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Composition of the wood oils of *Calocedrus macrolepis*, *Calocedrus rupestris* and *Cupressus tonkinensis* (Cupressaceae) from Vietnam

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ABSTRACT

In the present investigation we studied the essential oil contents and compositions of three individual plants from Cupressaceae family cultivated in Vietnam. The air-dried plants were hydrodistilled and the oils analysed by GC and GC-MS. The components were identified by MS libraries and their RIs. The wood essential oil of *Calocedrus rupestris* Aver, H.T. Nguyen et L.K. Phan., afforded oil whose major compounds were sesquiterpenes represented mainly by α -cedrol (31.1%) and thujopsene (15.2%). In contrast, monoterpene compounds mainly α -terpineol (11.6%) and myrtenal (10.6%) occurred in *Calocedrus macrolepis* Kurz. The wood of *Cupressus tonkinensis* Silba afforded oil whose major compounds were also the monoterpenes namely sabinene (22.3%), α -pinene (15.2%) and terpinen-4-ol (15.5%). The chemotaxonomic implication of the present results was also discussed.

Keywords: *Calocedrus macrolepis*; *Calocedrus rupestris*; α -cedrol; *Cupressus tonkinensis*; Essential oil Composition; Terpenes.

1. Introduction

Calocedrus Kurz is small genus of the Cupressaceae family with trans-oceanic distribution typical for many relict taxa of the Tertiary floras [1]. *Calocedrus macrolepis* Kurz., is a medium-size tree up to 25-35 m tall, with a trunk up to 2 m in diameter. The bark is orange-brown weathering greyish, smooth at first, becoming fissured and exfoliating in long strips on the lower trunk on old trees. The foliage is produced in flattened sprays with scale-like leaves 1.5–8 mm long; they are arranged in opposite decussate pairs, with the successive pairs closely then distantly spaced, so forming apparent whorls of four; the facial pairs are flat, with the lateral pairs folded over their bases. The upper side of the foliage sprays is glossy green without stomata, the underside is white with dense stomata [2]. *Calocedrus rupestris* Averyanov, H.T. Nguyen et L.K. Phan., the most recently discovered living species of *Calocedrus*, was identified in Vietnam and first described in 2004. *C. rupestris* is an evergreen, monoecious trees up to 25 m tall and 100 cm wide with a broadly rounded crown. Bark 8-12 mm thick, gray-brown to brown, fissured, fibrous and exfoliating in longitudinal strips, with numerous large resin ducts. Leaves are decussate, in whorls of 4, scale-like, broadly obtuse to obtuse at apex, base decurrent. Leaves are dimorphic along the branchlet [2].

There are few literature reports on the volatile constituents of some *Calocedrus* species. Published results indicate that α -pinene (57.2% and 61.7%), limonene (0.5% and 13.9%) and myrcene (11.2% and 6.2%) respectively were the main compounds of *C. macrolepis* and *C. formosana*, while δ -3-carene (15.2–20.2%), limonene (18.2–23.6%), α -pinene (8.7–15.8%), terpinolene (5.7–8.0%) and α -fenchyl acetate (3.5–9.7%) occurred in *C. decurens* [3]. Another report [4] identified limonene (31.3%), δ -3-carene (21.0%) and α -pinene (9.2%) as the major constituents of *C. decurrens* from Pacific northwest, USA. Also, α -pinene (44.2%) limonene (21.6%), β -myrcene (8.9%) and β -caryophyllene (8.2%) were the main volatiles of *C. formosana* [5]. Moreover, α -pinene (63.8%), totarol (9.9%) and ferruginol (8.9%) occurred as main compounds of *C. formosana* from Taiwan [6]. The main compounds of *C. decurrens* from Oregon, USA [7] were thymoquinone (35.9%), carvacrol (29.2%) and p-methoxythymol (11.0%).

The major compounds of *C. macrolepis* var. *formosana* wood were *p*-cymene (24.4%), terpinen-4-ol (16.6%) and α -terpineol (12.5%)^[8]; while another analysis identified α -pinene (44.2%), limonene (21.6%), β -myrcene (8.9%) and β -caryophyllene (8.2%) in abundance^[9]. Chen et al^[10] studied the volatile emission from the leaf of *C. macrolepis* var. *formosana* on different occasions and concluded that the major constituents in the leaf essential oil were β -caryophyllene (17.64%), limonene (16.38%) α -pinene (11.19%) and cadalene (8.29%). However, on a sunny midday, the major volatiles were α -pinene (19.88%), myrcene (16.10%), (-)-limonene (54.29%) and β -caryophyllene (8.46%), while on a rainy day, the main compounds were α -pinene (52.14%), myrcene (9.84%), (-)-limonene (31.21%) and β -caryophyllene (6.29%). Benzoic acid (23.3%), dodecan-7-ol (14.6%) and trimethylenehexane (7.3%) were the main constituents of previously studied *C. macrolepis* from Vietnam^[11].

Essential oils from some *Calocedrus* species were reported to possessed antifungal^[5, 9] and antitermitic^[5, 12] properties. The non-volatile compounds isolated from *C. macrolepis* include diterpenoids and lignans^[13].

The genus *Cupressus* is one of several genera within the family Cupressaceae that have the common name cypress. It is considered a polyphyletic group. Based on genetic and morphological analysis, the *Cupressus* genus is found in the Cupressoideae subfamily. As currently treated, these cypresses are native to scattered localities in mainly warm temperate regions in the Northern Hemisphere, including western North America, Central America, northwest Africa, the Middle East, the Himalayas, southern China and northern Vietnam^[1]. *Cupressus tonkinensis* Silba. is an evergreen, medium-sized tree, up to 15-25 cm in height. Bark is grey brown with longitudinal fissures. Leaves are scaly, closely inserted on twigs. Cones are unisexual, grouped on a stalk. Male cone is subglobular. This is an endangered species in Vietnam, only found in a narrow area of the Central Region^[14]. Little is known about the chemical constituents and biological potential of this plant. *C. tonkinensis* produced monoterpene-rich oils whose composition were α -pinene (23.1%), sabinene (21.0%) and terpinen-4-ol (14.4%) in the leaf; as well as α -pinene (42.5%), myrcene (10.2%) and cedrol (9.0%) in the stem^[15]. Another investigation identified sabinene (29.34%), α -pinene (25.4%), 4-terpineol (13.91%) and γ -terpinen (5.5%) as major compounds of its leaf oil^[14]. It could be seen that relatively fewer studies exists on the volatile contents of these plant species. This arouses our interest to investigate the volatile oils of these of poorly studied Vietnamese grown flora.

2. Materials and methods

2.1 Plants Collection

The woods of *C. rupestris* were obtained from Phong Nha-Ke Bang National Park, Quang Binh Province, Vietnam, in September 2010 while those of *C. macrolepis* were harvested from Khanh Vinh district, Khanh Hoa Province, in August 2011. The woods of *C. tonkinensis* were collected from Huu Lien Natural reserve, Lang Son Province, Vietnam, in August 2010. Voucher specimens DND 726, DND 779 and DND 721 respectively were deposited at the Botany Museum, Vinh University, Vietnam. Plant samples were air-dried prior to extraction.

2.2 Isolation of the oils

0.5 kg of air-dried sample of each species was shredded and their oils obtained by hydrodistillation for 4 h at normal pressure,

according to the Vietnamese Pharmacopoeia^[17]. The plant samples yielded a low content of essential oils: 0.20%, 0.25% and 0.15% (v/ w) respectively for *C. rupestris*, *C. macrolepis* and *C. tonkinensis*. All the oil samples were yellow coloured.

2.3 Analysis of the oils

Gas chromatography (GC) analysis was performed on an Agilent Technologies HP 6890 Plus Gas chromatograph equipped with a FID and fitted with HP-Wax and HP-5MS columns (both 30 m x 0.25 mm, film thickness 0.25 μ m, Agilent Technology). The analytical conditions were: carrier gas H₂ (1 mL/min), injector temperature (PTV) 250 °C, detector temperature 260 °C, column temperature programmed from 40 °C (2 min hold) to 220 °C (10 min hold) at 4 °C/min. Samples were injected by splitting and the split ratio was 10:1. The volume injected was 1.0 μ L. Inlet pressure was 6.1 kPa.

An Agilent Technologies HP 6890N Plus Chromatograph fitted with a fused silica capillary HP-5 MS column (30 m x 0.25 mm, film thickness 0.25 μ m) and interfaced with a mass spectrometer HP 5973 MSD was used for the GC/MS analysis. The conditions were the same as described above with He (1 mL/min) as carrier gas. The MS conditions were as follows: ionization voltage 70 eV; emission current 40 mA; acquisitions scan mass range of 35-350 amu at a sampling rate of 1.0 scan/s. The MS fragmentation patterns were checked with those of other essential oils of known composition with Wiley (Wiley 9th Version), NIST 08 Libraries (on ChemStation HP), with those in the literature, and also with standard substances.

2.4 Identification of constituents

The identification of constituents was performed on the basis of retention indices (RI) determined by co-injection with reference to a homologous series of *n*-alkanes (C₅-C₃₀), under identical experimental conditions. Further identification was performed by comparison of their mass spectra with those from NIST 08 and Wiley 9th Version and the home-made MS library built up from pure substances and components of known essential oils, as well as by comparison of their retention indices with literature values^[18, 19].

3. Results & Discussion

In the present investigation, we studied new essential oil compositions of three individual plants from Cupressaceae family cultivated in Vietnam. The wood essential oil of *C. rupestris* afforded oil whose major classes of compounds were sesquiterpenes hydrocarbon (34.8%) and oxygenated derivatives (38.5%). Monoterpenes were less common (9.8% and 8.5% respectively for hydrocarbons and oxygenated derivatives). The main constituents of the oil are α -cedrol (31.1%) and thujopsene (15.2%). Less predominant compounds were δ -cadinene (4.6%), α -selinene (4.4%) and carvacrol methyl ester (4.9%). The authors are unaware of any literature report on the volatile constituents of this species, and as such this may represent the first of its kind.

In contrast, oxygen-containing monoterpenes (59.7%) was the major class compounds identified in *C. macrolepis*. Moreover, sesquiterpenes (10.0%) are less common. α -Terpineol (11.6%) and myrtenal (10.6%) are the main constituents of the oil. In addition, the less predominant compounds were bornyl acetate (5.6%) and carvacrol methyl ester (5.6%). Though monoterpene compounds were the main constituents of *C. macrolepis* from USA^[3], there are

both qualitative and quantitative variations between this study and the said report. The present investigation had an abundance of oxygen-containing monoterpene compounds while monoterpene hydrocarbons and non-terpenoids [3, 11] were the main compounds present in previous studies of oils of *C. macrolepis*. From Table 2, it could be seen that the oils of *C. macrolepis* is thought to exist in three chemotypic forms namely α -pinene/myrcene forms; benzoic acid/dodecan-7-ol form and α -terpineol/myrtenal form. Noteworthy observation is that α -cedrol, thujopsene, α -terpineol and myrtenal were not previously reported to be major constituents of any *Calocedrus* volatiles (Table 2). Moreover, compounds such as α -pinene, δ -3-carene, limonene, myrcene, thymoquinone, carvacrol, *p*-methoxythymol, γ -cadinene, benzoic acid, dodecan-7-ol, totarol and ferruginol, which are the major compounds of previously

studied *Calocedrus* species are either absent or detected in low quantities in the present investigation.

The From Table 3, it could be seen that *C. tonkinensis* produce monoterpene-rich (65.2% hydrocarbon and 19.5% oxygenated derivatives) oils whose composition were sabinene (22.3%), α -pinene (15.2%) and terpinen-4-ol (15.5%). The quantitative amount of sesquiterpene compounds is low (8.8%). The oil also features significant amounts of γ -terpinene (5.7%), elemol (5.0%) and β -myrcene (4.7%). The abundance of monoterpene compounds (sabinene, α -pinene and terpinen-4-ol) is in agreement with results previously obtained from the leaf and stem of this plant [14, 15]. Further analysis is necessary before a conclusion could be drawn on the chemotaxonomy of the oil of *C. tonkinensis*.

3.1 Tables

Table 1: Percentage composition and fragrance compounds of *Calocedrus rupestris* and *Calocedrus macrolepis*

Compounds ^a	RI (Cal.)	RI (Lit.)	Percent composition	
			<i>C. r</i>	<i>C. m</i>
Butanoic acid	772	763	1.1	-
Tricyclene	927	921	3.5	-
α -Pinene	939	932	3.5	1.5
Camphene	953	046	-	1.8
Sabinene	976	969	1.2	0.1
Myrcene	990	988	0.2	1.1
2,6-Dimethylhepten-2-ol	996	989	-	0.9
<i>p</i> -Cymene	1024	1020	0.6	0.2
Limonene	1032	1024	0.4	-
1,8-Cineole	1034	1026	-	0.3
Acetophenone	1060	1059	-	0.1
Camphenilone	1082	1078	-	0.4
α -Terpinolene	1089	1086	-	3.0
α -Phenylethanol	1110	1106	-	0.8
<i>endo</i> -Fenchol	1112	1114	-	1.3
<i>exo</i> -Fenchol	1122	1118	-	0.1
<i>allo</i> -Ocimene	1128	1128	0.4	-
1-Terpineol	1134	1130	-	2.1
<i>trans</i> -Pinocarveol	1135	1135	0.3	-
β -Terpineol	1140	1140	-	4.2
<i>trans</i> -Pinocamphone	1160	1158	-	3.2
Borneol	1165	1165	0.3	-
<i>trans</i> -Lilanol oxide (pyranoid)	1175	1170	-	0.5
Terpinen-4-ol	1177	1174	1.6	2.0
1-(4-methylphenyl)-Ethanone	1183	1181	-	0.7
α -Terpineol	1189	1186	0.5	11.6
Myrtenal	1195	1195	-	10.6
γ -Terpineol	1203	1199	-	2.2
Verbenone	1205	1204	0.3	3.9
Carvacrol methyl ether	1245	1241	4.9	5.6
Piperitone	1252	1249	-	4.4
Bornyl acetate	1289	1287	-	5.6
Thymol	1291	1289	-	1.5
Cuminol (<i>p</i> -Cymen-7-ol)	1293	1291	-	0.1
Carvacrol	1298	1298	0.4	-
α -Cubebene	1351	1345	1.9	-
α -Copaene	1377	1374	0.6	-
<i>iso</i> -longifolene	1387	1389	0.4	-
Methyl eugenol	1407	1410	0.2	-
β -Caryophyllene	1419	1417	1.9	-
<i>cis</i> -Thujopsene	1431	1429	15.2	-
α -Humulene	1454	1452	0.3	-
β -Chamigrene	1475	1476	0.9	-
α -Selinene	1494	1498	4.4	-

<i>cis</i> -Cadina-1,4-diene	1496	1495	2.3	0.1
α -Murolene	1500	1500	0.5	-
Lepidozene	1502	1502	0.5	-
β -Bisabolene	1506	1505	-	0.4
(<i>E,E</i>)- α -Farnesene	1506	1505	1.3	-
Acetoveratrone	1520	1520	-	2.1
δ -Cadinene	1525	1522	4.6	6.5
Elemol	1550	1548	0.5	-
(<i>E</i>)-Nerolidol	1563	1561	-	0.2
Spathulenol	1579	1577	0.6	-
Caryophyllene oxide	1583	1582	1.0	0.1
α -Cedrol	1601	1600	31.1	-
β -Cedre-9-one	1633	1630	1.3	-
<i>epi</i> - α -Cadinol	1633	1638	-	0.4
α -Cadinol	1654	1652	2.1	-
10- <i>nor</i> -Calamene-10-one	1702	1702	1.9	0.2
Benzyl benzoate	1760	1759	-	7.8
Tetradecanoic acid	1770	1769	-	0.2
<i>n</i> -Octadecane	1800	1800	-	0.1
<i>n</i> -Nonadecane	1900	1900	-	0.3
Hexadecanoic acid	1960	1959	-	0.3
<i>n</i> -Eicosane	2000	2000	-	2.2
<i>n</i> -Heneicosane	2100	2100	-	0.3
<i>n</i> -Docosane	2200	2200	-	0.6
<i>cis</i> -Totarol methyl ester	2208	2208	0.3	0.6
<i>trans</i> -Ferruginol	2325	2331	-	0.1
Total			93.0	92.3
Monoterpene hydrocarbons			9.8	7.7
Oxygenated monoterpenes			8.5	59.7
Sesquiterpene hydrocarbons			34.8	9.1
Oxygenated sesquiterpenes			38.5	0.9
Diterpenoids			0.3	0.7
Non-terpenoids			1.1	14.2

^a Elution order on HP-5MS column Ri (Cal.) = Retention indices on HP-5MS capillary column; RI (Lit.) = Literature Retention indices (see Materials and method); Tr, trace amounts < 0.1%; *C. m* = *Calocedrus macrolepis*; *C. r* = *Calocedrus rupestris*

Table 2: Main compounds of volatile oils of studied *Calocedrus rupestris* plants

Species (part)	Origin	Major constituents	References
<i>C. macrolepis</i> var. <i>formosana</i> (wood)	Taiwan	<i>p</i> -cymene (24.4%), terpinen-4-ol (16.6%) and α -terpineol (12.5%)	⁸
“ (leaf)	Taiwan	α -pinene (44.2%), limonene (21.6%), β -myrcene (8.9%) and β -caryophyllene (8.2%)	⁹
“ (leaf)	Taiwan	β -caryophyllene (17.64%), limonene (16.38%) α -pinene (11.19%) and cadalene (8.29%)	¹⁰
“ (leaf)	“	α -pinene (19.88%), myrcene (16.10%), (-)-limonene (54.29%), and β -caryophyllene (8.46%)	“
“ (leaf)	“	α -pinene (52.14%), myrcene (9.84%), (-)-limonene (31.21%) and β -caryophyllene (6.29%)	“
“ (unknown)	China	limonene (12.4%), α -pinene (5.8%), α -cadinol (5.1%)	²⁰
<i>C. formosana</i> (seed)	Taiwan	α -pinene (63.8%), totarol (9.9%) and ferruginol (8.9%)	⁶
“ (leaf)	Taiwan	α -pinene (44.2%) limonene (21.6%), β -myrcene (8.9%) and β -caryophyllene (8.2%)	⁵
“ (leaf)	Taiwan	α -pinene (61.7%) limonene (13.9%) and myrcene (6.2%)	³
<i>C. decurrens</i> (leaf)	USA	δ -3-carene (15.2–20.2%), limonene (18.2–23.6%), α -pinene (8.7–15.8%), terpinolene (5.7–8.0%) and α -fenchyl acetate (3.5–9.7%)	“
<i>C. decurrens</i> (heartwood)	Oregon, USA	thymoquinone (35.9%), carvacrol (29.2%), <i>p</i> -methoxythymol (11.0%)	⁷
“ (unknown)	USA	limonene (31.3%), δ -3-carene (21.0%) and α -pinene (9.2%)	⁴
<i>C. macrolepis</i> (leaf)	China	α -pinene (57.2%) and myrcene (11.2%)	³
“ (stem)	Vietnam	benzoic acid (23.3%) and dodecan-7-ol (14.6%) and trimethylhexane (7.3%)	¹¹
“ (wood)	Vietnam	α -terpineol (11.6%) myrtenal (10.6%), bornyl acetate (5.6%) and carvacrol methyl ester % (5.6%)	This study
<i>C. rupestris</i> (wood)	Vietnam	α -cedrol (31.1%) and thujopsene (15.2%)	This study

Table 3: Percentages of volatile composition of *C. tonkinensis*

Compounds ^a	RI (Cal.)	RI (Lit.)	Percent (%)
Tricyclene	927	921	0.1
α -Thujene	931	924	3.9
α -Pinene	939	932	15.2
Camphene	953	946	0.3
Sabinene	976	969	22.3
β -Pinene	980	974	0.7
β -Myrcene	990	988	4.7
α -Phellandrene	1006	1002	0.1
α -Terpinene	1016	1014	3.7
<i>p</i> -Cymene	1024	1020	1.3
β -Phellandrene	1030	1025	0.9
Limonene	1032	1024	3.9
(E)- β -Ocimene	1052	1044	0.2
γ -Terpinene	1061	1054	5.7
<i>cis</i> -Sabinene hydrate	1070	1065	1.8
α -Terpinolene	1089	1086	2.2
1-Terpineol	1134	1130	0.5
Camphor	1146	1141	Tr
Terpinen-4-ol	1177	1174	15.5
α -Terpineol	1189	1186	0.8
<i>cis</i> -Piperitol	1196	1195	0.2
<i>trans</i> -Piperitol	1208	1207	0.4
Bornyl acetate	1289	1287	0.1
α -Terpinenyl acetate	1349	1346	0.2
Isolatedene	1373	1374	0.1
β -Elemene	1391	1389	0.1
β -Caryophyllene	1419	1417	0.4
dehydro-Aromadendrene	1463	1460	0.1
<i>epi</i> -Bicyclosesquiphellandrene	1478	1487	0.2
Germacrene D	1485	1484	0.3
β -Selinene	1490	1489	0.2
γ -Cadinene	1514	1513	0.2
δ -Cadinene	1459	1522	0.2
Elemol	1550	1548	5.0
Caryophyllene oxide	1583	1582	0.2
α -Cedrol	1601	1600	0.2
γ -Eudesmol	1632	1630	0.6
β -Eudesmol	1651	1649	1.0
<i>ent</i> -Pimara-8(14),15-diene	1939	1939	0.8
Podocarpa-8,11,13-triene	2054	2054	3.0
<i>trans</i> -Totarol	2314	2314	1.3
<i>cis</i> -Ferruginol	2371	2370	0.1
Total			97.7
Monoterpene hydrocarbons			65.2
Oxygenated monoterpenes			19.5
Sesquiterpene hydrocarbons			1.8
Oxygenated sesquiterpenes			7.0
Diterpenoids			5.6

^a Elution order on HP-5MS column Ri (Cal.) = Retention indices on HP-5MS capillary column; RI (Lit.) = Literature Retention indices (see Materials and method); Tr, trace amounts < 0.1%;

4. Conclusion

Therefore, we may deduce the following chemical classifications for the volatile contents of known *Calocedrus* species.

- (i) Oils rich in α -pinene/limonene/myrcene (*C. macrolepis* var. *formosana*, *C. formosa* and *C. macrolepis*)
- (ii) Oil whose major constituents are limonene/ δ -3-carene/ α -pinene (*C. decurrens*)
- (iii) Oils containing abundance of myrtenol/myrtenyl / γ -cadinene (*C. macrolepis* var. *formosana*)
- (iv) Oil whose major constituents are α -terpineol/myrtenal/ bornyl acetate (*C. macrolepis*)
- (v) Oil with significant quantities of α -cedrol/ thujopsene/ δ -cadinene (*C. rupestris*).
- (vi) Oil rich in *p*-cymene/terpinen-4-ol/ α -terpineol (*C. macrolepis* var. *formosana*)
- (vii) Oil with abundance of thymoquinone/carvacrol/ *p*-methoxythymol (*C. decurrens*)

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