

Identification of Essential Oil Composition of Four *Picea* Mill. (Pinaceae) Species from Canada

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Abstract: The aim of this study was to determine the volatile composition of essential oil of four *Picea* Mill. species (*Picea pungens* Engelm., *Picea mariana* (Mill.) Britton, *Picea glauca* (Moench) Voss., *Picea rubens* Sarg.) needles. The volatile components extracted from these four species needles were analyzed by using headspace solid phase microextraction (HS-SPME)/gas chromatography-mass spectrum (GC-MS) and 31, 34, 27 and 24 compounds were identified representing 91.77%, 92.70%, 92.38% and 94.06% of the total oil, respectively. The major constituents were found to be bornylacetate (29.40%), camphor (26.43%), β -myrcene (7.47%) and camphene (7.01%) in *P. pungens*; camphene (22.03%), bornylacetate (21.64%), α -pinene (16.62%) and borneol (7.79%) in *P. mariana*; bornylacetate (31.25%), limonene (17.27%), α -pinene (15.85%); and camphene (13.65%) in *P. glauca* and borneol (12.38%), α -pinene (10.36%), germacrene D (9.86%) and δ -cadinene (8.25%) in *P. rubens*. This study sought to detect new phytochemical data on the *Picea* genus to help chemotaxonomy and usable of studied species.

Key words: *Picea*, essential oil, Pinaceae, Canada, HS-SPME/GC-MS.

1. Introduction

The genus *Picea* D. Don ex Loudon belonging to the Pinaceae family is represented in Canada by six species: *Picea engelmannii* Parry ex Engelm. (Engelmann's spruce), *Picea glauca* (Moench) Voss. (white spruce), *Picea mariana* (Mill.) Britton, Sterns & Poggenburg (black spruce), *Picea rubens* Sarg. (red spruce), *Picea sitchensis* (Bong.) Carr. (sitkaspruce) and *Picea pungens* Engelm. (Colorado spruce). These trees are native to the Rocky Mountains of the United States from Colorado to Wyoming, and showed as a popular ornamental tree found far beyond its native range [1]. *P. glauca* is native to boreal forests in North America and Wyoming [2]. *Picea* taxa referred as Canadian, skunk, cat, western white, black hills, Alberta white and Porsild spruce [3]. *P. mariana*

(black spruce) is native to northern Northern America and widely distributed throughout Eastern Canada [4]. *P. rubens* is native to Eastern North America, ranging from Eastern Quebec to Nova Scotia, and from New England south in the Adirondack Mountains and Appalachians to Western North Carolina [4]. *P. pungens* has many cultivar forms that they are often grown as ornamental trees in gardens and parks; it is also grown for the Christmas tree in many countries [5].

Aromatic, fragrant and medicinal plants have been known since antiquity and their essential oils are used in many industrial fields [6-8] and have antibacterial, antifungal and antioxidant properties [8]. The content and the chemical composition of volatile oil isolated from *Pinaceae* family depends on the geographic origin [9], the part of the plant material (needles, twigs, cones) and the isolation and determination techniques used for analysis of essential oils [10], the essential oil ingredients are also strongly affected by soil type and

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air pollution [11]. The constituents emitted by conifer trees are generally detected by analyzing essential oils, used from foliage and cones of plant parts [12]. It is well known that the essential oils of conifers possess antifungal, antibacterial, antioxidant and cytotoxic effects [13]. *P. abies* (L.) H. Karst (Norway spruce) can be used to avoid the erosion of the soil, and is of great economic importance and even in phytotherapy [14]. Essential oil of Norway spruce fir was used in Europe in the treatment of catarrhal diseases of children, by inhalation with hot water [15].

Essential oil constituents of coniferous are poorly known in literature, although there have been some studies on the chemical composition of the coniferous species [16]. The aim of this study is to examine the chemical composition from needles of *Picea* species to detect new phytochemical data to help chemotaxonomy and potential usable of studied species.

2. Materials and Methods

2.1 Plant Harvesting and Analysis of Gas Exchange

P. pungens was collected in vicinity of Westmount in Canada on December 5, 2012, 150-200 m, Kilic 4444; *P. mariana* was collected in vicinity of Silver lake from Waterloo in Canada on December 5, 2012, 300-350 m, Kilic 4445; *P. glauca* in vicinity of Victoria park, Waterloo in Canada on December 5, 2012, 300-350 m, Kilic 4446; *P. rubens* was collected in vicinity of Hamilton in Canada on December 5, 2012, 300-350 m, Kilic 4447.

2.2 Analysis of Gas Exchange

5 g powder of pine needle was carried out by a head space solid phase microextraction (HS-SPME) method using a divinyl benzene/carboxen/polydimethylsiloxane fiber, with 50/30 μm film thickness; before the analysis the fiber was conditioned in the injection port of the gas chromatography (GC) as indicated by the manufacturer. For each sample, 5 g powder of needle previously homogenized was 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 analytes 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. [17].

A Varian 3800 gas chromatograph directly interfaced with a Varian 2000 ion trap mass spectrometer (VarianSpa, 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 μm film thickness. 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 range, 40 m/z to 200 m/z; scan rate, 1/us. The compounds were identified using the National Institute of Standards and Technology (NIST) library, mass spectral library and verified by the retention indices which were calculated as described by Van Den Dool and Kratz [18]. The identified constituents are listed in Table 1.

3. Results and Discussion

The volatile components of *P. pungens*, *P. mariana*, *P. glauca* and *P. rubens* were analyzed by HS-SPME/GC-MS; bornylacetate (29.40%), camphor (26.43%), β -myrcene (7.47%) and camphene (7.01%) were determined as the main compounds of *P. pungens*; camphene (22.03%), bornylacetate (21.64%), α -pinene (16.62%) and borneol (7.79%) were detected as major constituents of *P. mariana*; bornylacetate (31.25%), limonene (17.27%), α -pinene (15.85%) and

Table 1 The identified constituents of *Picea* taxa.

| Constituents | *RRI | <i>P. pungens</i> | <i>P. mariana</i> | <i>P. glauca</i> | <i>P. rubens</i> |
|-----------------------------|-------|-------------------|-------------------|------------------|------------------|
| α -pinene | 1,022 | 4.06 | 16.62 | 15.85 | 10.36 |
| Camphene | 1,049 | 7.01 | 22.03 | 13.65 | 1.44 |
| β -pinene | 1,068 | - | - | - | 3.63 |
| β -myrcene | 1,090 | 7.47 | 2.89 | - | - |
| Limonene | 1,106 | - | 2.55 | 17.27 | - |
| β -phellandrene | 1,127 | - | - | - | 2.05 |
| α -terpinolene | 1,153 | 0.30 | - | 0.98 | - |
| 1.8-cineole | 1,156 | 0.48 | - | - | - |
| β -cymene | 1,180 | - | 0.09 | - | - |
| α -cubebene | 1,258 | - | 0.07 | 0.06 | - |
| Copaene | 1,287 | 0.14 | 0.17 | 0.09 | 0.51 |
| Bicycloelemene | 1,297 | - | - | 0.05 | - |
| Benzene, 1-methyl-4 | 1,312 | 0.06 | - | - | - |
| α -bourbonene | 1,324 | - | 0.05 | - | - |
| Sabinenehydrate | 1,351 | 0.20 | - | - | - |
| Fenchylacetate | 1,362 | - | 0.58 | 0.28 | - |
| Bicyclo (4.4.0) dec-1-ene | 1,373 | 0.10 | 0.46 | 0.25 | 3.83 |
| Linalool | 1,387 | 0.24 | - | 0.20 | - |
| β -elemene | 1,395 | 0.42 | 0.93 | 0.30 | - |
| 6-octenal | 1,403 | - | - | 0.42 | - |
| β -elemene | 1,408 | - | - | - | 0.89 |
| Caryophyllene | 1,434 | 0.36 | 0.93 | 0.72 | 3.08 |
| Bicyclo (3.1.0) hexan-2-ol | 1,438 | 0.40 | - | - | - |
| Bicyclo (2.2.1) heptan-2-ol | 1,455 | - | - | 0.17 | - |
| γ -muurolene | 1,461 | 0.14 | 0.33 | - | 2.78 |
| Bornylacetate | 1,479 | 29.40 | 21.64 | 31.25 | 5.68 |
| Isobornylacetate | 1,489 | - | 1.64 | 0.36 | 1.79 |
| Exo-methylcamphenillol | 1,498 | 0.57 | - | - | - |
| α -caryophyllene | 1,506 | - | - | 0.35 | - |
| Camphor | 1,508 | 26.43 | 1.41 | - | 5.39 |
| δ -cadinene | 1,512 | - | 2.43 | 0.91 | 8.25 |
| α -amorphene | 1,522 | - | 2.09 | - | 6.62 |
| γ -cadinene | 1,528 | 0.33 | - | - | 1.88 |
| Ethanol | 1,538 | 0.40 | - | 0.33 | - |
| Germacrene D | 1,535 | 0.33 | 1.13 | 0.73 | 9.86 |
| 4-carene | 1,547 | - | - | 0.60 | 1.45 |
| 1.3-cyclohexadiene | 1,560 | - | 1.31 | - | - |
| 3-cyclohexene-1-methanol | 1,562 | 0.71 | - | 0.24 | - |
| Borneol | 1,578 | 3.57 | 7.79 | 4.34 | 12.38 |
| 6-octen-1-ol | 1,586 | 0.23 | 0.34 | 0.61 | - |
| Cyclopentene | 1,615 | - | 0.15 | - | - |
| Santolinaepoxide | 1,639 | - | 0.06 | - | - |
| Calamenene | 1,648 | 0.03 | - | - | - |
| 2.6-octadien-1-ol | 1,667 | - | - | - | 0.41 |
| 2-cyclohexen-1-ol | 1,680 | 0.12 | - | - | - |
| 2-cyclohexene-1-one | 1,714 | 1.52 | 0.08 | - | - |
| Verbenone | 1,742 | 0.08 | - | - | - |
| Cyclohexene | 1,798 | - | 0.04 | - | - |

(Table 1 continued)

| Constituents | *RRI | <i>P. pungens</i> | <i>P. mariana</i> | <i>P. glauca</i> | <i>P. rubens</i> |
|---------------------------|-------|-------------------|-------------------|------------------|------------------|
| δ -cadinene | 1,809 | 0.21 | 0.20 | - | - |
| 1,6-cyclodecadiene | 1,838 | 0.04 | 0.07 | - | - |
| Naphthalene | 1,885 | 5.47 | 3.65 | 1.44 | 5.72 |
| Bicyclo (4.4.0) dec-1-ene | 1,968 | - | 0.19 | 0.11 | 0.56 |
| α -cadinol | 2,029 | - | 0.06 | - | - |
| Caprolactam | 2,252 | - | 0.21 | - | - |
| Diethylphythalate | 2,296 | - | - | - | 0.28 |
| Phenol | 2,455 | - | 0.06 | - | 0.79 |
| 1,3-benzenediamine | 2,565 | 0.95 | 0.45 | 0.82 | 4.43 |
| Total | | 91.77 | 92.70 | 92.38 | 94.06 |

*RRI: relative retention index.

camphene (13.65%) were found to be the main constituents of *P. glauca*; borneol (12.38%), α -pinene (10.36%), germacrene D (9.86%) and δ -cadinene (8.25%) were detected as the main compounds of *P. rubens* (Table 1).

In the old foliage essential oil of *P. engelmannii* -pinene (2.3%) and β -pinene (1.2%) were reported in much smaller amounts, with myrcene (12.2%) and camphor (14.9%) dominating; whereas, in the juvenile foliage essential oil borneol (5.2%), camphene hydrate (5.0%) and piperitone (4.6%) were reported as major components [19]. Similarly in this study, α -pinene (16.62%, 15.85% and 10.36%) was detected as main constituents of *P. mariana*, *P. glauca* and *P. rubens*, respectively; whereas α -pinene (4.06%) was detected low amounts from *P. pungens* essential oil. Borneol was determined as the main constituents of *P. mariana* (7.79%) and *P. rubens* (12.38%); alternatively borneol was found to be little in *P. pungens* (3.57%) and *P. glauca* (4.34%). It is noteworthy that camphor was determined as major components of *P. pungens* (26.43%) but this compound was not detected in *P. glauca* and was reported in much smaller amounts in *P. rubens* (5.39%) and *P. mariana* (1.41%) (Table 1). The main compounds of *P. abies* L. were: α -pinene, camphene, limonene, myrcene, bornyl acetate, δ -cadinene, muurolene, cadinol, muurolol and manool [20]. In our study, α -pinene in *P. mariana* (16.62%), *P. glauca* (15.85%) and *P. rubens* (10.36%); bornylacetate in *P.*

mariana (21.64%), *P. glauca* (31.25%) and *P. pungens* (10.36%); camphene in *P. mariana* (22.03%), *P. glauca* (13.65%) and *P. pungens* (7.01%) were detected as main constituents. On the other hand β -myrcene (7.47%), limonene (17.27%) and borneol (12.38%) were determined only in *P. pungens*, *P. glauca* and *P. rubens*, respectively. Whereas, bornylacetate in *P. rubens* (5.68%), β -myrcene in *P. mariana* (2.89%), limonene in *P. mariana* (2.55%) was found to be in much smaller amounts. On the other hand, β -myrcene was not detected from *P. glauca* and *P. rubens*; in addition limonene was absent from *P. pungens* and *P. rubens* oils (Table 1).

Pinaceae species which were natively grown in Turkey were investigated by Tumen et al. [21]; α -pinene (17.90%, 30.91%, 14.76%, 45.36% and 47.09%) was the main constituent of *P. pinea*, *P. brutia*, *P. slyvestris*, *P. nigra* and *P. halepensis*, respectively; contrary to other species, β -pinene (32.7%) was found as major compound in *Picea orientalis* [21]. In our study, α -pinene (16.62%, 15.85%, 10.36%) was the major constituent of *P. mariana*, *P. glauca* and *P. rubens*, respectively (Table 1). In another coniferous study, caryophyllene (27.60%) and α -pinene (12.96%) in *P. resinosa* Sol. ex Aiton; α -pinene (33.29%) and β -pinene (16.24%) in *P. flexilis* E. James; acetic acid (31.12%) and bicyclo (2.2.1) heptan-2-one (21.45%) in *Pinus nigra* J.F. Arnold; α -pinene (32.96%) and β -myrcene (27.72%) in *Pinus strobus* L.; α -pinene (25.56%) and

caryophyllene (13.21%) in *Pinus parviflora* Siebold and Zucc.; 3-carene (36.54%) and *p*-cymene (18.03%) in *P. mugo* Turra subsp. *mugo* were identified as main components [16]. It is noteworthy that there have been some similarities and differences in respect to main components among *P. taxa* and our study with *Picea* species. Among the monoterpenes limonene (69.5%) in *P. pinea* and in *Cedrus libani* (22.7%), β -pinene (39.6%) in *P. brutia* was detected in higher amounts [21]. On the other hand, limonene (17.27%) was found only in *Picea glauca* in higher amounts; it is noteworthy that limonene was not detected in *Picea pungens* and *Picea rubens* (Table 1). In the essential oil juvenile foliage of *Picea engelmannii* α -pinene (23.3%) and β -pinene (29.7%) dominate. In the old foliage essential oil of *Picea engelmannii* α -pinene (2.3%) and β -pinene (1.2%) exist in much smaller amounts, with myrcene (12.2%) and camphor (14.9%) dominate [20]; in our study, there are some qualitative and quantitative differences from cited investigation. β -pinene was not determined in *P. pungens*, *P. mariana* and *P. glauca* oils; β -pinene was reported small amounts in *P. rubens* (3.63%). Germacrene D was found to be high amounts only in *P. rubens* (9.86%) (Table 1).

4. Conclusions

In conclusion, the major compounds of studied samples were found to be bornylacetate (29.40%), camphor (26.43%) and β -myrcene (7.47%) in *P. pungens*; camphene (22.03%), bornylacetate (21.64%) and α -pinene (16.62%) in *P. mariana*; bornylacetate (31.25%), limonene (17.27%) and α -pinene (15.85%) in *P. glauca* and borneol (12.38%), α -pinene (10.36%) and germacrene D (9.86%) in *P. rubens*; our findings and the data obtained from literature show considerable variation in the means of essential oil compositions; bornylacetate (29.40%) and camphor (26.43%) were detected the chemotypes of *Picea pungens*; camphene (22.03%) and bornylacetate (21.64%) were determined the chemotypes of *Picea*

mariana; bornylacetate (31.25%) and limonene (17.27%) were identified the chemotypes in *Picea glauca*; borneol (12.38%) and α -pinene (10.36%) were found to be chemotypes of *Picea glauca* (Table 1). In addition, obtained chemical data from this study might be helpful in potential usefulness and chemotaxonomy of *Picea taxa*. Moreover, these results may constitute a significant connection between antibacterial, antifungal, antioxidant, etc. activities and chemical composition for the future development of *Picea* extract as an antibacterial, antifungal, antioxidant, etc. agents can be used as a potential preservative in food products.

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