

Volatile Constituents of *Juniperus communis* L., *Taxus canadensis* Marshall. and *Tsuga canadensis* (L.) Carr. from Canada

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Abstract: The essential oil composition leaves of *Juniperus communis* L., *Taxus canadensis* Marshall. and *Tsuga canadensis* (L.) Carr. from Canada were investigated by head space solid phase microextraction (HS-SPME) and gas chromatography/mass spectrometry (GC-MS). Thirty-three, 30 and 31 components were identified representing 95.78%, 93.89%, 96.14% of the oil, respectively. Limonene (26.12%), benzene (15.62%), β -myrcene (9.08%) and β -pinene (7.30%) were found to be the main constituents of *J. communis*; 1-propanone (36.38%), morpholine (10.95%), methylamine (9.10%) and methanone (8.14%) were detected main components of *Taxus canadensis*; bornylacetate (26.84%), α -pinene (23.74%), camphene (11.93%) and limonene (6.02%) were determined as major constituents of *Tsuga canadensis*. The chemical distributions of the essential oil compounds in the genus pattern were discussed in means of chemotaxonomy and natural products.

Key words: *Juniperus*, *Taxus*, *Tsuga*, essential oil, Canada, HS-SPME, GC-MS.

1. Introduction

Medicinal and aromatic plant taxa have been used for centuries as remedies for human diseases, because medicinal and aromatic plants contain chemical components of therapeutic significance [1]. According to the World Health Organization (WHO) in 2008, about more than 80% of the world's population relies on traditional medicine for their primary healthcare needs [2]. Volatile oils of plant taxa are valuable natural products used as raw materials in perfumes, cosmetics, aromatherapy, phototherapy, spices and nutrition fields [3]. Furthermore, the essential oils are used in traditional medicine for their antiseptic actions are constituted 1% of plant secondary metabolites and are mainly represented by terpenoids, phenylpropanoids or

benzenoids, fatty acids and amino-acid derivatives [4]. Some studies in the literature indicate that the Cupressaceae may be a rich source of anti-tick compounds and that further investigation of cypress taxa are used for repellent and insecticide. *J. communis* L. is traditionally used for healing urinary infections and *J. oxycedrus* L. is used as a remedy for dermatological infections. Furthermore, the berries of *J. communis* and the oil from wood of *J. oxycedrus* are common ingredients of several dermatologic preparations, having monographs cited in several National Pharmacopoeias [5-7]. *J. turbinata* Guss. oil was investigated and finally the activity of the oil against foodborne pathogens and spoilage micro-organisms was recently reported [8]. Oil of *Juniperus* L. are used in aromatherapy, through inhalation, massage, ingestion to create good health and beauty and used in perfume industries. German authorities reported that *Juniperus* tea is diuretic and urinary antiseptic [9], also *Juniper* berry tea used as a

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digestive aid, both stimulating appetite and relieving flatulence. Moreover, the oils of *Juniperus* also help increase the flow of digestive fluids, improve digestion, eliminate gas, stomach cramping [10], steam inhalant for bronchitis, control arthritis and irritating to microbes [11]. Adams et al. [12] studied with different origins and different taxa of *Juniperus*; eventually they reported the following compositions: α -pinene in *J. phoenicea* from Greece and Spain; α -pinene/ β -phellandrene/ α -terpinyl acetate in *J. phoenicea* var. *turbinata* from coastal Spain and *J. phoenicea* subsp. *eu-mediterranea* from coastal Portugal as main constituents.

The genus *Taxus* ranges from temperate North America into subtropical Central America, and from temperate Eurasia to subtropical Southeast Asia [13]. The main constituents identified in the leaf oil of Indian yew *Taxus wallichiana* 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%); furthermore, *Taxus wallichiana* has anticonvulsant, analgesic and antipyretic activities [14].

Juniperus communis, *Taxus canadensis* and *Tsuga canadensis* lack detailed in terms of phytochemical investigation. Therefore, in this paper we report on the composition of essential oils isolated from leaves of *Juniperus communis*, *Taxus canadensis* and *Tsuga canadensis* grown on Canada, that might be helpful in potential usefulness and chemotaxonomical importance of studied taxa.

2. Material and Methods

2.1 Plant Source

Juniperus communis was collected in vicinity of Guelph/Waterloo/Canada, May 20, 2012, 350-400 m., Kilic 4448; *Taxus canadensis* was collected in vicinity of Kitchener/Waterloo/Canada, May 20, 2012, 350-400 m., Kilic 4449; *Tsuga canadensis* in vicinity of Cambridge/Waterloo/Canada, May 20, 2012, 300-350 m, Kilic 4450.

2.2 HS-SPME Procedure

In this study, plant samples were analysed using head space solid phase microextraction (HS-SPME) and gas chromatography/mass spectrometry (GC-MS) method (Brand name: Agilent 7980 A/5970 C). 5 g powder of plant leaves were carried out by HS-SPME using a divinyl benzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber, with 50/30 μ m film thickness; before the analysis the fiber was pre conditioned in the injection port of the gas chromatography (GC) as indicated by the manufacturer. For each sample, 5 g of leaves, previously homogenized, were weighed into a 40 mL vial; the vial was equipped with a "mininert" valve. The vial was kept at 35 °C with continuous internal stirring and the sample was left to equilibrate for 30 min; then, the SPME fiber was exposed for 40 min to the headspace while maintaining the sample at 35 °C. After sampling, the SPME fiber was introduced into the GC injector, and was left for 3 min to allow the analyzes thermal desorption. In order to optimize the technique, the effects of various parameters, such as sample volume, sample headspace volume, sample heating temperature and extraction time were studied on the extraction efficiency as previously reported by Verzera et al. [15].

2.3 GC-MS Analysis

A Varian 3800 gas chromatograph directly interfaced with a Varian 2000 ion trap mass spectrometer (Varian Spa, Milan, Italy) was used with injector temperature, 260 °C; injection mode, splitless; column, 60 m, CP-Wax 52 CB 0.25 mm i.d., 0.25 μ m film thickness (Chrompack Italys.r.l., Milan, Italy). The oven temperature was programmed as follows: 45 °C held for 5 min, then increased to 80 °C at a rate of 10 °C/min, and to 240 °C at 2 °C/min. The carrier gas was helium, used at a constant pressure of 10 psi; the transfer line temperature, 250 °C; the ionisation mode, electron impact (EI); acquisition ion range, 40 m/z to 200 m/z; scan rate, 1 us⁻¹. The compounds were identified

using the National Institute of Standards and Technology (NIST) library (NIST/WILEY/EPA/NIH), mass spectral library and verified by the retention indices which were calculated as described by Van den Dool and Kratz [16]. The relative amounts were calculated on the basis of peak-area ratios. The identified constituents of studied taxa are listed in Table 1.

3. Results and Discussion

In this study essential oil composition needless of *Juniperus communis*, *Taxus canadensis* and *Tsuga canadensis* from Canada were investigated by HS-SPME and GC-MS. Limonene (26.12%), benzene

(15.62%), β -myrcene (9.08%) and α -pinene (7.30%) in *Juniperus communis*; 1-propanone (36.38%), morpholine (10.95%), methylamine (9.10%) and methanone (8.14%) in *Taxus canadensis*; bornylacetate (26.84%), α -pinene (23.74%), camphene (11.93%) and limonene (6.02%) were determined as major constituents in *Tsuga canadensis* (Table 1). Changes in the composition of an essential oil can be caused by environmental factors, such as the soil or climate in which the plants are grown, and by different harvesting methods or distillation techniques, but the major components of the plant generally not changes by cited factors.

Table 1 Chemical composition of the studied samples.

Constituents	RRI*	<i>Juniperus communis</i>	<i>Taxus canadensis</i>	<i>Tsuga canadensis</i>
α -pinene	1,023	4.60	-	23.74
1-decene	1,025	-	0.15	-
Camphene	1,050	-	-	11.93
β -pinene	1,070	7.30	-	3.34
β -myrcene	1,090	9.08	-	-
α -phellandrene	1,094	-	-	4.07
Limonene	1,107	26.12	-	6.02
1-dodecene	1,114	-	0.51	-
β -phellandrene	1,128	-	-	4.37
β -ocimene	1,142	1.17	-	-
α -terpinolene	1,153	0.83	-	1.00
<i>p</i> -cymene	1,179	-	-	0.98
α -cubebene	1,258	0.13	-	-
Copaene	1,287	0.28	-	0.11
Isolodene	1,342	0.29	-	-
Sabinenehydrate	1,351	-	-	0.14
Trans (2,3)-epoxynonane	1,363	-	0.30	-
Bicyclo (4,4,0) dec-1-ene	1,373	0.33	-	-
γ -elemene	1,375	-	-	0.10
Linalool	1,384	1.88	-	0.04
Decenal	1,396	-	0.13	-
Thujone	1,405	5.91	-	-
β -elemene	1,408	0.62	-	-
Caryophyllene	1,433	1.23	-	1.91
Bicyclo (3,1,0) hexan-2-ol	1,438	-	-	0.17
Propanoicacid	1,457	-	0.24	-
α -amorphene	1,460	0.31	-	-
Bornylacetate	1,477	2.79	-	26.84
α -muurolene	1,485	0.20	-	-
Dimethylsulfide	1,488	-	0.23	-
Cyclohexen	1,490	-	-	0.19
Propanedinitrile	1,494	-	-	0.21

**Volatile Constituents of *Juniperus communis* L., *Taxus canadensis* Marshall,
and *Tsuga canadensis* (L.) Carr. from Canada**

(Table 1 continued)

Constituents	RRI*	<i>Juniperus communis</i>	<i>Taxus canadensis</i>	<i>Tsuga canadensis</i>
Camphor	1,506	1.11	-	2.64
δ-cadinene	1,512	1.04	-	-
Trans-γ-bisabolene	1,515	-	-	0.19
Ethanol	1,523	-	1.17	0.29
Benzene	1,530	15.62	-	-
Germacrene D	1,535	0.70	-	-
Prapanoate	1,539	-	0.18	-
Cis-sabinol	1,547	2.82	-	-
1-Butanol	1,550	-	0.99	-
Cycloheptane	1,554	-	-	1.22
Phosphinicacid	1,560	-	2.60	-
3-cyclohexene-1-methanol	1,562	-	-	0.17
Borneol	1,576	0.27	-	1.47
Benzene, 1-methoxy-2	1,580	1.61	-	-
β-citronellol	1,587	0.11	-	0.15
2-propanoic acid	1,649	-	0.48	-
1,3-cyclopentadiene	1,656	-	-	0.98
Silane	1,694	-	0.45	-
2-cyclohexene-1-one	1,714	0.04	-	2.46
1-propanone	1,715	-	36.38	-
Butylatedhydroxytoluene	1,728	-	0.54	-
Morpholine	1,740	-	10.95	-
γ-butalactone	1,764	-	0.66	-
1-propenone	1,767	-	0.64	-
δ-cadinene	1,809	0.40	-	-
β-farnesene	1,823	-	-	0.04
Isolongifolene	1,829	-	0.69	-
Butenol	1,853	-	0.25	-
Naphthalene	1,885	4.82	1.43	-
4,9-decadien-2-amine	1,907	-	1.02	-
Phenylacetate	1,913	-	3.51	-
Cyclomethanol	1,920	2.15	-	-
Caryophylleneoxide	1,936	-	4.05	0.05
Hexadecanoicacid	1,951	-	2.80	-
Benzene-1-methyl-4	1,960	0.63	-	-
1-octanol	2,038	-	3.21	-
β-eudesmol	2,067	0.16	-	-
Caprolactam	2,250	0.05	0.55	0.07
Diethylphthalate	2,281	-	0.51	0.02
Methanone	2,331	-	8.14	-
Dibutylphthalate	2,517	-	1.05	-
Methylamine	2,519	-	9.10	-
1,3-benzenediamine	2,560	1.18	0.98	1.19
Cyclomethanol	1,920	2.15	-	-
Caryophylleneoxide	1,936	-	4.05	0.05
Hexadecanoicacid	1,951	-	2.80	-
Benzene-1-methyl-4	1,960	0.63	-	-
Total		95.78	93.89	96.14

RRI*: relative retention index.

In *Juniperus phoenicea* L. monoterpene hydrocarbons (70.19%) were found to be the major group of compounds, the most abundant components found in the leaf oil were α -pinene (49.15%), α -phellandrene (7.39%), myrcene (5.24%) and β -pinene (3.58%) [17]. On the other hand, in our study limonene (26.12%), benzene (15.62%), β -myrcene (9.08%) and α -pinene (7.30%) were detected as main components in the oil of *Juniperus chinensis*. The essential oil obtained from the needles of *J. communis* of Greek origin was subjected to GC/MS analysis and 56 compounds were identified accounting for more than 96% of the oil. The major component was α -pinene (41.25%), while the predominant minor constituents were sabinene (17.4%), limonene (4.2%), terpinen-4-ol (2.7%), β -myrcene (2.6%) and β -pinene (2.0%) [18]. Similarly, in this study limonene (26.12%), β -myrcene (9.08%) and β -pinene (7.30%) were determined main constituents in *J. communis*; on the other hand, benzene (15.62%) was determined as main constituent only in this study with *J. communis* (Table 1). Shaw [19] reported that bornylacetate, α -pinene, camphene, limonene, tricyclene, β -pinene and β -myrcene were determined as major constituents in the essential oil of *Tsuga canadensis*. Similarly, in this study bornylacetate (26.84%), α -pinene (23.74%), camphene (11.93%) and limonene (6.02%) were determined as major constituents in *Tsuga canadensis* (Table 1). The leaves of Indian yew *Taxus wallichiana*, analysed by GC and GC-MS, eventually the main constituents identified in the leaf oil were (*E*)-2-octen-1-ol (14.5%), *n*-pentacosane (8.1%), caryophyllene oxide (7.1%) and 1-octanol (6.5%) [14]. Whereas in our study with *Taxus canadensis*, (*E*)-2-octen-1-ol (14.5%) and *n*-pentacosane were not determined; caryophyllene oxide (4.05%) and 1-octanol (3.21%) were reported minor amount; 1-propanone (36.38%), morpholine (10.95%) and methylamine (9.10%) were detected in high content (Table 1).

Essential oil composition of six *Pinus* L. taxa (Pinaceae) from Canada (*P. resinosa* Sol. ex Aiton, *P.*

flexilis E. James, *P. nigra* J.F. Arnold, *P. strobus* L., *P. parviflora* Siebold & Zucc. and *P. mugo* Turra subsp. *mugo*) were investigated by HS-SPME/GC-MS and caryophyllene (27.60%), α -pinene (12.96%), 3-carene (12.93%) and naphthalene (9.37%) in *P. resinosa*; α -pinene (33.29%), β -pinene (16.24%), and germacrene D (6.13%) in *P. flexilis*; acetic acid (31.12%), bicyclo (2.2.1) heptan-2-one (21.45%) and borneol (8.64%) in *P. nigra*; α -pinene (32.96%), β -myrcene (27.72%) and β -pinene (8.01%) in *P. strobus*; α -pinene (25.56%), caryophyllene (13.21%), germacrene D (6.71%), limonene (6.21%) and camphene (5.68%) in *P. parviflora*; 3-carene (36.54%), *p*-cymene (18.03%), α -pinene (9.00%) and limonene (5.09%) in *P. mugo* subsp. *mugo* were identified as main components [20, 21]. In this study limonene (26.12%), β -myrcene (9.08%) and α -pinene (7.30%) in *Juniperus communis*; α -pinene (23.74%), camphene (11.93%) and limonene (6.02%) were determined as major constituents in *Tsuga canadensis*; it is noteworthy that 1-propanone (36.38%), morpholine (10.95%), methylamine (9.10%) and methanone (8.14%) was found as main constituent only in *Taxus canadensis* essential oil (Table 1). In addition, the volatile components four *Picea* Mill. species (*P. pungens* Engelm., *P. mariana* (Mill.) Britton, *P. glauca* (Moench) Voss., *P. rubens* Sarg.) also were analyzed by HS-SPME/GC-MS. The main components were bornylacetate (29.40%), camphor (26.43%), β -myrcene (7.47%) and camphene (7.01%) in *P. pungens*. The main components were camphene (22.03%), bornylacetate (21.64%), α -pinene (16.62%) and borneol (7.79%) in *P. mariana*. The main components were bornylacetate (31.25%), limonene (17.27%), α -pinene (15.85%) and camphene (13.65%) in *P. glauca*. The main components were borneol (12.38%), α -pinene (10.36%), germacrene D (9.86%) and δ -cadinene (8.25%) in *P. rubens*. [20, 21]. Bornylacetate was detected the major compounds of *Tsuga canadensis*; It is noteworthy that in the composition of *Taxus canadensis* bornylacetate was not

determined or determined with very low amounts (2.79%) in study pattern of *Juniperus communis* (Table 1). α -pinene (16.62%, 15.85% and 10.36%) was detected one of the major compounds of *Picea mariana*, *Picea glauca* and *Picea rubens* respectively [22]. Similarly, α -pinene (7.30% and 23.74%) was the main compound of *Juniperus communis* and *Tsuga canadensis* (Table 1).

In conclusion, findings showed that the studied taxa had a considerable variation in essential oil composition and this study demonstrates the occurrence of the limonene, benzene and β -myrcenechemotype in *Juniperus communis*; 1-propanone, morpholine and methylamine chemotype in *Taxus canadensis*; bornylacetate (26.84%), α -pinene (23.74%) and camphor chemotype in *Tsuga canadensis*.

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