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Articulo / Article

Chemical constituents of essential oils from three Vietnamese species of *Pinus*

[Constituyentes químicos de los aceites esenciales de tres especies Vietnamitas de *Pinus*]

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Abstract: This paper reports the chemical composition of essential oils obtained from *Pinus dalatensis* Ferré, *Pinus kwangtungensis* Chun ex. Tsiang and *Pinus armandii* subsp. *xuannhaensis* L.K. Phan. The oils were studied by gas chromatograpgy (GC) and gas chromatography coupled to mass spectrometry (GC-MS). The main constituents of *P. dalatensis* were the terpene hydrocarbons namely α-pinene (38.2%), β-pinene (25.3%), β-myrcene (11.0%) and β-caryophyllene (10.5%), while α-cedrol (19.2%) was the only significant compound of *P. armandi* subsp. *xuannhaensis*. *P. kwangtungensis* showed β-pinene (26.3%), α-pinene (18.0%), limonene (16.1%) and β-myrcene (10.4%) as the dominant compounds. The volatile constituents of *P. dalatensis* and *P. armandi* subsp. *xuannhaensis* are being reported for the first time.

Keywords: *Pinus dalatensis*, *Pinus kwangtungensis*, *Pinus armandii* subsp. *xuannhaensis*, hydrodistillation, terpenes

Resumen: En este artículo se reportan los constituyentes químicos de los aceites esenciales de *Pinus dalatensis* Ferré, *Pinus kwangtungensis* Chun ex. Tsiang y *Pinus armandii* subsp. *Xuannhaensis* L.K. Phan que se analizaron mediante cromatografía de Gases (GC) y por Cromatografía de Gases acoplada a la Espectrometría de Masas (GC-EM). Los principales constituyentes de *P. dalatensis* fueron los hidrocarburos terpénicos, a saber, α-pineno (38.2%), β-pineno (25.3%), β-mirceno (11.0%) y β-cariofileno (10.5%). Por otro lado, α-cedrol (19.2%) fue el único compuesto significativo de *P. armandi* subsp. *Xuannhaensis* mientras que el aceite de *P. kwangtungensis* estuvo dominado por β-pineno (26.3%), α-pineno (18.0%), limoneno (16.1%) y β-mirceno (10.4%). Los constituyentes volátiles de *P. dalatensis* y *P. armandi* subsp. *xuannhaensis* se informa por primera vez.

Palabras clave: *Pinus dalatensis*, *Pinus kwangtungensis*, *Pinus armandii* subsp. *xuannhaensis*, hidrodestilación, terpenos.

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ABBREVIATION LIST

v/w- volume by weight; GC Gas chromatography; GC-MS Gas chromatography coupled to mass spectrometry; SPME Solid-phase microextraction

INTRODUCTION

In continuation of our study on the chemical constituents of Vietnamese flora (Chinh *et al*., 2017; Huong *et al*., 2017), we report the chemical compounds identified in the essential oils hydrodistilled from three *Pinus* (Pinaceae) plants. *Pinus dalatensis* Ferre' is a medium-sized evergreen tree growing to 30 to 40 m. It is an endemic plant with restricted habitats at higher altitudes in Vietnam highland (Businský, 1999; Rácz & Huyen, 2007; Phong *et al*., 2015; Phong *et al*., 2016). A phytochemical study on extracts of *P. dalatensis* leaves led to the isolation of caryolane sesquiterpenoid, labdane diterpenoids, serratane triterpenoid, diacylated flavonoid glucoside, stilbenoid and sterols. The sterol displayed cytotoxicity effect on SK-LU-1, MCF-7 and Hep-G2 cell lines with IC_{50} values of 141.22, 127.81 and 166.84 μM, respectively (Sa *et al*., 2017). No previous study could be found on the chemical compounds present in the essential oil, but extracts from the plant are known to contain fatty acids (Imbs & Pham, 1996).

Pinus kwangtungensis Chun ex. Tsiang is a tree that grows up to 30 m tall. It has often been confused, and even united, with *Pinus fenzeliana*, however, the two species are not considered here to be conspecific (Thai, 2012). *Pinus kwangtungensis* is a five-needled pine, inhabiting isolated mountain tops, cliffs or slopes in the montane areas of southern China and northern Vietnam (Zhang *et al*., 2007). The plant is known to contained polycyclic aromatic hydrocarbons (Kuang *et al*., 2014). Previous phytochemical study on *P. kwangtungensis* produced (4S,5R,9S,10R)-6-oxo-labd-7,13-dien-16,15-olid-19-oic acid, 15(S)-n-butoxypinusolidic acid and β-D-glucopyranosyl-(4S,5R,9S,10R)-labda-8(17),13-dien-15,16-olid-19-oate (Hu *et al*., 2017). In addition, lambertianic acid and cassipourol 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 (Hu *et al*., 2017). The authors are aware of only two reports describing the volatile contents of *P. kwangtungensis*. α-Pinene (16.41%), β-caryophyllene (14.50%) and δ-cadinene (8.09%) were characterized from *P. kwangtungensis* (Thai & Hong, 2014). Moreover, lambertianic acid (25.0%), abietic acid (20.0%), β-myrcene (16.1%), isopimaric acid (12.0%) and neoabietic acid (10.9%) were fatty acids and monoterpene reported previously from the volatile oil of *P. kwangtungensis* (Song *et al*., 1995).

Pinus armandii subsp. *xuannhaensis* L.K. Phan is a new five needle pine discovered recently from Xuan Nha Nature Reserve. The subspecies is considered as a narrow endemic to Vietnam and is assessed as endangered (Loc *et al*., 2014; Tam *et al*., 2015; Duy *et al*., 2016). Presently there is no literature information on the volatile and non-volatile components of this plant. However, the chemical compositions of essential oils from a related species, *P. armandii*, have been studied. Previously, α-pinene (21.8%), abietic acid (20.1%), isopimaric acid (14.0%) and lambertianic acid (12.1%) were characterized from *P. armandi* (Song *et al*., 1995), while another authors (Tsitsimpikou *et al*., 2001) reported large quantity β-caryophyllene (36.3%) and γ-muurolene (40.7%). The cone oil extracted by hydrodistillation has its principal components to be α-pinene (20.92%) and D-limonene (15.78%) while the oil extracted by SPME showed α-pinene (41.59%), D-limonene (17.8%), β-caryophllene (11.02%) as the principal components (Yang *et al*., 2010). Also, quantitative amount of 3-carene (23.84%), β-pinene (18.69%) and α-pinene (14.74%) were identified from the bark oil of *P. armandii* (He *et al*., 2009) while another investigated sample (Chen *et al*., 2007) contained limonene (29.27%), benzene (17.81%) and α-pinene (16.08%). The main volatile compounds of resins of *P. armandii* (Li *et al*., 2006) were α-pinene (52.494%) and β-piene (39.269%). In summary, α-pinene seems to be a chemotaxonomy marker of *P. armandii* essential oils.

MATERIALS AND METHOD

Plants collection

Sample of *P. dalatensis* Ferré were collected from Đà Lạt, City, Lâm Đồng Province, Vietnam, in May 2015 while *P. kwangtungensis* Chun ex. Tsiang were harvested from Pà Cò-Hang Kia Nature Reserve, Hòa Bình Province, in June 2015. However, *P. armandii* subsp. *xuannhaensis* were obtained from Xuân Nha Nature Reserve, Sơn La, Province, in July 2015. Voucher specimens VN 12, VN 15, VN 16 respectively were deposited at the Vinh University Herbarium, Vietnam. Plant samples were air-dried prior to extraction.

Distillation of the essential oils

Briefly, 500 g each of the air-dried pulverized samples were carefully introduced into a 5 L flask and distilled water (5 L) was added until it covers the sample completely. Hydrodistillation was carried out with a Clevenger-type distillation unit designed according to the specification (Vietnamese Pharmacopoeia, 1997). The distillation time was 3 h and conducted at normal pressure. The oils were kept refrigerated (4º C) until analysis.

Analysis of the essential oils

GC analysis of the oils were done with a Agilent HP 6890 Plus instrument equipped with HP-5MS column (30 m x 0.25 mm; 0.25 μm film thickness) using N_2 as carrier gas (1 mL/min). Injection (1 μL) was performed in split mode, split ratio 10:1. The injection port temperature was 250° C; the oven temperature programme was 40° C (2 min), then raised to 220° C at 4° C/min (10 min). Each analysis was performed in triplicate. Retention indices (RI) values for each component were determined relative to the retention times of an homologous *n*-alkane series (C_6-C_{40}) on the HP-5MS column. The relative amounts of individual components were calculated based on the GC peak area (FID response) without using correction factors.

GC/MS analysis of the oils was performed using an Agilent Technologies HP 6890N Plus Chromatograph fitted with HP-5MS column (30 m x 0.25 mm; 0.25 μm film thickness) and interfaced with a mass spectrometer HP 5973 MSD. The conditions were the same as described above for GC. The MS was operated at 70 eV while the emission current was set at 40 mA. The acquisitions scan mass range of 35-350 amu at a sampling rate of 1.0 scan/s.

Identification of the constituents

The identification of constituents was performed on the basis of their retention indices (RI) determined by co-injection with reference to a homologous series of *n*-alkanes, under identical experimental conditions. Further identification was performed by comparison of their mass spectra with those from NIST database (NIST, 2011).

RESULTS AND DISCUSSION

The yield of essential oils were 0.30% (v/w, *P. dalatensis*), 0.33% (v/w, *P. kwangtungensis*) and 0.24% (v/w, *P. armandi* subsp. *xuannhaensis*), calculated on dry weight basis. The identity and percentages of the chemical constituents presented in the oil and their retention indices on HP-5MS column are shown in Table 1. The classes of compounds identified in *P. dalatensis* were monoterpene hydrocarbons (78.8%) and sesquiterpene hydrocarbons (16.5%). The main constituents of the oil were the terpene hydrocarbons namely α-pinene (38.2%), β-pinene (25.3%), β-myrcene (11.0%) and β-caryophyllene (10.5%). No previous report exists on the volatile compounds of *P. dalatensis*.

P. armandii subsp. *xuannhaensis* comprised mainly of oxygenated sesquiterpenes (37.7%), monoterpene hydrocarbons (20.2%), oxygenated monoterpenes (17.4%) and oxygenated sesquiterpenes (10.5%). α-Cedrol (19.2%) was the only significant compound of the oil while bicycloelemene (5.8%), α-terpineol (5.5%), δ-3-carene (5.0%), caryophyllene oxide (4.2%), β-pinene (4.5%) and α-pinene (4.1%) were present in significant quantity. Although, no previous report could be seen on the volatile compounds of *P. armandii* subsp. *xuannhaensis,* however, α-pinene, the observed chemotaxonomy marker of *P. armandii* essential oils (Song *et al*., 1995; Li *et al*., 2006; He *et al*., 2009; Yang *et al*., 2010) occurred in a much lower quantity in the present studied oil sample.

Monoterpene compounds comprising of hydrocarbons derivatives (71.9%) and oxygenated form (18.8%) were identified in abundance in *P. kwangtungensis.* The significant constituents of the oil were identified to be β-pinene (26.3%), α-pinene (18.0%), limonene (16.1%) and β-myrcene (10.4%). The present oil sample does not contained diterpenes such as lambertianic acid, abietic acid and neoabietic acid that were found in previously oil sample (Song *et al*., 1995).

The oil and oleoresin compositions of several different *Pinus* species have been studied and it has been shown that their composition exhibited considerable qualitative and quantitative variations both between and within species. There is a group of *Pinus* essential oils which contained large amount of monoterpene compounds, although the identities of terpenes varied from one species to another. The main monoterpene constituents of *P. carribea* (Moronkola *et al*., 2007) were limonene and β-phellandrene, while α-pinene, β-phellandrene and β-pinene were the main compounds of *P. peuce* (Hajdari *et al*., 2016). The essential oils of *Pinus densiflora, P. parviflora, P. rigida, P. strobus*, and *P. thunbergii* were mainly composed of α-pinene and β-pinene (Jeon & Lee, 2012). The main compounds of *P. wallichiana*, *P. monticola* and *P. strobus* were monoterpene hydrocarbons namely α-pinene, β-pinene, limonene and myrcene (Dambolena *et al*., 2016). Likewise, *P. pinaster* and *P. halepensis* essential oils were characterized by a high percentage of α-pinene (Ghanmi *et al*., 2006). The oils of *P. pumila* contained caranes as major components, whereas those of *P. parviflora* contained camphanes as major components (Kurose *et al.*, 2007). Also, limonene was the dominant compound of *P. pinea* (Demirci *et al.*, 2015). In addition, β-pinene, α-pinene and limonene were determined as main components of *P. brutia*, *P. nigra* and *P. pinea*, respectively (Yener *et al.*, 2014). Moreover, the significant compounds in *P. strobus* were identified as α-pinene, β-myrcene and β-pinene, while 3-carene, *p*-cymene and α-pinene were identified as main components of *P. mugo* subsp. *mugo* (Kılıç & Koçak, 2014).

Table 1
Chemical constituents of the studied oil samples

Compounds [b]	**RI**[c]	**RI**[d]	**Percentages** [a]		
			P. d	***P. asx***	***P. k***
(*E*)-3-Hexanol	855		-	0.3	-
α-Thujene	930	921	0.3	-	-
α-Pinene	939	932	38.2	4.1	18.0
Camphene	953	946	1.3	1.1	0.6
β-Pinene	980	978	25.3	4.5	26.3
β-Myrcene	990	988	11.0	0.7	10.4
δ-3-Carene	1011	1004	-	5.0	-
o-Cymene	1024	1022	0.1	0.9	0.5
β-Phellandrene	1028	1028	0.4	-	-
Limonene	1032	1030	2.4	3.9	16.1
1,8-Cineole	1034	1032	-	-	0.8
Perillene	1106	1102	-	-	0.9
α-Pinene oxide	1113	1105	-	-	0.5
endo-Fenchol	1123	1116	0.1	1.0	-
α-Campholenal	1137	1130	-	0.5	0.4
cis-Limonene oxide	1144	1138	-	-	0.5
trans-Pinocarveol	1154	1140	-	1.6	4.5
trans- Limonene oxide	1148	1141	-	-	0.2
trans-Sabinol	1150	1141	0.5	-	-
Pinocarvone	1160	1154	0.2	0.6	0.6
Borneol	1167	1167	0.1	0.8	0.3
p-Mentha-1,5-dien-8-ol	1168	1168	-	0.3	-
Terpinen-4-ol	1177	1177	-	-	1.4
m-Cymen-8-ol	1189	1185	-	2.1	-
α-Terpineol	1190	1187	-	5.5	1.4
p-Cymen-8-ol	1194	1188	-	1.4	0.3
Myrtenol	1206	1192	0.2	1.0	1.9
Myrtenal	1209	1197	0.2	1.0	1.8
Verbenone	1209	1204	-	0.5	0.9
trans-Carveol	1217	1219	-	0.4	1.1
cis-Carveol	1220	1220	-	-	0.2
(*Z*)-3-Hexenyl-2-methylbutanoate	1231	1238	-	1.3	-
(*E*)-2-Hexenyl-2-methylbutanoate	1238	1240	-	0.9	-
Carvone	1257	1244	-	-	0.9

p-Mentha-1,8-dien-7-ol	1301	1302	-	-	0.2
Neral	1318	1308	-	0.7	-
Bicycloelemene	1327	1327	-	5.8	-
δ-Elemene	1340	1337	-	0.9	-
α-Copaene	1377	1374	0.2	-	0.6
β-Caryophyllene	1419	1417	10.5	0.9	0.2
α-Humulene	1454	1452	1.7	0.6	-
γ-Muurolene	1480	1482	1.2	1.4	-
Germacrene D	1485	1484	1.0	-	-
α-Muurolene	1500	1500	0.3	-	0.3
γ-Cadinene	1514	1513	0.1	0.9	-
δ-Cadinene	1525	1522	0.2	-	-
(*E*)-Nerolidol	1563	1561	-	0.9	-
Spathulenol	1578	1577	-	1.1	-
Caryophyllene oxide	1583	1581	1.9	4.2	3.0
α-Cedrol	1601	1600	-	19.2	0.4
Humulene oxide	1642	1640	0.2	2.2	0.5
τ-Muurolol	1646	1646	0.2	1.8	-
α-Cadinol	1654	1652	0.2	2.9	-
epi-α-Cadinol	1666	1680	-	3.4	-
Farnesol	1718	1722	-	0.6	-
Benzyl benzoate	1760	1759	-	0.8	-
Benzyl salicylate	1866	1866	-	0.6	-
Total			**99.0**	**88.3**	**95.7**
Monoterpene hydrocarbons			**78.8**	**20.2**	**71.9**
Oxygenated monoterpenes			**1.3**	**17.4**	**18.8**
Sesquiterpene hydrocarbons			**16.4**	**10.5**	**0.8**
Oxygenated sesquiterpenes			**2.5**	**37.7**	**3.9**
Non-terpenes			**-**	**2.5**	**-**

[a] Standard deviation (SD ±) were insignificant and were excluded from the Table; [b] Elution order on HP-5MS column; [c] Retention indices on HP-5MS column; [d] Literature retention indices; - Not identified; *P.d, P. dalatensis*; *P.asx, P. armandii* subsp. *xuannhaensis*; *P.k, P. kwangtungensis*

Previous reports have also indicated another group of essential oils of *Pinus* plants with the abundance of sesquiterpene compounds. The oils of *P. bungeana* and *P. koraienesis* contained sesquiterpene hydrocarbons (β-caryophyllene and germacrene D) as the main components (Jeon & Lee, 2012). The oils of *P. merkusii*, *P. petula* and *P. rudis* contained larger amounts of caryophyllanes than those of the cadinane group (Kurose *et al.*, 2007), while β-caryophyllene occurred in *P. taeda* (Adams *et al*., 2014) and *P. halapensis* (Yener *et al.*, 2014). *Pinus sylvestris* possessed an oil rich in caryophyllene oxide and manoyl oxide rather than the usual monoterpene hydrocarbons (Tsitsimpikou *et al.*, 2001). The main constituents in *P. merkusii* were β-caryophyllene, caryophyllene oxide and α-humulene (Kılıç & Koçak, 2014).

However, some *Pinus* essential oils were can be classified into another group known to consist of mixture of monoterpene and sesquiterpene compounds. The oil of *P. heldreichii* contained mixture of limonene, germacrene D and β-caryophyllene (Simic *et al.*, 1996). The main components of *P. nigra* were *α*-pinene, β-pinene, β-caryophyllene and germacrene D (Seziki *et al.*, 2010). Also, sizeable proportions of β-caryophyllene, α-pinene and 3-carene in *P. resinosa*; α-pinene, β-pinene and germacrene D in *P. flexilis*; as well as α-pinene, β-caryophyllene and germacrene D in *P. parviflora* (Kılıç & Koçak, 2014) have been reported.

Another observation was the presence of diterpenoid compounds in *Pinus* oils. *Pinus torreayana* oil was dominated by 4-epi-isocembrol, cembrene and thunbergol while *P. contorta* var. *contorta* was characterized by the high percentage of pimarinal and manool (Ioannou *et al.,* 2014). Interestingly, there is a group of essential oil of *Pinus* plants in which the presence of non-terpenoid compounds has been documented. Palmitic acid featured prominently in the essential oil of *P. roxburghii* (Labib *et al*., 2017). In addition, acetic acid and bicyclo[2.2.1]heptan-2-one were the main constituents of *P. nigra* (Kılıç & Koçak, 2014).

It is well known that there is the existence of intra-specific variations in the essential oil of *Pinus* essential oils. For example, while β-caryophyllene was identified as the main compound in *P. taeda* (Adams *et al*., 2014), another authors have reported the high contents of β-phellandrene, tricyclene, β-myrcene, β-pinene and α-pinene (Teixeira *et al*., 2016). In a report, *P. nigra* contained acetic acid and bicyclo[2.2.1]heptan-2-one (Kılıç & Koçak, 2014) when compared with data from other studies in which the oil was predominantly consist of α-pinene, β-pinene, limonene, β-caryophyllene and germacrene D (Seziki *et al*., 2010; Yener *et al.*, 2014).

The present report may represent the first of its kind aimed at the characterization of the volatile compounds of *P. dalatensis* and *P. armandii* subsp. *xuannhaensis*. It was observed that *P. dalatensis* and *P. kwangtungensis* oils were dominated by monoterpene hydrocarbon compounds. High contents of oxygenated sesquiterpene are present in *P. armandi* subsp. *xuannhaensis* with substantial amount of monoterpene compounds. According to the previous postulates (Teixeira *et al*., 2016), the main fraction of the essential oils from pine species are mostly monoterpene hydrocarbons, which is in agreement with the results obtained in the present study.

The essential oils of *Pinus* species have shown antifungal (Dambolena *et al*., 2016), cytotoxicity to cancer cell lines (Hoai *et al*., 2015), antioxidants (Yener *et al.*, 2014), antibacterial (Adams *et al*., 2014; Demirci *et al.*, 2015), antifungal (Ghanmi *et al*., 2006) and anti-inflammatory (Labib *et al*., 2017) activities. This may be attributed to the activity of the main compounds or synergy between the main and some minor constituents of the oils.

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