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The stem essential oil of *Taxus chinensis* (Rehder & E.H. Wilson) Rehder (Taxaceae) from Vietnam

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Abstract
This paper report the volatile compounds identified in the essential oil hydrodistilled from the stem of *Taxus chinensis* (Rehder & E.H. Wilson) Rehder (Taxaceae) from Vietnam. The chemical constituents of the oil were analysed by gas chromatography (GC) and gas chromatography coupled with mass spectrometry. The yield of the yellow oil was 0.12% (v/w), calculated on a dry weight basis. Monoterpene hydrocarbons (42.1%), oxygenated monoterpenes (17.7%) and oxygenated sesquiterpenes (25.8%) were the main classes of compounds identified in the oil. The major constituents of the oil were α-pinene (34.8%) and caryophyllene oxide (17.1%).

Keywords *Taxus chinensis*, essential oil composition, monoterpenes, sesquiterpenes

1. Introduction
The species of *Taxus* are more geographically than morphologically separable. All species are poisonous; most contain the anti-cancer agent taxol [1]. *Taxus chinensis* or Chinese yew belongs to the family *Taxaceae*. The plan is an evergreen, coniferous shrubs or small tree that grows to mature heights of 60 feet (20 m) tall. The trunk of *T. chinensis* can measure up to 60 in (150 cm) in diameter. *T. chinensis* grows with a rounded or pyramidal crown of numerous, ascending to erect branches. The needle leaves are distichous with a short-lanceolate to oblong shape, measuring 0.6 to 0.8 in (15- 20 mm) long and 0.08 to 0.13 in (2 - 3.2 mm) broad. The bark is thin, red-brown or purple-brown to gray in color. The slender twigs are round, finely grooved along the leaf bases. The seeds are slightly flattened ovoid in shape about 0.2 to 0.32 in (5-8 mm) long and 0.14 to 0.2 in (3.5 - 5 mm) broad. The young green seeds turn dark brown at maturity [2, 3].
Some of the biologically active compounds isolated from *T. chinensis* include 13,15-epoxy-13-epi-taxayunnasin A and taxchinin N, were isolated from the leaves and stems [4], paclitaxel or taxol [5] and 2- deacetoxy-7,9-dideacetyltaxinine J [6]. Sciadopitysin isolated from *T. chinensis* inhibits the Aβ aggregation and reduce Aβ-induced toxicity in the primary cortical neurons [7]. Paclitaxel and 2α-benzoxy-4α,9α,10β,13α-tetraacetoxytax-11-ene showed anti-cancer property [8]. The taxoids, 2,20-*O*-diacetyltaxumairol N and 14*β*-hydroxy-10-deacetyl-2-*O*-debenzoylbacatin III, isolated from the needles and stems of *T. chinensis*, exhibited weak cytotoxicity activity against T-24 and QGY-7701 cancer cell lines [9]. Flavonoid compounds such as amentoflavone, quercetin and ginkgetin were also isolated from *T. chinensis* [10].
The chemical constituents of essential oils from *T. chinensis* have not been the subject of literature discussion. However, the volatile constituents identified from some other *Taxus* oil samples have been published. The dominant compounds of the leaves of *T. media* were benzene propanenitrile (21.30%), 1,4-dioxane-2,3-diol (20.13%), 3-bromo-3-methyl-butyric acid (17.92%) and 1-hydroxy-2-butanone (9.85%) while *T. chinensis* var. *mairei* was dominated by benzene propanenitrile (49.39%) and 1-hydroxy-2-butanone (12.72%) [11]. Also, palmitic acid (35.66%) and 9-hexadecenoic acid (11.28%) were also identified from the leaf oil of *T. chinensis* var. *mairei* [12]. Likewise, abundance of hexadecanoic acid (21.19%) and tetradecanoic acid (8.87%) in the leaf of *T. chinensis* var. *mairei* has been reported [13]. Moreover, *cis*-vaccenic acid (36.73% and 36.96%) and (*E*)-palmitoleic acid (23.66% and 24.05%) were also identified in the essential oil of leaves of *T. chinensis* var. *mairei* [14]. Recent study indicated that *n*-hexadecanoic acid (5.97%-19.37%) and phthalic acid, mono (2-ethylhexyl) ester (0.93%-26.38%) were the main compounds in the aerial stem oils of

T. chinensis var. *mairei* [15]. 1-Octen-3-ol (32.4%), (*E*)-2-hexen-1-ol (8.2%), caryophyllene oxide (7.2%) and hexahydrofarnesyl acetone (6.8%) were identified as the main compounds in the oil of *T. baccata* L. from western Turkey and 1-octen-3-ol (20.7 %), 1-hexanol (10.9%) and (*E*)-2-hexen-1-ol (7.3%) were present in the oil of *T. baccata* L. from southern Turkey [16]. Hexahydrofarnesyl acetone (18.3%) and myrtenol (18.3%) were the main volatiles of the needles and branches of *T. baccata* [17]. A population of *T. baccata* were known to contained 1-en-3-ol (23.48%), (3*Z*)-hex-3-en-1-ol (11.46%), and myrtenol (11.38%) [18]. In addition, 3-hexen-1-ol (8.57 %), 2-hexenal (7.24 %) and 1-octen-3-ol (6.10 %) were the major components of *T. wallichiana* var *mairei* [19]. The main constituents identified in the leaf oil of Indian yew *T. wallichiana* were (*E*)-2-octen-1-ol (14.5%), *n*-pentacosane (8.1%) and caryophyllene oxide (7.1%) [20]. The aliphatic alcohol, l-octen-3-ol (40%) occurred as the major component *T. Canadensis* [21]. Moreover, 1-propanone (36.38%), morpholine (10.95%), methylamine (9.10%) and methanone (8.14%) were detected as the main components of *T. canadensis* from Canada [22]. The composition of the stem oil of *T. cuspidate* [23] were ethyl linoleate (9.0%), longiborneol (7.9%) and 13-diepoxy-14,15-bisnorlabdane (7.0%).

The biological activities of essential oils from some *Taxus* plants have been reported. However, no such information could be found for *T. chinensis.* Essential oil of *T. media* leaves possessed stronger antimicrobial activity than the essential oil of *T. chinensis* var. *mairei* leaves against some bacteria [11]. The stem oil of *T. cuspidata* showed antioxidant capacity against DPPH radical, nitric oxide, superoxide, and hydroxyl radicals [23]. Moreover, the oil displayed concentration-dependent reducing power ability and remarkable ferric ion-induced lipid peroxidation inhibitory effect in bovine brain extract [23]. The leaf oil of *T. cuspidata* displayed moderate antimicrobial action against *Bacillus cereus* ATCC 13061, *Staphylococcus aureus* ATCC 12600, *Listeria monocytogenes* ATCC 7644, *Salmonella typhimurium* ATCC 43174 and *Escherichia coli* ATCC 43889 with the minimum inhibitory concentration (MIC) in the range of 125-500 μg/mL [24].

In continuation of our research on the volatile composition and biological activities of essential oils from Vietnamese plants [25], this study reports the chemical constituent identified in *T. chinensis* stem essential oil.

2. Materials and methods

2.1 Plant collection and identification

Mature stem barks of *T. chinensis* were collected from Pù Mát National Park, Nghệ AN Province, Vietnam, in August 2018. Botanical identification was carried out by Dr. Dai. A voucher specimen DND 539 was deposited at the Botany Museum, Vietnam.

2.2 Hydrodistillation experiment

Five hundred gram of air-dried stem samples was shredded and the essential oils were obtained by hydrodistillation for 3h at normal pressure, according to the established procedure [26]. Pulverised stem were loaded into 5 L flask. Distilled water was added until the sample was covered completely. The sample was then subjected to hydrodistllation in Clevenger-type apparatus. The oil sample was collected from the arm of the distiller into previously weighed sample bottle.

2.3 Analysis of the oil samples

Gas chromatography (GC) analysis was performed on an Agilent Technologies HP 7890A Plus Gas chromatograph equipped with a FID and fitted with HP-5MS column (30 m x 0.25 mm, film thickness 0.25 μm, Agilent Technology). The analytical conditions were: carrier gas He (1 mL/min), injector temperature (PTV) 250 ºC, detector temperature 260 ºC, column temperature programmed from 60 ºC (2 min hold) to 220 ºC (10 min hold) at 4ºC/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. The relative amounts of individual components were calculated based on the GC peak area (FID response) without using correction factors.

An Agilent Technologies HP 7890A Plus Chromatograph fitted with a fused silica capillary HP-5 MS column (30 m x 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 70 eV; emission current 40 mA; acquisitions scan mass range of 35-350 amu at a sampling rate of 1.0 scan/s.

2.4.1 Identification of constituents of oil samples

The identification of constituents from the GC/MS spectra of *T. chinensis* was performed on the basis of retention indices (RI) determined with reference to a homologous series of *n*-alkanes (C_4-C_{40}), under identical experimental conditions. In some cases, co-injection with known compounds under the same GC conditions was employed. The mass spectral (MS) fragmentation patterns were checked with those of other essential oils of known composition [27] and with those in the literature as described previously [25].

3. Results & Discussion

3.1. Yield of the essential oil

The average yields of the essential oils of *T. chinensis* was 0.12% (v/w, ± 0.01), calculated on a dry weight basis. The oil was light yellow coloured.

3.2 Chemical constituents of the oil

Table 1 reveals the identities of the compounds, percent composition and the retention indices on HP-5MS column. Monoterpene hydrocarbons (42.1%), oxygenated monoterpenes (17.7%) and oxygenated sesquiterpenes (25.8%) represent the main classes of compounds present in the oil. The sesquiterpene hydrocarbon compounds were less common (0.9%). The major constituents of the oil were α-pinene (34.8%) and caryophyllene oxide (17.1%). There are significant amounts of *trans*-verbenol (5.0%), verbenone (4.6%), β-pinene (2.4%), humulene epoxide II (2.3%) and *trans*-sabinol (2.1%).

Two reports could be seen on the compositions of essential oils from the stems of *Taxus* plants. The oil of *T. chinensis* var. *mairei* [15] contained n-hexadecanoic acid and phthalic acid mono ester-2-ethylhexyl ester which were not identified in *T. chinensis* stem oil. In addition, ethyl linoleate, longiborneol and diepoxy-14,15-bisnorlabdane, the main constituents of stem oil of *T. cuspidate* [23] were conspicuously absent in the oil under investigation. A noteworthy observation was that α-pinene and caryophyllene oxide described as the main constituents of *T. chinensis* oil were not reported previously to be major compounds of the volatiles of other *Taxus* oil samples. Some terpenoid compounds identified in previous analysed *Taxus* oils were not present in *T. chinensis* stem oil. These include hexahydrofarnesyl acetone and myrtenol from *T. baccata* [17, 18] and longiborneol in *T. cuspidate* [23]. Interestingly, a lesser quantity of caryophyllene oxide was identified in the leaf oil of *T.*

wallichiana grown in India [20]. A survey of literature [11-23] indicates that terpene compounds were less common and are not the major contributor to the volatiles of other *Taxus* oil samples. The exception was found in the leaf oil of *T. baccata* analysed in Serbia [17]. However, monoterpene and sesquiterpene compounds were the predominant class of compounds identified in *T. chinensis* stem oil.

Therefore, from the present and previous studies, the volatiles of *Taxus* plants could be classified into five groups based on the constituents occurring in higher quantity. The classes are:

i. Oils dominated by non-terpene compounds (aromatic and halogenated constituents) such as leaves of *T. media*, *T. chinensis* var. *mairei* [11] and *T. canadensis* [22].
ii. Oils containing large amount of fatty acids as seen in *T. chinensis* var. *mairei* (leaf) [12-14], *T. chinensis* var. *mairei* (stem) [15] and *T. cuspidata* stem [23].
iii. Oils whose main compounds were aliphatic alcohol such as *T. baccata* leaf [16, 18], *T. wallichiana* var *mairei* [19, 20] and *T. canadensis* leaf [21].
iv. Oils in which oxygenated monoterpenes and sesquiterpenes predominates but represented by *T. baccata* leaf [17].
v. Oils which has large amounts of terpene hydrocarbons exemplified by *T. chinensis* (this study).

The observed variations in the chemical constituents of essential oils from various *Taxus* plants are obviously due to the nature of the plant, parts being analysed as well as environmental and climatic condition between the various origins of analysis.

Table 1: Compounds identified in *Taxus chinensis* essential oil

Sr. No	RT (min)	Compounds[a]	RI[b]	RI [c]	Percent
1	9.95	α-Thujene	927	921	0.4
2	10.30	α-Pinene	938	932	34.8
3	10.78	Camphene	954	946	1.2
4	10.92	Thuja-2,4(10)-diene	958	950	0.2
5	11.49	Sabinene	977	968	0.3
6	11.66	β-Pinene	983	978	2.4
7	13.17	o-Cymene	1028	1026	1.1
8	13.31	Limonene	1032	1030	1.9
9	13.83	(*E*)-β-Ocimene	1047	1044	0.2
10	16.77	α-Campholenal	1132	1132	1.0
11	17.36	*trans*-Sabinol	1149	1147	2.1
12	17.49	*trans*-Verbenol	1153	1151	5.0
13	18.14	Pinocarvone	1171	1169	1.0
14	18.66	Terpinen-4-ol	1185	1187	0.3
15	18.91	p-Cymen-8-ol	1193	1190	0.6
16	19.34	Myrtenal	1205	1205	1.7
17	19.82	Verbenone	1219	1217	4.6
18	20.07	*trans*-Carveol	1226	1224	0.5
19	20.95	Carvone	1252	1250	0.3
20	22.34	Bornyl acetate	1281	1281	0.6
21	25.98	*cis*-β-Elemene	1401	1402	0.3
22	27.12	Carvone hydrate	1438	1440	0.2
23	29.16	β-Selinene	1502	1502	0.2
24	29.43	α-Muurolene	1511	1511	0.2
25	30.01	Photosantalol	1531	1534	1.5
26	31.99	Spathulenol	1597	1591	1.0
27	32.19	Caryophyllene oxide	1604	1602	17.1
28	32.93	Humulene epoxide II	1630	1636	2.3
29	33.69	Caryophylla-3(15),7(14)-dien-6-ol	1657	1660	0.3
30	33.75	*epi*-α-Muurolol	1659	1660	0.2
31	34.12	α-Cadinol	1672	1672	0.3
32	34.61	14-Hydroxy-9-*epi*-(*E*)-caryophyllene	1689	1690	1.0
33	36.42	(*Z*)-Lanceol	1756	1758	0.4
34	38.48	Farnesyl acetone	1838	1840	1.6
Total					86.5
Monoterpene hydrocarbons (Sr. No. 1-9)					42.1
Oxygenated monoterpenes (Sr. No. 10-20)					17.7
Sesquiterpene hydrocarbons (Sr. No. 21-24)					0.9
Oxygenated sesquiterpenes (Sr. No. 25-34)					25.8

[a] Elution order on HP-5MS column; [b] Retention indices on HP-5MS column; [b] Literature retention indices [Reference 27], RT, retention time on HP-5Ms column; Sr. No serial number

4. Conclusions

The main compounds of *T. chinensis* stem oil were identified as α-pinene and caryophyllene oxide. This study may represent the first attempt aimed at characterization of the constituents of *T. chinensis* essential oils.

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