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Terpene Composition of Three Species of Gymnosperms from Vietnam

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ABSTRACT

In the present investigation, we studied new 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 Gas chromatography (GC) and Gas chromatography couples with Mass spectrometry (GC-MS). The components were identified by comparison of their retention indices on HP-5 MS capillary column with literature reference and MS pattern with authentic library's collection. *α*-Pinene (36.0%), *α*-cedrol (18.3%) and thujopsene (5.7%) were the main constituents of *Cupressus tonkiensis* Silba. Monoterpenes were the quantitative significant compounds of *Sabina chinensis* (L.) Antoine. These are bornyl acetate (19.5%), sabinene (17.3%) and *α*-pinene (15.8%). Moreover, the main compounds of *Thuja orientalis* L., were identified as *α*-pinene (34.1%), *α*-cedrol (16.5%), *β*-caryophyllene (5.4%) and *β*-selinene (5.2%). The chemotaxonomy implication of these results was also discussed.

Keywords: *Cupressus tonkiensis*; *Sabina chinensis*; *Thuja orientalis*; Bornyl Acetate; *α*-Cedrol; *α*-Pinene; Sabinene

1. Introduction

The genus *Cupressus* is one of several genera within the family Cupressaceae that has 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 [1]. 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 [2]. *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 [3]. Little is known about the chemical constituents and biological potential of this plant. Leaves and stems of *C. tonkinensis* produce monoterpene-rich oils whose composition is *α*-pinene (23.1%), sabinene (21.0%) and terpinen-4-ol (14.4%) in the leaf while, *α*-pinene (42.5%), myrcene (10.2%) and cedrol (9.0%) were identified in the stem [4]. Another investigation identified sabinene (29.34%), *α*-pinene (25.4%), 4-terpineol (13.91%) and *γ*-terpinene (5.5%) as major compounds of its leaf oil [3].

Sabina chinensis (L.) Antoine is an evergreen tree that grows up to 20 m high. The leaves are basal while the yellow flowers are unisexual [5]. *S. chinensis* is one of the important species used in Chinese garden and has a long history. It has many uses and more than 100 cultivars. The first report on the main constituents of *S. chinensis* identified sabinene (46.73%), *α*-pinene (17. 03%), terpinen-4-ol (5.34%) and limonene (4.48%) as major constituents [6]. Another analysis [7] reported an abundance of *α*-pinene (21.1%), sabinene (18.2%) and limonene (9.2%) in the oil. Cui *et al*. [8] demonstrated that the main substances in volatile oil *S. chinensis* were bornyl acetate (38.1%), *α*-phellandrene (24.0%) and *p*-menth-l-en-4-o1 (12.4%). Another report [9] described the main composition of *S. chinensis* to be sabinene (20.99%), limonene (19.78%) and bornyl acetate (11.68%).

The oil composition of two other species in the genus has been documented. The major compounds of *S. virginiana* [10] from Japan were limonene (32.9%), safrole (23.0%), asarone (15.9%) and *α*-pinene (5.2%). *S. chi-*

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nensis cv. Kaizuca leaf oil, as reported by Cui *et al*. [10] contained bornyl acetate (46.5%), limonene (30.0%) and β-pinene (7.9%), while limonene (24.56%) and β-myrcene (8.04%) are the prominent compounds from sample analysed by Hao *et al*. [9]. The volatile oil from *S. chinensis* had greater effects of bacteriostasis and air decontamination [7,8,11]. It is also indicated that constituents such as bornyl acetate, phellandrene, limonene and alpha-pinene and beta-pinene could also contribute to the antimicrobial activity of the volatile oils [11].

Thuja occidentalis (syn. *Platycladus orientalis* (L.) Franco or *Biota orientalis* (L.) Endl.) is an evergreen coniferous tree, in the cypress family Cupressaceae, which is native to the northeast of the United States and the southeast of Canada, but widely cultivated as an ornamental plant. *T. occidentalis* has fan-like branches and scaly leaves. It is only a small tree, growing to a height of 10 - 20 m. The bark is red-brown, furrowed and peels in narrow, longitudinal strips. The cones are slender, yellow-green ripening brown, 10 - 15 mm broad, with 6 - 8 overlapping scales. The branches may take root if the tree falls. Plants are susceptible to attacks by honey fungus [12]. Plants are monoecious, male catkins being produced at the tips of branches and female cones at the base [12]. The essential oil from the leaf of *P. orientalis* is an important natural product which is used in fragrance, air freshener, deodorizer, insectifuge, and aromatherapy.

The essential oil composition of the leaf of *T. orientalis* was investigated in Xuzhou, China [13], where its main components were α-pinene (40.2%), 3-carene (13.2%), and cedrol (9.3%). The data reported from Iran [14-16] showed that the main constituents were α-pinene (15.0% - 30.0%), 3-carene (10.5% - 21.7%), and cedrol (6.1% - 20.3%). Previous studies in China [17-20] had revealed that the main constituents were α-pinene (8.4% - 24.9%), 3-carene (2.6% - 12.2%), and cedrol (0.7% - 31.4%). In a recent report [21], a population of *T. orientalis* afforded oils whose major compounds were α-pinene (37.6% - 68.1%), cedrol (3.4% - 18.1%), 3-carene (0.1% - 29.4%), β-caryophyllene (2.0% - 10.2%) and α-caryophyllene (1.7% - 7.1%) while a sample contained cedrol (15.0%), 3-carene (12.2%) and β-caryophyllene (8.0%), yet another sample had cedrol (19.2%), β-caryophyllene (14.3%) and α-caryophyllene (11.3%) while the last sample contained cedrol (23.7%), terpinenyl acetate (19.4%), 3-carene (10.6%) and α-pinene (10.2%). α- and β-Thujone, fenchone, camphor and sabinene, as well as the diterpenes beyerene and rimuene were the main compounds in the work of Tsiri *et al*. [22] and Chizzola *et al*. [23]. In their own investigation, Guleria *et al*. [24] identified α-pinene (29.2%), δ-3-carene (20.1%), α-cedrol (9.8%), caryophyllene (7.5%), α-humulene (5.6%) and limonene (5.4%) in the oil of the plant. The dominant constituents in essential oil of *T. orientalis* from most localities are a-pinene, cedrol, and 3-carene. The previous studies also reveal a large variation in the essential oil composition of *T. orientalis*.

In the present paper, we report the essential oil compositions of three gymnosperms namely *Cupressus tonkinensis* Silba., *Sabina chinensis* (L.) Antoine and *Thuja occidentalis* L., from Vietnam. This is part of our extensive research on the volatile composition of Vietnamese flora as they are made available [25].

2. Materials and Methods

2.1. Plants Collection

Leaves of *C. tonkiensis* were collected from Huu Lien Natural reserve, Lang Son Province, Vietnam, in August 2010. The leaves of *S. chinensis* and *T. orientalis* were collected from Nghe An Province in August 2011. Voucher specimens DND 721, DND 194 and DND 196, respectively have been deposited at the Botany Museum, Vinh University, Vietnam. Plant samples were air-dried prior to extraction.

2.2. Isolation of the Volatile 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 [26]. The plant samples yielded a low content of essential oils: 0.20%, 0.18% and 0.20% (v/w; respectively for *C. tonkiensis*, *S. chinensis* and *T. orientalis*). Oil samples were light yellow colored.

2.3. Gas Chromatography (GC) Analysis

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 × 0.25 mm, film thickness 0.25 μm, Agilent Technology). The analytical conditions were: carrier gas H_2 (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 μL. Inlet pressure was 6.1 kPa.

2.4. Gas Chromatography/Mass Spectrometry Analysis/(GC-MS)

An Agilent Technologies HP 6890N Plus Chromatograph fitted with a fused silica capillary HP-5 MS column (30 m × 0.25 mm, film thickness 0.25 μm) and interfaced with a mass spectrometer HP 5973 MSD was used for the GC/MS analysis, under the same conditions as those used for GC 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 70eV; emission current 40 mA; acquisitions scan mass range of 35 - 350 amu at a sampling rate of 1.0 scan/s.

2.5. 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 under identical experimental conditions. Further identification was performed by comparison of their mass spectra with those from NIST 08 Libraries (on ChemStation HP) 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 [27,28].

3. Results and Discussion

The identities of compounds identified in the oil samples could be seen in **Table 1**. A total of 33 compounds were identified in the oil of *C. tonkinensis*, representing 94.0% of the oil content. The oil composed largely of monoterpenes (50.1%) and sesquiterpenes (40.7%) represented largely by α-pinene (36.0%) and α-cedrol (18.3%), along with minor quantities of thujopsene (5.7%) and δ-3-carene (3.1%). Monoterpene compounds namely α-pinene, sabinene, terpinen-4-ol and myrcene were the representa-

Table 1. Terpene composition of the oil samples.

Compounds[a]	RI[b]	RI[c]	*C.p*	*S.c*	*T.o*
Butanoic acid	802	763	3.1	-	-
Tricyclene	927	921	-	0.5	0.2
α-Thujene	931	924	-	1.1	-
α-Pinene	939	932	36.0	15.8	34.1
Camphene	953	946	0.8	0.8	0.8
Verbenene	962	961	-	-	0.3
Sabinene	976	969	0.6	17.3	1.2
β-Pinene	980	974	1.8	0.6	3.1
β-Myrcene	990	988	1.8	5.7	4.3
α-Phellandrene	1006	1002	-	0.1	Tr
δ-3-Carene	1013	1008	3.1	-	0.2
α-Terpinene	1016	1014	-	1.9	0.5
p-Cymene	1024	1020	0.3	0.1	0.1
Limonene	1032	1024	-	6.0	4.9
(*E*)-β-Ocimene	1052	1044	-	Tr	0.2
γ-Terpinene	1061	1054	-	2.8	0.3
cis-Sabinene hydrate	1070	1065	-	0.2	-
α-Terpinolene	1089	1086	0.4	1.5	3.7
Linalool	1100	1095	1.0	0.2	Tr
n-Nonanal	1106	1100	-	-	Tr
cis-*p*-Menth-2-en-1-ol	1117	1118	-	0.2	-
allo-Ocimene	1128	1128	-	-	0.1
trans-Pinocarveol	1139	1135	0.5	-	-
Camphor	1146	1141	0.6	-	-
Terpinen-4-ol	1177	1174	1.0	1.6	0.1
Methyl chavicol	1196	1195	-	0.4	-
Verbenone	1205	1204	0.5	-	-
Carvacrol methyl ether	1245	1241	1.7	-	-
Linalyl acetate	1257	1254	-	-	Tr
2-Decenal	1259	1260	-	-	Tr
1-Decanol	1276	1266	-	-	Tr
Bornyl acetate	1289	1287	-	19.5	0.7
Linalyl propanoate	1335	1334	-	Tr	Tr
α-Cubebene	1351	1345	1.2	-	-

Continued

Neryl acetate	1362	1359	-	-	0.1
α-Ylangene	1375	1373	-	-	Tr
α-Copaene	1377	1374	-	0.1	0.2
β-Bourbonene	1385	1387	-	Tr	0.1
β-Elemene	1397	1398	1.4	0.1	0.3
Methyl eugenol	1407	1410	-	0.3	-
α-Cedrene	1409	1410	-	-	0.3
α-Gurjunene	1410	1409	0.6	-	-
β-Caryophyllene	1419	1417	0.6	-	5.4
cis-Thujopsene	1431	1429	5.7	-	-
β-Gurjunene	1434	1431	-	0.1	-
α-Guaiene	1440	1437	1.0	-	-
Aromadendrene	1441	1439	-	-	0.6
Widdrene	1444	-	-	-	0.3
α-Humulene	1454	1452	0.6	-	-
trans-β-Farnesene	1455	1454	-	-	0.1
allo-Aromadendrene	1460	1458	-	-	0.2
β-Acoradiene	1470	1469	-	-	0.2
ar-Curcumene	1475	1479	-	-	0.1
γ-Gurjunene	1477	1475	-	0.1	-
γ-Muurolene	1480	1478	0.8	-	-
Germacrene D	1485	1484	-	0.7	0.9
α-Amorphene	1486	1483	-	Tr	0.8
β-Selinene	1490	1489	-	-	5.2
Zingiberene	1494	1493	1.8	-	1.6
cis-Cadina-1,4-diene	1496	1495	1.1	-	0.3
α-Selinene	1498	1498	-	-	0.5
α-Muurolene	1500	1500	-	0.2	0.2
γ-Patchoulene	1502	1502	-	-	0.1
Lepidozene	1502	-	-	-	0.2
Cuparene	1505	1504	2.2	-	-
β-Bisabolene	1506	1505	-	-	0.3
(*E*, *E*)-α-Farnesene	1506	1505	0.9	-	-
γ-Cadinene	1514	1513	-	0.1	0.3
trans-γ-Bisabolene	1517	1514	-	-	0.1
δ-Cadinene	1525	1522	2.1	0.6	0.7
Elemol	1550	1548	0.6	6.4	0.1
3-Hexen-1-ol, benzoate	1551	-	-	0.1	0.2
Dodecanoic acid	1567	1565	-	-	Tr
Germacrene-D-4-ol	1574	1574	-	-	0.2
Spathulenol	1579	1577	-	-	1.3
Caryophyllene oxide	1583	1582	-	0.1	1.6
α-Cedrol	1601	1600	18.3	-	26.5
β-Oplopenone	1608	1607	-	0.4	-
Tetradecanal	1611	1611	-	-	Tr
γ-Eudesmol	1632	1630	-	0.4	-
τ-Muurolol	1646	1640	1.6	1.5	0.3
β-Eudesmol	1651	1649	0.7	-	-
α-Cadinol	1654	1652	-	-	0.5
α-Bisabolol	1686	1685	0.4	-	-

Continued

Franesol[d]	1718	-	-	-	0.1
Benzyl benzoate	1760	1759	-	Tr	-
Tetradecanoic acid	1770	-	-	-	-Tr
1,2-Benzenedicarboxylic acid	1917	1917	-	1.0	2.9
16-Hexadecanolide	1928	-	-	-	0.2
ent-Pimara-8(14),15-diene	1939	-	-	0.3	-
Hexadecanoic acid	1970	1959	-	Tr	Tr
Abietatriene	2050	2055	-	0.7	0.2
Phytol	2125	1942	-	-	Tr
Totarol	2314	2314	-	0.1	-
cis-Ferruginol	2371	2370	-	0.1	0.1
(*Z*)-9-Octadecenamide	2398	2396	-	-	0.1
Total			**94.0**	**90.0**	**98.4**
Monoterpene hydrocarbons			**44.8**	**53.6**	**54.2**
Oxygenated monoterpenes			**5.3**	**23.5**	**0.9**
Sesquiterpene hydrocarbons			**20.0**	**2.0**	**19.2**
Oxygenated sesquiterpenes			**20.7**	**8.8**	**20.6**
Diterpenes			**-**	**1.2**	**0.1**
Non-terpenes			**3.1**	**1.1**	**3.4**

[a]Elution order on HP-5MS capillary column; [b]Retention indices on HP-5MS capillary column; [c]Literature Retention indices (see Experimental); [d]Correct isomer not identified; - Not identified and not present in Literature; Tr, Trace amount, <0.1%; *C. p = Cupressus tonkinensis*; *S. c = Sabina chinensis*; *T. o = Thuja orientalis*.

tive compounds of previous studies on *C. tonkinensis* [3, 4]. The *α*-pinene content of this oil competes favourably with previous studies while the contents of other monoterpenes above are significantly lower. Cedrol was previously present in the stem oil but not in the leaf [4].

A total of 45 compounds could be identified from the leaf oil of *S. chinensis*, representing 90.0% of the oil content. Also, monoterpenoids (77.1%) were in abundance in the oil. The major constituents among the monoterpenes were bornyl acetate (19.5%), sabinene (17.3%) and *α*-pinene (15.8%). The large amount of *α*-pinene and sabinene is in agreement with previous reports [6-9], but differs from its lower contents of tricyclene, *β*-myrcene, *α*-phellandrene, limonene and *p*-menth-l-en-4-o1. Sesquiterpenes are less common (10.8%) represents mainly by elemol (6.4%) and *τ*-muurolol (1.5%) as compounds above 1%. There seems to be homogeneity in the oil composition of *S. chinensis*. Bornyl acetate was among the main compounds of *S. chinensis* cv. Kaizuca [10]. Limonene is a common compound of the oil of the genus *Sabina* [6-10].

Monoterpenes (55.1%) and sesquiterpenes (39.8%) are classes of compounds identified in the 69 constituents of the oil of *T. orientalis*. The main compounds were *α*-pinene (34.1%) and *α*-cedrol (16.5%). Other significant compounds were *β*-caryophyllene (5.4%), *β*-selinene (5.2%), limonene (4.9%) and *α*-terpinene (3.7%). The predominant compounds were *α*-pinene and cedrol. This result was similar to those previously reported [13-21,24, 29,30-32], which revealed that the main constituents were a-pinene (15.0% - 68.1%), cedrol (3.4% - 39.06%) and 3-carene (0.1% - 34.55%). However, the content of 3-carene in this sample is too low when compared with previous studies (**Table 2**). Other compounds such as *β*-caryophyllene, *α*-caryophyllene and terpinenyl acetate [21], *α*-and *β*-thujone, fenchone, sabinene, camphor, beyerene and rimuene [22,23], fenchol and *β*-santalene [32] and cedrene [19], that are characteristics of previous reports are either observed in low amounts (e.g. *δ*-3-carene, sabinene) or completely absent (e.g. *α*- and *β*-thujone, fenchone, fenchol, terpinenyl acetate, *β*-santalene, *α*-caryophyllene, cedrene, beyerene and rimuene) in this present study (**Tables 1** and **2**).

The previous studies also revealed a large variation in the essential oil composition of *T. orientalis*. Therefore, the oils of *T. orientalis* could be classified as follows:

1) group characterized by a predominant *α*-pinene, *δ*-3-carene and cedrol [13-18,20,21,24,29 and this study]

2) group dominated by cedrol, *δ*-3-carene and *β*-caryophyllene [21]

3) group with contents of cedrol, *β*-caryophyllene and *α*-caryophyllene

4) *α*-pinene, *δ*-3-carene and sabinene group [14]

5) *α*-thujone, *β*-thujone and fenchone rich oil [22]

Table 2. Major constituents of essential oils of *T. orientalis*.

Origin	Major Constituents	Ref
Vietnam (l)	α-cedrol (16.5%), α-pinene (34.1%), β-caryophyllene (5.4%) and β-selinene (5.2%)	This study
China (l)	α-pinene (40.2%), 3-carene (13.2%) and cedrol (9.3%)	13
Iran (w)	α-pinene (15.0%), 3-carene (10.5%) and cedrol (20.3%)	14
Iran (l)	α-pinene (15.2%), 3-carene (12.0%), cedrol (11.7%) and sabinene (10.0%)	14
Iran (f)	α-pinene (23.5%), 3-carene (23.8%) and sabinene (11.1%)	14
Iran (l)	α-pinene (21.9%), α-cedrol (20.3%), δ-3-carene (10.5%) and limonene (7.2%)	15
Iran (f)	α-pinene (52.4%), δ-3-carene (14.2%), α-cedrol (6.5%) and β-phellandrene (5.1%)	15
Iran (l)	α-pinene (30.0%), δ-3-carene (21.7%), β-caryophyllene (6.9%), and cedrol (6.1%)	16
Iran (st)	δ-3-carene (24.3%), α-pinene (15.4%), cedrol (17.7%), sabinene (8.2%), α-humulene (6.1%)	16
Iran (f)	α-pinene (38.7%), δ-3-carene (20.4%) and α-fenchene (5.0%)	16
China (l)	α-pinene (8.4%), 3-carene (12.2%) and cedrol (10.7%)	17
China (l/tw)	cedrol (30%) and α pinene (5%)	18
China (l)	cedrene (41.25%), cedrol (39.06%), cedrol (31.43%)	19
China (l)	α-pinene (24.9%), 3-carene (2.6%) and cedrol (12.7%)	20
China (l)	α-pinene (37.6% - 68.1%), cedrol (3.4% - 18.1%), 3-carene (0.1% - 29.4%), β-caryophyllene (2.0% - 10.2%) and α-caryophyllene (1.7% - 7.1%)	21
China (l)	cedrol (15.0%), 3-carene (12.2%) and β-caryophyllene (8.0%)	21
China (l)	cedrol (19.2%), β-caryophyllene (14.3%) and α-caryophyllene (11.3%)	21
China (l)	cedrol (23.7%), terpinenyl acetate (19.4%), 3-carene (10.6%) and α-pinene (10.2%)	21
Poland	α- and β-thujone, fenchone, sabinene, beyerene and rimuene[a]	22
Austria (tw)	camphor (27.8%), fenchone (18.9%), α-thujone (6.8%) and β-thujone (5.3%)	23
China (l)	α-pinene (29.2%), δ-3-carene (20.1%), α-cedrol (9.8%), caryophyllene (7.5%), α-humulene (5.6%) and limonene (5.4%)	24
Pakistan (l)	α-pinene (40.6%), 3-carene (26.0%) and cedrol(10.7%)	29
Pakistan (f)	α-pinene (41.8% - 45.61%), 3-carene (32.69% - 34.55%) and cedrol (1.82% - 4.50%)	30
Iran (dp)	α-pinene, β-pinene, sabinene, δ-3-carene and terpinolene[a]	31
Nigeria (l)	β-santalene (12.19%), cedrol (11.66%), fenchol (10.66%) and β-elemene (7.81%)	32

[a]Quantitative data not available; l = leaf; f = fruit; st = stem; dp = different parts; tw = twig.

6) camphor, fenchone and α-thujone group [23]
7) α-pinene, β-pinene and sabinene group [31]

4. Conclusion

Quantitative and qualitative variations were observed between these oils of Cupressaceae family cultivated in Vietnam and elsewhere. This may be attributed to factors such as the place of collection, age and nature of the plant, climatic conditions, handling procedures etc.

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