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Essential oil composition of *Taxus wallichiana* Zucc. from the Northern Himalayan region of India†

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ABSTRACT: The leaves of Indian yew *Taxus wallichiana*, growing in Gulmarg, Jammu and Kashmir, on hydrodistillation, gave 0.025% oil on a fresh weight basis. GC and GC-MS analysis of the oil resulted in the identification of 62 constituents, representing 93.3% of the oil. The main constituents identified in the leaf oil were (*E*)-2-octen-1-ol (14.5%), *n*-pentacosane (8.1%), caryophyllene oxide (7.1%), 1-octanol (6.5%), hexanoic acid (5.5%) and (*Z*)-3-hexenol (4.1%). Copyright © 2006 John Wiley & Sons, Ltd.

KEY WORDS: *Taxus wallichiana* Zucc.; Taxaceae; essential oil composition; (*E*)-2-octen-1-ol; *n*-pentacosane; caryophylleneoxide; 1-octanol; hexanoic acid; (*Z*)-3-hexenol

Introduction

Taxus is a genus of evergreen trees or shrubs, distributed in the Northern Hemisphere, i.e. Europe, North America, Northern India, China and Japan. Some species are grown in gardens for their graceful foliage and scarlet fruits. The typical tree-like form is not much planted for ornamental purpose due to its slow growth, but shrubby garden forms are popular. In India one species of Himalayan yew, *Taxus wallichiana* Zucc. (sometimes referred to as *T. baccata*), is found as an evergreen tree, in the temperate Himalayas at altitudes between 1800 and 3300 m and in the hills of Meghalaya and Manipur at an altitude of 1500 m.[1]

In contrast to the other yews, the Himalayan yew has a remarkable history of medicinal uses. It is also interesting to note that Himalayan yew does not contain the toxic taxine, which is present in the European yew, *T. baccata*. A medicinal tincture made from the young shoots of Himalayan yew has long been used for the treatment of headache, giddiness, feeble and falling pulse, coldness of the extremities, diarrhoea and severe biliousness. The leaves are credited with emmenagogue and anti-spasmodic properties. They are employed for the treatment of hysteria, epilepsy and nervousness, and as a lithic in calculas complaints. The aqueous extract of leaves shows a depressant effect on the central nervous system in rats, indicating the presence of a tranquilizing effect. In a study on the antifertility activity of the leaves, some fractions of the petroleum ether, alcoholic and aqueous extracts inhibited pregnancy in albino rats, and some others exhibited partial or complete resorption at term.[1] *T. baccata* has been reported to be the source of the drug Zarnab, which is very frequently prescribed in the Unani system of medicine.[1–3] All parts of the tree are poisonous; it has been used as a poison since the time of Julius Caesar. However, the fleshy aril part of the plant is nonpoisonous, hence it is eaten by tribal populations. It is credited with carminative, expectorant and stomachic properties. Extracts of *T. baccata* can be added to cosmetics, such as hair-lotions, rinses, beauty and shaving creams and dentifrices.[1] It is also used as a colouring matter and its wood is burnt for incense.[4]

After the novel discovery of anticancer drug Taxol from *T. brevifolia*, by Wani and Wall[5] in 1971, tremendous work has been carried out on the chemical investigation of almost all parts (needles, bark, roots, seeds and heartwood) of several yew species,[4,6–18] resulting in the isolation and characterization of over 300 taxoids. Besides the widespread occurrence of taxoids, the yew also contains steroids like ecdysteroids, responsible for insect moulting activity, terpenoids like rhodoxanthin, responsible for the red colour of the aril surrounding the yew seeds, biflavones like amentoflavones, which bind the GABA receptor at the benzodiazepine binding site, lignans with different biological activities, sugar derivatives and other important compounds like uncharacterized pro-anthocyanidins. Compounds of this class probably colour the red paste made from the bark of Himalayan yew, used by the Brahmins of India to mark their foreheads with a red dot. To the best of our knowledge essential oil composition and

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glycosidically bound volatile compounds of *T. canadensis* have been reported by Jean *et al*.[19] and *T. baccata* by Erdemoglu *et al*.,[20] which prompted us to carry out detailed GC and GC/MS analysis of hitherto uninvestigated leaf volatiles of *T. wallichiana*.

Experimental

Plant material

The leaves of *Taxus wallichiana* Zucc. were collected from wild-growing trees from Gulmarg, Jammu and Kashmir (J&K) in the month of July 1999 after identification by Dr A. R. Naqshi, Centre of Plant Taxonomy, University of Kashmir, Srinagar (J&K). A voucher specimen has been deposited in the herbarium of the Centre of Plant Taxonomy, University of Kashmir, Srinagar (J&K).

Isolation of essential oil

A sample of fresh leaves of *T. wallichiana* Zucc. was subjected to hydrodistillation in a conventional Clevenger-type apparatus for 3 h. The oil was dried over anhydrous sodium sulfate and stored at 4 °C until analysis.

Gas chromatography (GC)

GC analysis of the oil was performed on a Perkin-Elmer GC 8500, equipped with a flame ionization detector, using BP-1 (polydimethyl siloxane) and BP-20 (Carbowax 20 M) columns (30 m × 0.32 mm, film thickness 0.25 μm). Nitrogen was used as the carrier gas at 10 psi inlet pressure. Temperature programming was from 60 to 220 °C at 5 °C/min and then held isothermal at 220 °C for 5 min, then heated at 3 °C/min to 245 °C and held isothermal at 245 °C for 5 min for the BP-1 column and from 60 to 220 °C at 5 °C/min and then held isothermal at 220 °C for 13 min for the BP-20 column. The injector and detector temperatures were 250 and 300 °C, respectively. Samples (0.1–0.2 ml) were injected neat with a split ratio of 1:80.

Gas chromatography/mass spectrometry (GC/MS)

GC/MS data were obtained on a Perkin-Elmer Turbo Mass spectrometer instrument using a PE-Wax column (60 m × 0.32 mm i.d., film thickness 0.25 μm). Carrier gas, helium; temperature programming, 5 min at 70 °C, rising at 2 °C/min to 120 °C and 3 °C/min to 240 °C.

Identification of compounds

Duplicate analysis was performed. Quantitative results are mean data derived from GC analysis on two columns. The individual peaks were identified by comparison of their retention indices on the BP-1 and BP-20 columns with literature values.[19–21] The final confirmation of constituents was made by computer matching of the mass spectra of peaks with Wiley and NIST libraries mass spectral database and literature.[21,22] Chirality analysis was not performed. Relative amounts of individual components are based on GC peak areas obtained without FID response factor correction. The retention indices were obtained from gas chromatograms by logarithmic interpolation between bracketing *n*-alkanes. The homologous series of *n*-alkanes (C8–C22, Poly Science, Niles, USA) were used as standards.

Results and discussion

The volatile oil was obtained by conventional hydrodistillation of fresh leaves of Indian Himalayan yew, *Taxus wallichiana*, which gave viscous oil at 0.025% yield on a fresh weight basis. GC and GC/MS analysis enabled the identification of a total of 62 constituents in the leaf oil of Himalayan yew from Kashmir, India. The relative concentrations of the volatile components identified are presented in Table 1 according to their elution order on the BP-1 column. The main constituents of the oil were found to be (*E*)-2-octen-1-ol (14.5%), *n*-pentacosane (8.1%), caryophyllene oxide (7.1%), 1-octanol (6.5%), hexanoic acid (5.5%) and (*Z*)-3-hexenol (4.1%). The other minor constituents present in the oil were geraniol (2.4%), anisaldehyde (2.3%), α-pinene (2.1%), *n*-heptanol (2.1%), geranyl-*n*-heptanoate (1.8%), *trans*-pinene hydrate (1.8%), methyl salicylate (1.9%), camphor (1.4%), *cis*-3-hexenyl acetate (1.6%), geranyl tiglate (1.6%), isopropyl *n*-octanoate (1.3%), benzaldehyde (1.3%) and amyl anisoate (1.2%). On comparing our results with those reported by Jean *et al*.[19] for Canadian yew *Taxus canadensis* Marsh, significant differences were observed in the percentage and chemical composition of the leaf oil constituents. In *T. canadensis*, 1-octen-3-ol (44.6%), (*E*)-2-hexenal (24.1%) were the major constituents, while hexanal (2.1%), (*Z*)-3-hexenol (2.0%), occidentalol (1.4%) and hexanol (1.0%) were the minor constituents. Careful analysis of both the oils showed that, out of 62 and 18 compounds identified in the Indian and Canadian yew oils respectively, only six constituents were common in both the oils. Out of these six common constituents, the amount of (*Z*)-3 hexenol (4.1 and 2.0%) was 2 times, eugenol (0.9 and 0.2%) 4–5 times and octanol (6.5 and 0.6%) and geraniol (2.4 and 0.2%) 10–12 times higher in Indian yew oil than Canadian yew. On the other hand, amounts of *n*-hexenol (1.0 and 0.6%) and myrtenol (0.7 and 0.3%) were about twice as high in Canadian oil than in Indian yew oil. This drastic variation in the leaf essential oils of Indian and Canadian yew may be attributed due to variation in their environmental conditions and geographical origin, which significantly affect the chemical composition of the essential oils.[23] The major constituents of Indian yew

Table 1. Essential oil composition of *Taxus wallichiana* Zucc. from the northern Himalayan region of India

Sample no.	Compound	Percentage	RRI	
			BP-1	BP-20
1	(*Z*)-3-Hexenol	4.1	839	1388
2	(*E*)-2-Hexenol	0.1	851	1363
3	*n*-Hexenol	0.6	854	1306
4	1-Hepten-3-ol	0.2	870	1356
5	*n*-Heptanal	0.1	880	1170
6	*n*-Heptan-2-ol	0.1	888	1294
7	Santolinatriene	0.6	901	—
8	(*Z*)-3-Hexenyl format	0.1	912	1254
9	α-Pinene	2.1	932	1032
10	Benzaldehyde	1.3	945	1488
11	*n*-Heptanol	2.1	960	1398
12	(*E*)-2-Octen-1-ol	14.5	968	—
13	β-Pinene	0.9	971	1116
14	*n*-Octanal	0.6	983	1277
15	(*Z*)-3-Hexenyl acetate	1.6	989	—
16	(*E*)-2-Hexenyl acetate	0.1	996	—
17	Hexanoic acid	5.5	1010	1857
18	(*Z*)-β-Ocimene	0.5	1029	1204
19	(*Z*)-2-Octen-1-ol	1.2	1039	—
20	(*E*)-2-Octenal	0.5	1047	1433
21	1-Octanol	6.5	1059	1526
22	Methyl benzoate	0.8	1074	1589
23	(*E*)-3-Heptenyl acetate	0.2	1081	—
24	*n*-Nonanal	1.8	1084	—
25	(*Z*)-Sabinene hydrate	0.2	1096	—
26	(*Z*)-Pinene hydrate	0.1	1103	—
27	Octyl formate	1.5	1112	—
28	Camphor	1.4	1128	—
29	(*E*)-Pinene hydrate	1.8	1133	—
30	(*E*)-Verbenol	0.3	1138	—
31	Benzyl acetate	0.2	1141	1708
32	(*E*)-2-Nonenal	0.6	1145	1551
33	Benzoic acid	0.7	1159	—
34	(*E*)-2-Nonenol	0.3	1161	1688
35	*n*-Nonanal	2.7	1168	1635
36	Methyl salicylate	1.9	1179	1766
37	Myrtenol	0.3	1185	—
38	*n*-Octyl acetate	1.2	1194	1468
39	Isopropyl-*n*-octanoate	1.3	1213	—
40	Anisaldehyde	2.3	1236	1980
41	Geraniol	2.4	1244	1792
42	Sabinyl acetate	0.9	1263	1665
43	Eugenol	0.9	1360	2097
44	(*E*)-2-Hexenyl-*n*-hexanoate	0.7	1379	1679
45	Anisyl acetate	0.6	1387	—
46	Dodecanal	0.4	1395	—
47	(*Z*)-α-Bergamotene	0.2	1414	—
48	β-Caryophyllene	0.3	1420	1615
49	(*E*)-α-Bergamotene	0.1	1434	—
50	α-Humulenen	0.1	1449	1642
51	(*E*)-β-Farnesene	0.9	1454	—
52	(*Z*)-3-Hexenyl benzoate	0.5	1553	2126
53	Caryophyllene oxide	7.1	1567	2006
54	Globulol	0.8	1591	2110
55	Geranyl tiglate	1.6	1649	—
56	(*E*,*E*)-Farnesol	0.6	1707	—
57	*n*-Amyl anisoate	1.2	1727	2393
58	Geranyl-*n*-heptanoate	1.8	1827	2138
59	Geranyl benzoate	0.9	1937	—
60	*n*-Eicosane	0.1	1980	—
61	Docosane	0.2	2197	2206
62	*n*-Pentacosane	8.1	2500	2500
	Total	93.3	—	—

oil, (*E*)-2-octen-1-ol, widely occurs in apple, bilberry, guava, grape, melon, peas, potato, chicken (heated), cognac, rum, black tea, cloud berry, mushroom and kelp (www.leffingwell.com/alkenol.htm), and its odour is like green citrus.

The second major constituent of Indian yew oil, *n*-pentacosane, has also been reported from many common plants such as *Elettaria cordamomum* (L.) Malons (seeds), *Ginkgo biloba* (leaf) *Plantago ovata* Forssk (seed coat) and *Populus balsamifera* (plant; www.ars-grin.gov:8080/npgspub/xsql/duke/chemdisp.xsql?chemical=N-PENTACOSANE). Its presence in *Salvia glutinosa* and *S. pratensis* leaf has been reported to an extent of 2.0–8.0%,[24] while in the cuticle of termite it is present to an extent of 16–21%.[25] The third major constituent of Indian yew oil, caryophyllene oxide, very commonly occurs in most of essential oils, and industry claims its occurrence in rosemary, orange juice, lime, cranberry and cinnamon. It is used in beverages, ice cream, candy, condiments, baked goods, frozen dairy desserts, meat products, cigarettes and smokeless tobacco due to its flavouring and adjuvant properties.[26] Caryophyllene also possess antiedemic, antifeedent, anti-inflammatory, anti-tumor and insecticidal activities (www.tobaccodocuments.org/profiles/additives/caryophyllene_oxide.html).[26–28] The fourth major constituent of Indian yew, 1-octanol, is a common compound of several essential oils, such as green tea, grapefruit, California orange and bitter orange.[29] It possesses a sweet, penetrating, aromatic odour. It is used in the manufacture of perfumes and other chemicals to mask industrial odours (www.hgmp.mrc.ac.uk/software/EMBOSS/Apps/octanol.html).

On the other hand hexanoic acid, the fifth major constituent of Indian yew, possesses a characteristic rotting flesh, roman cheese and rancid butter odour. It is the major constituent (48%) of the volatile odour compound of false truffle, *Truncocolumella citrine* (www.intox.org/databank/documents/chemical/chem581/cie581.htm). It also occurs at 11–14 $\mu g\ kg^{-1}$ in Italian cheese.[30] Similarly, the sixth major compound of Indian yew, (*Z*)-3-hexenol, possess an odour of green grass and green leaves. It is one of the most important flavour and fragrance materials used extensively in all types of fruit and vegetable compositions. It occurs in various fruits, and juices. The esters of this alcohol, (*Z*)-3-hexenyl acetate (1.6%), (*Z*)-3-hexenyl benzoate (0.5%) and (*Z*)-3-hexenyl formate (0.1%), are also important flavour and fragrance raw materials.[22]

From the above it is evident that most of the major constituents of *T. wallichiana* oil possess a significant wide range of flavour and fragrance applications and may find application in the flavour and fragrance industry.

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