CHEMICAL COMPOSITION OF THE NEEDLE AND CONE ESSENTIAL OILS OF *Pinus fenzeliana* (Pinaceae) FROM VIETNAM

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ABSTRACT

The needle and cone essential oils of *Pinus fenzeliana* Hand-Mazz. (*Pinus kwangtungensis* Chun & Tsiang) collected in Hang Kia-Pa Co Nature Reserve, Hoa Binh province were obtained by hydrodistillation and analyzed by a combination of GC-FID, GC-MS and NMR (Gas Chromatography/Gas Chromatography-Mass Spectrometry/Nuclear Magnetic Resonance (NMR) analysis). The oil yields were 0.2% and 0.37% respectively (v/w on dry weight basis). Twenty-seven compounds were detected in both needle and cone oils, accounting for 98.0% and 98.2% of the whole composition, respectively. The main components identified in the needle oil were α-pinene (20.7%), β-pinene (11.3%), δ-3-carene (32.2%), limonene (8.0%) and E-β-caryophyllene (7.1%). The cone oil contained mainly α-pinene (19.3%), β-pinene (45.4%), δ-3-carene (3.5%), limonene (12.7%) and E-β-caryophyllene (3.4%). This is the first report on the chemical composition of the cone essential oil of *P. fenzeliana* from Vietnam.

Keywords: *Pinus fenzeliana*, α-pinene, β-pinene, δ-3-carene, limonene and E-β-caryophyllene.

INTRODUCTION

The genus *Pinus* (Pinaceae) comprises approximately 100 species widely distributed from the northern hemisphere to Central and South America, Malaysia and Indonesia (Vo, 2004; Farjon, 2010). Farjon (2010) reported that the genus *Pinus* is divided into two subgenera, *Strobus* and *Pinus*. The subgenus *Strobus*, also known as Haploxylon, is composed of conifers in Asia and North America, while the subgenus *Pinus*, also known as Diploxylon, is composed of conifers in Central Europe and the Mediterranean. According to Pham (1991), Phan (2003), Nguyen (2004), Nguyen et al. (2005), there are 6−7 pine species, including 3 five-needle pine species, *Pinus dalatensis* de Ferre, *Pinus kwangtungensis* Chun & Tsiang and *Pinus cernua* Aver., K. S. Nguyen & T .H. Nguyen recognized for Vietnam.

The species Thông pà cò was for a long time identified as *P. kwangtungensis* Chun & Tsiang by some Vietnamese authors (Pham, 1991; Phan, 2003; Vo, 2004; Nguyen et al., 2005). Farjon (2010) reported *Pinus fenzeliana* Hand-Mazz as an accepted valid name for *P. kwangtungensis* Chun & Tsiang, *Pinus wangii* Hu & W. C. Cheng var. *kwangtungensis* (Chun & Tsiang) Silba, *P. kwangtungensis* Chun & Tsiang var. *variifolianan* Li & Y. C. Zhong, *Pinus fenzeliana* Hand.-Mazz. var. *annamiensis* Silba and *Pinus eremitana* Businský.

In this paper, we use the name *Pinus fenzeliana* for the species Thông pà cò. This species is a tree with a wide canopy, stem up to 20 m tall and 0.7 m d.b.h. with bark gray. Winter buds are dark brown, the sap is milky white. Leaves are needle-shaped with 4–5 needles per fascicle, up to 7 cm long. Female cones are solitary. Cones appear in March-April and open in October the following year. The species grows often as a scattered tree on rocky slopes of limestone mountains at an elevation of 900–1,600 m, sometime forms monodominant primary forests, or low mountain tropical monsoon evergreen forests mixed with *Pseudotsuga sinensis, Forkienia hodginsii, Xanthoxyparis vietnamensis,*

Podocarpus pilgeri, Nageia fleuryi and *Cephalotaxus mannii*. This species has very limited capacity for natural regeneration and seedlings often do not tolerate shade. *P. fenzeliana* is primarily found in Ha Giang, Cao Bang, Bac Kan, Son La, Hoa Binh and Thanh Hoa provinces of Vietnam and is found also in South China. It provides good timber used for constructions and resin used to make glue. It is a prospect tree for afforestation with current conservation status identified as vulnerable species (VU) (MOST & VAST, 2007; Vo, 2004; Nguyen et al., 2005., Kohsuke et al., 2007).

Since ancient times, pine essential oils have been used in traditional medicine as a respiratory disinfectant and as an ingredient in embalming the bodies of kings and mandarins. Pine essential oils have also been used as materials in the production of valuable drugs and cosmetics, herbicides, solvents for paints, varnishes and mineral processing (MOST & VAST, 2007; Loannou et al., 2014; La et al., 2002).

The chemical composition of pine essential oils has been reported only in a limited number of studies. Hu et al. (2017) reported that 15 diterpenoids were isolated from the methanol extract of the needles and twigs of *P. kwangtungensis*, including 3 new compounds (4S,5R,9S,10R)-6- oxo-labd-7,13-olic-19-oic acid (1), 15(S)-n-butoxypinusolidic acid (2) and β-d-glucopyranosyl- (4S,5R,9S,10R)-labda-8(17),13-diene-15,16-olid-19-oate (3). Among the isolates, lambertianic acid (10) and cassipourol (15) showed inhibitory activities against human protein tyrosine phosphatase 1 B (PTP1B), a target for the treatment of type-II diabetes and obesity, with IC_{50} values of 25.5 and 11.2 μM, respectively. The oleoresin of *P. kwangtungensis* was reported to contain mainly myrcene (16.1%), isopimaric acid (12.0%), lambertianic acid (25.0%), abietic acid 20.4%) and neoabietic acid 10.9% (Song et al., 1995).

The essential oils from the needles of 46 pine species, including 37 and 17 taxa of the subgenera *Pinus* and *Strobus,* respectively, were analyzed by means of GC-FID and GC-MS, with more than 190 compounds detected

and quantified. Most of the pine essential oils were characterized by a relative abundance of monoterpenes and sesquiterpenes such as α-pinene, β-pinene, limonene, δ-3-carene and β-phellandrene. Sesquiterpenoids were primarily represented by germacrene D and β-caryophyllene. Diterpene contents varied in a wide range (from zero in *P. sylvestris* to 67.3% in *Pinus pinaster*). Monoterpenes and sesquiterpenes dominated most species of the subgenus *Strobus,* whereas *Pinus aristata* contained almost only monoterpenes.

Monoterpenes were characterized by high α-pinene content, except for *Pinus gerardiana, Pinus monophylla, Pinus strobifomis* and *Pinus wallichiana* with β-pinene being the major component. The essential oils from the needles of *P. aristata* and *Pinus parviflora* were dominated by δ-3-carene and β-phellandrene but germacrene D was the predominant component in the sesquiterpenoid fraction. Diterpenes only occurred at low concentrations, except for *Pinus piomila* which accounted for 13.6% (Loannou et al., 2014).

Figure 1. The species *Pinus fenzeliana* Hand-Mazz. in Hang Kia-Pa Co Nature Reserve.
A, B: Cone-bearing branchlet and seed cones; C: Leafy branchlet and seed scales; D: Needles;
E: Seed cone; F: Seed, abaxial view; G: Seed, adaxial view

The essential oil of *P. kwangtungensis* collected in Xuan Nha Nature Reserve (Moc Chau district) was reported to contain α-pinene (16.41%), L-limonene (6.65%), β-caryophyllene (13.21%), δ-cadinene (8.09%) (Tran et al., 2007). After the new species *Pinus cernua* (= *P. armandi* ssp. *xuannhaensis* L. K. Phan) was published by Averyanov et al. (2017), we realized we had not identified this species correctly and the correct name of this species was *P. cernua.* The oil composition of different parts of *P. cernua* was identified by GC, GC/MS and NMR as follows: from the needles including α-pinene (12.5%), β-pinene (28.4%), myrcene (47%) and limonene (4.6%); from the cones including α-pinene (44.1%), β-pinene (8.1%), myrcene (11.5%) and E-β-caryophyllene (6.1%); from the twigs including α-pinene (17.9%), β-pinene (9.8%), myrcene (32.8%), limonene (22%), δ-cadinene (3.1%) and E-β-caryophyllene (2.3%) (Tran et al., 2019).

The essential oil from twigs of *P. fenzeliana* (= *P. kwangtungensis*) collected at Pa Co-Hang Kia Nature Reserve, Hoa Binh province was determined by GC/MS. The main constituents were α-pinene (18%), β-pinene (26.3%), β-myrcene (10.4%), limonene (16.1%), trans-pinocarverol (4.5%) and caryophyllen oxide (3%) (Tran et al., 2018).

This paper reports the chemical composition of essential oils from *P. fenzeliana* (= *P. kwangtungensis*) collected in Hang Kia-Pa Co Nature Reserve, Hoa Binh province using combined analysis by GC-FID, GC-MS and NMR.

MATERIALS AND METHODS

Plant material

Needles and cones of *P. fenzeliana* were collected in Hang Kia-Pa Co Nature Reserve, Hoa Binh province in May 2022. Plant material was identified by Nguyen Quang Hung in the Institute of Ecology and Biological Resources (IEBR). A voucher specimen (TNTV-PC 20) was deposited at the Herbarium of the IEBR, Vietnam Academy of Science and Technology

(VAST). Needles and cones of *P. fenzeliana* were hydrodistilled separately (5 h) using a Clevenger-type apparatus (MOH, 1977). Both oil samples were submitted to GC (RI), GC-MS and ^{13}C NMR analyses, following a method developed at the University of Corsica.

Gas Chromatography (GC) analysis

Analyses were carried out using a Clarus 500 Perkin Elmer Chromatograph (Courtaboeuf, France), equipped with a flame ionization detector (FID) and two fused-silica capillary columns (50 m × 0.22 mm, film thickness 0.25 µm), BP-1 (polydimethyl siloxane) and BP-20 (polyethylene glycol). The oven temperature was programmed from 60−220 ºC at 2 ºC/min and then held isothermal at 220 ºC for 20 min; injector temperature: 250 ºC; detector temperature: 250 ºC; carrier gas: helium (0.8 mL/min); split: 1/60; injected volume: 0.5 µl. Retention indices (RI) were determined relative to the retention times of a series of *n*-alkanes (C8-C29) with linear interpolation («Target Compounds» software from Perkin Elmer).

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

Samples were analysed with a Perkin Elmer TurboMass detector (quadrupole), directly coupled to a Perkin-Elmer Autosystem XL (Courtaboeuf, France), equipped with a DB-1 (polydimethylsiloxane) fused-silica capillary column (60 m × 0.22 mm i.d., film thickness 0.25 µm). The oven temperature was programmed from 60 to 230 ºC at 2 º/min and then held isothermal at 230º for 45 min; injector temp., 250 ºC; ion-source temp., 150 ºC; carrier gas, He (1 mL/min); split ratio, 1:80; injection volume, 0.2 µL; ionization energy, 70 eV. The electron ionization (EI) mass spectra were acquired over the *m/z* range of 35–350 Da.

Nuclear Magnetic Resonance (NMR) analysis

All ^{13}C-NMR spectra were recorded on a Bruker AVANCE 400 Fourier transform spectrometer (Bruker, Wissembourg, France) operating at 100.623 MHz for ^{13}C, equipped with a 5 mm probe, in CDCl$_3$, with all shifts

referred to internal TMS. The following parameters were used: pulse width = 4 µs (flip angle 45°); relaxation delay D1 = 0.1 s, 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 3,000 for each sample (40 mg in 0.5 mL of $CDCl_3$).

Identification of individual components

Identification of the individual components was carried out: (i) by comparison of their GC retention indices (RI) on polar and apolar columns, with those of reference compounds;[1,2] (ii) on computer matching against commercial mass spectral libraries;[1,3,4] (iii) on comparison of the signals in the ^{13}C-NMR spectra of the mixtures with those of reference spectra compiled in the laboratory spectral library, with the help of a laboratory-made software.[5] This method allows the identification of individual components of the essential oil at content as low as 0.4–0.5%.

Identification of the individual components was carried out: (i) by comparison of their GC retention indices (RI) on polar and apolar columns, with those of reference compounds (Konig et al., 2001; Babushok et al., 2011); (ii) on computer matching against commercial mass spectral libraries (Konig et al., 2001; Adams, 2007; NIST, 2014) (iii) on comparison of the signals in the ^{13}C-NMR spectra of the mixtures with those of reference spectra compiled in the laboratory spectral library, with the help of a laboratory-made software (Tomi et al., 1995; Ouattara et al., 2014; Bazzali et al., 2016). This method allows the identification of individual components of the essential oil at content as low as 0.4–0.5%.

RESULTS AND DISCUSSION

Chemical composition of *Pinus fenzeliana* essential oils

The oil yields from the needles and cones of *P. fenzeliana* from Vietnam were 0.20% and 0.37% respectively (v/w on a dry weight basis). Both essential oils were colorless liquids having densities lighter than water. Compared to the previous study by Thai et al. (2018), the essential oil yield from the needles of *P. fenzeliana* reached 0.3% which was slightly higher than that in the present study (0.2%) and both of them were lower than the essential oil yield from the cone (0.37%). The chemical composition of the essential oils from the needles and cones was summarized in Table 1.

Twenty-seven compounds were identified in the needle oil, accounting for 98% of the whole composition. The main components were α-pinene (20.7%), β-pinene (11.3%), δ-3-carene (32.2%), limonene (8%) and E-β-caryophyllene (7.1%). The abundant minor components were β-myrcene (2%), (E)-β-ocimene (1.2%), α-terpineol (2.8%), germacrene D (2.8%) and α-humulene (1.3%). The rest of the components were presented at low contents (less than 1%). The composition of the needle oil was dominated by monoterpenes hydrocarbons (85.1%), followed by oxygenated monoterpenes (6.5%) and sesquiterpenes hydrocarbons (6.0%). While oxygenated sesquiterpenes and the other constituents only accounted for 0.4% and 0.2%.

Twenty-seven compounds were detected in the cone oil, accounting for 98.2% of the whole composition. The main components were α-pinene (19.3%), β-pinene (45.4%), δ-3-carene (3.5%), limonene (12.7%) and E-β-caryophyllene (3.4%). The other components with appreciable content were β-myrcene (2%), (E)-β-ocimene (1.2%), α-terpineol (2.8%), germacrene D (2.8%) and α-humulene (1.3%). The rest of the components accounted for less than 1%. The composition of the cone oil was represented by monoterpenes hydrocarbons (80.8%) and sesquiterpenes hydrocarbons (11.8%). Meanwhile, oxygenated monoterpenes, oxygenated sesquiterpenes and the other constituents only accounted for 3.6%, 1.2% and 0.6%.

Table 1. Chemical composition of the needle and cone essential oils
of *Pinus fenzeliana* from Vietnam

Components	RIaL	RIpL	RIa	RIp	Cones (%)	Needles (%)	Identification
(E)-3-Hexenol	837	1373	836	1389	-	0.3	RIs, MS
α-Pinene	934	1025	933	1020	19.3	20.7	RIs, MS, ^{13}C NMR
Camphene	947	1068	946	1070	0.5	0.6	RIs, MS, ^{13}C NMR
Sabinene	968	1122	967	1126	0.3	0.5	RIs, MS
β-Pinene	973	1110	973	1116	45.4	11.3	RIs, MS, ^{13}C NMR
β-Myrcene	983	1161	982	1166	1.4	2.0	RIs, MS, ^{13}C NMR
α-Phellandrene	999	1168	999	1170	tr	0.1	RIs, MS
δ-3-Carene	1007	1149	1008	1155	3.5	32.2	RIs, MS, ^{13}C NMR
α-Terpinene	1011	1178	1011	1185	0.1	0.3	RIs, MS
p-Cymene	1015	1270	1013	1276	0.1	0.2	RIs, MS
β-Phellandrene	1021	1209	1024	1215	0.9	0.7	RIs, MS, ^{13}C NMR
Limonene	1024	1198	1024	1205	12.7	8.0	RIs, MS, ^{13}C NMR
(E)-β-Ocimene	1038	1250	1037	1254	-	1.2	RIs, MS, ^{13}C NMR
γ-Terpinene	1050	1245	1050	1249	0.1	0.6	RIs, MS, ^{13}C NMR
p-Cymenene	1074	1437	1074	1441	-	0.1	RIs, MS
Terpinolene	1079	1282	1081	1288	0.9	2.5	RIs, MS, ^{13}C NMR
Fenchol	1101	1570	1099	1586	0.2	0.1	RIs, MS
Undecane	1100	1100	1101	1100	-	0.1	RIs, MS
trans-Pinocarveol	1126	1661	1126	1659	0.4	-	RIs, MS
Pinocarvone	1140	1575	1139	1571	tr	-	RIs, MS
Borneol	1153	1700	1152	1704	0.2	-	RIs, MS
Terpinen-4-ol	1164	1601	1164	1604	0.6	0.7	RIs, MS, ^{13}C NMR
α-Terpineol	1176	1694	1174	1699	4.7	2.8	RIs, MS, ^{13}C NMR
Myrtenol	1182	1790	1181	1795	0.3	-	RIs, MS
Verbenone	1184	1720	1185	1707	0.1	-	RIs, MS
(E)-Anethole	1265	1826	1262	1829	0.1	-	RIs, MS
β-Bourbonene	1382	1523	1386	1510	0.1	-	RIs, MS
(E)-β-Caryophyllene	1419	1598	1421	1597	3.4	7.1	RIs, MS, ^{13}C NMR
α-Humulene	1449	1667	1453	1668	0.6	1.3	RIs, MS, ^{13}C NMR
α-Amorphene	1466	1693	1472	1688	-	0.2	RIs, MS
Germacrene D	1476	1708	1478	1708	1.9	2.6	RIs, MS, ^{13}C NMR
δ-Cadinene	1514	1756	1516	1757	-	0.6	RIs, MS, ^{13}C NMR
Caryophyllene oxide	1570	1986	1573	1978	0.4	-	RIs, MS
T-Cadinol	1626	2170	1628	2184	-	0.6	RIs, MS, ^{13}C NMR
α-Cadinol	1640	2227	1640	2230	-	0.6	RIs, MS, ^{13}C NMR
Total					98.2	98.0	
Monoterpene hydrocarbons					85.1	80.8	
Oxygenated monoterpenes					6.5	3.6	
Sesquiterpene hydrocarbons					6.0	11.8	
Oxygenated sesquiterpene					0.4	1.2	
Miscellaneous					0.2	0.6	

Notes: RIaL = RI literature, apolar column, Babushok et al., 2011; RIpL = RI literature, polar column, Babushok et al., 2011; RIa: Retention indices on apolar column; RIp: Retention indices on polar column.

The main components of the needle and cone essential oils were α-pinene, β-pinene, δ-3-carene and limonene which were found in the essential oils of many *Pinus* species. These monoterpenes can be used as a starting material for the synthesis of aromatic compounds and other polyterpenes. These compounds all have anti-inflammatory and antibacterial activities. Limonene and δ-3-carene help to protect plants from pests. In addition to its uses in cosmetics and skincare formulations, limonene and δ-3-carene are also used to improve heart health and repair damaged bones. The other constituents were present at low content, of which cymene and anethole with benzenoid nucleus have been widely used in the food and cosmetic industries (La et al., 2002; Loannou et al., 2014).

The main components of both essential oils displayed relatively similarities but differences in amounts such as α-pinene (19.3–20.7%), β-pinene (11.3–45.4%), δ-3-carene (3.5–32.2%), limonene (8–12.7%) and E-β-caryophyllene (3.4–7.1%). Some components with low contents including *trans*-pinocarveol (0.4%), myrtenol (0.3%), borneol (0.25%), verbenone (0.1%) and E-anethole (0.1%) were only presented in the cone oil whereas (E)-β-ocimene (1.2%), δ-cadinene (0.6%), T-cadinol (0.6%), α-cadinol (0.6%) were only found in the needle oil. In comparison to the sample previously reported, the main constituents of the twig and needle oils of *P. fenzeliana* including α-pinene (18%), β-pinene (26.3%), limonene 16.1% were similar to that of the needle oil in the present study, except for the absence of δ-3-carene (Thai et al., 2018).

The needle and cone oils of *P. fenzeliana* were characterized by a high content of α-pinene, β-pinene and limonene which were similar to that of *P. cernua*, but myrcene (1.4−2.0%) were much lower than that of *P. cernua* (11.5−47.0%), and δ-3-carene was absent in *P. cernua* (Thai et al., 2019).

The needle and cone essential oils of *P. fenzeliana* had some main components which were similar to the essential oil of *Pinus heldreichii* from Greece including α-pinene (11.1%), β-pinene (3.6%),

δ-3-carene (18.6%), limonene (23.7%) and to the essential oil of *Pinus nigra* subsp. *laricio* from England including α-pinene (18.0%), β-pinene (0.9%), δ-3-carene (16.1%), lismonene (9.7%) (Loannou et al., 2014).

CONCLUSION

The needle and cone essential oils of *P. fenzeliana* collected in Hang Kia-Pa Co Nature Reserve, Hoa Binh province were obtained with yields of 0.2% and 0.37% respectively (v/w on a dry weight basis). Both essential oils were colorless liquids having densities lighter than water.

Twenty-seven compounds were identified in the needle oil, accounting for 98% of the whole composition. The main components were α-pinene (20.7%), β-pinene (11.3%), δ-3-carene (32.2%), limonene (8.0%) and E-β-caryophyllene (7.1%).

Twenty-seven compounds were detected in the cone oil, accounting for 98.2% of the whole composition. The main components were α-pinene (19.3%), β-pinene (45.4%), δ-3-carene (3.5%), limonene (12.7%) and E-β-caryophyllene (3.4%). This is the first report on the chemical composition of the cone essential oil of *P. fenzeliana* from Vietnam.

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