oil samples were recorded with the following parameters: pulse width = 4 µs (flip angle 45°); acquisition time = 2.7 s for 128K data table with a spectral width of 25 000 Hz (250 ppm); CPD mode decoupling; digital resolution = 0.183 Hz/pt. The number of accumulated scans was 3000 (50 mg of oil in 0.5 mL of CDCl\(_3\)).

2.5 Identification of Components
Identification of the individual components was based: (i) by comparison of their GC retention indices (RIs) on polar and apolar columns, determined relative to the retention times of a series of \(n\)-alkanes with linear interpolation with those of reference compounds (ii) on computer search using digital libraries of mass spectral data and comparison with literature \[18-23\], (iii)on comparison of the signals in the \(^{13}\)C NMR spectra of essential oils with those of reference spectra compiled in the laboratory spiral library, with the help of a laboratory-made software. In the investigated samples individual components were identified by NMR at content as low as 0.3-0.4% \[24-27\].

3. Results & Discussion
Subjected to hydrodistillation, wood of \textit{Cunninghamia konishii} furnished a pale yellow oil with a yield of 0.48% (v/w). The wood oil sample was analyzed by combination of chromatographic (GC/retention indices) and spectroscopic techniques, GC-MS and \(^{13}\)C NMR following a computerized
method developed in our laboratory [24-27]. Forty six compounds, accounting for 88.4% of the whole composition have been identified (Table 1). All the main components have been identified by the three techniques. Most minor components have been identified by GC(RI) and GC-MS. A few minor components that co-eluted on the capillary column used to perform GC-MS analysis, have been identified by their RIs and by $^{13}$C NMR.

The investigated sample of *C. konishii* wood oil may be considered quantitatively as a sesquiterpene-rich essential oil. Indeed, the identified monoterpene hydrocarbons accounted for only 3.3% of the whole composition. Oxygenated monoterpens were present at higher content, 21.3%, with α-terpineol (9.6%) and borneol (4.4%) as main monoterpenols. Sixteen sesquiterpene hydrocarbons have been identified, identification of most of them being ensured by $^{13}$C NMR. Sixteen sesquiterpene hydrocarbons have been identified, identification of most of them being ensured by $^{13}$C NMR. 

<table>
<thead>
<tr>
<th>N°</th>
<th>Components</th>
<th>RI lit$^a$</th>
<th>RIa$^b$</th>
<th>Rlp$^c$</th>
<th>% Identification</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>α-Thujene</td>
<td>932</td>
<td>924</td>
<td>1028</td>
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<td>2</td>
<td>α-Pinene</td>
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<td>α-Fenchene</td>
<td>941</td>
<td>943</td>
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<tr>
<td>4</td>
<td>Camphene</td>
<td>950</td>
<td>945</td>
<td>1072</td>
<td>0.3 RI, MS</td>
</tr>
<tr>
<td>5</td>
<td>Sabine</td>
<td>973</td>
<td>966</td>
<td>1125</td>
<td>0.5 RI, MS, $^{13}$C NMR</td>
</tr>
<tr>
<td>6</td>
<td>β-Pinene</td>
<td>978</td>
<td>972</td>
<td>1114</td>
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<tr>
<td>7</td>
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<td>982</td>
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<tr>
<td>8</td>
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<td>1012$^a$</td>
<td>1005</td>
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<tr>
<td>9</td>
<td>p-Cymene</td>
<td>1015</td>
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<td>β-Phellandrene</td>
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<tr>
<td>13</td>
<td>Fenchone</td>
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<td>1069</td>
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<td>14</td>
<td>Fenchol</td>
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<tr>
<td>15</td>
<td>Terpin-3-en-1-ol</td>
<td>-</td>
<td>1118</td>
<td>nd</td>
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<td>16</td>
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</tr>
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<td>1162</td>
<td>1843</td>
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</tr>
<tr>
<td>21</td>
<td>α-Terpineol</td>
<td>1176</td>
<td>1174</td>
<td>1692</td>
<td>9.6 RI, MS, $^{13}$C NMR</td>
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<td>1180</td>
<td>1697</td>
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<td>Piperitone</td>
<td>1226</td>
<td>1231</td>
<td>1734</td>
<td>tr RI, MS</td>
</tr>
</tbody>
</table>

Comparison of the chemical composition of the investigated sample in the present work with those reported in the literature suggested some comments:

- Cedrol is much more abundant in the Taiwanese oil samples (53.0 and 58.3%) than in the Vietnamese ones (29.8-37.0% in published samples and 11.2% in the present work). In contrast, the content of α-cedrene is higher in our sample (18.4%) than in Taiwanese samples (6.6 and 11.8%) and in other Vietnamese ones (3.4-5.3%).
- Camphor and borneol were present at appreciable contents in all the samples.
- α-Terpineol was the main component of a Vietnamese oil sample (36.6%, Hà Giang province); α-Pinene was abundant (25.6%) only in one Taiwanese wood oil sample although it was the major component of leaf oil samples;
- Fenchol was one of the major components of two Vietnamese oil samples (16.1 and 27.5%) while its content was very low in all other investigated samples (0.1-1.8%);
- The three eudesmol isomers accounted for 2.8-8.8% in the present oil sample. They were much more abundant than in all the other samples (0.0-1.9%);
- Safrole, β-funebrene, α-muurolene, β-bisabolene, β-elemol and sesquithuriferol were reported for the first time as components of *C. konishii* wood oil.
4. Conclusions
In conclusion, according to our results and published data, it appears that Cunninghamia konishii essential oil isolated from wood harvested in four Vietnamese provinces exhibited various chemical compositions dominated either by monoterpens (α-terpinol, β-fenchyl alcohol) or by sesquiterpenes (cedrol, α-cedrene). Therefore, it should be informative to investigate the chemical variability of Vietnamese C. konishii wood oil (chemical variability of genetic or pedo-climatic origin). This study could be carried out after normalization of the harvest procedure, essential oil isolation and analysis of oil samples by complementary analytical techniques.

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6. References

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