Journal of Agricultural Science and Technology B 4 (2014) 67-73
Earlier title: Journal of Agricultural Science and Technology, ISSN 1939-1250

Essential Oil Composition of Six *Pinus* L. Taxa (Pinaceae) from Canada and Their Chemotaxonomy

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Received: November 26, 2013 / Published: January 20, 2014.

Abstract: In this study six *Pinus* L. taxa from Canada (*P. strobus* L., *P. parviflora* Siebold & Zucc., *P. mugo* Turra subsp. *mugo*, *P. resinosa* Sol. ex Aiton, *P. flexilis* E. James and *P. nigra* J. F. Arnold) were studied to determine on chemical characters of studied taxa. For this purpose, essential oil from needles of the six *Pinus* taxa were investigated by HS-SPME/GC-MS. 38, 33, 39, 28, 31 and 46 compounds were respectively identified from each species representing 95.90%, 95.07%, 95.79%, 96.20%, 93.05% and 96.25% of the oil. The results have given some clues on the chemotaxonomy of this genus and are of usable potentials of the plants as renewable resources. Although the essential oil composition of studied taxa showed chemical divergences because of climatical, seasonal, geographical and geological factors, but the major compounds of plant derivatives are generally similar and the major compounds are chemotaxonomical markers for studied taxa.

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

1. Introduction

Pine oils are widely used as fragrances in cosmetic industry, as flavoring additives for food and beverages, and as scenting agents in a variety of household products and intermediates in the synthesis of perfume chemicals [1]. Essential oils which were obtained from aromatic and medicinal plants have been known since antiquity to possess biological activity, most notably antibacterial, antifungal and antioxidant properties. With growing interest in the use of essential oils in both the food and pharmaceutical industries, the systematic and potential usefulness of plant extracts studies has become increasingly important [2, 3]. Cones of some coniferous taxa were used in industry [4] as renewable source of essential oils. Besides economic value of essential oils, they play an important role in the plant defense system against fungus and insect attacks. Some studies have been carried out to observe the effect on the seasonal, genotypic and environmental variability of the chemical contents in *Pinus* L. taxa [5-7]. The effects of geographical variations in the needle oil composition [8] and chemical composition of *P. nigra* have been published [9].

Although there have been some studies on the antioxidant activity, terpenoids, steroids, anti-HIV activity, procyanidins, etc. of the Pinaceae cones, essential oil constituents cones of the Pinaceae family are poorly known yet [10]. Pine is used in ethnomedical practice throughout the world; Indians use a boiled extract of the inner bark from *P. strobus* as an astringent for diarrhea or in cough remedies. In 19th century in North America, *P. sylvestris* was used as a diuretic and to induce perspiration and thus help break a fever [11]. *P. brutia* is used in folk medicine in Turkey, and recently the antimicrobial activity of tar obtained from the roots and stems of *P. brutia* against *Staphylococcus aureus*, *Streptococcus*

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pyrogenes, *Escherichia coli* and *Candida albicans* was reported [12]. Previous studies on *Pinus* species determined the diterpenoids [13], triterpenoids [14], flavonoids and lignans [15]. Moreover, pine oils are used for medicinal purposes in aromatherapy as carminative, rubefacient, emmenagogue and abortifacient agents. Pines are among the most important forest trees in the Mediterranean region that, pine oils were studied from the geographical [16], seasonal [17], genotypic [18] and environmental [19] points of view.

This paper reports the chemical composition of the essential oil of six *Pinus* taxa which were collected in vicinity region of Canada. The aim of the present study is to provide chemical data that might be helpful in potential usefulness, to summarize the available information in order to facilitate and guide future research and to examine potential chemotaxonomic significance of studied taxa.

2. Materials and Methods

2.1 Plant Material

P. resinosa (4441); *P. flexilis* (4442) and *P. nigra* (4443) were collected from Wilfrid Laurier University Campus, Waterloo, Canada, on May 5, 2012, at an altitude of 350-400 m, by Kilic. *P. strobus* (4451) and *P. parviflora* (4452) were collected in vicinity of Niagara Falls, Ontario, Canada, May 22, 2012, 150-250 m, by Kilic. *P. mugo* subsp. *mugo* (4453) was collected in vicinity of Botany Hill Park, Toronto, Canada, May 22, 2012, 350-400 m, Kilic.

2.2 HS-SPME Procedure

Aerial parts of plant sample previously triturated by a liquidizer and 5 g powder of needles were carried out by a (HS-SPME) head space solid phase microextraction method using a divinyl benzene/carboxen/polydimethylsiloxane (DVB/CAR/ PDMS) fiber, with 50/30 lm 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 of needles, previously homogenized, were weighed into a 40 mL vial. The vial was equipped with a "mininert" valve and 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 and sample headspace volume, sample heating temperature and extraction time were studied on the extraction efficiency as previously reported by Verzera et al. [20].

2.3 GC-MS Analysis

A Varian 3800 GC directly inter faced with a Varian 2000 ion trap mass spectrometer was used with injector temperature, 260 °C; injection mode, splitless; column, 60 m, CP-Wax 52 CB 0.25 mm i.d., 0.25 lm 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); acquisit ion range, 40 m/z to 200 m/z; scan rate, 1 u/s. The compounds were identified using the National Institute of Standards and Technology (NIST) library (NIST/Wiley/EPA/NIH) and mass spectral library, and verified by the retention indices which were calculated as described by Dool and Kratz [21]. The relative amounts were calculated on the basis of peak-area ratios. The identified constituents were listed in Tables 1 and 2.

3. Results and Discussion

In this study, 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

Table 1 Chemical compositions of *P. strobus, P. parviflora* and *P. mugo* subsp. *mugo*.

Constituents	RRI	*P. strobus*	*P. parviflora*	*P. mugo* subsp. *mugo*
α-pinene	1022	32.96	25.56	9.00
Camphene	1050	2.60	5.68	0.47
β-pinene	1068	8.01	-	1.33
3-carene	1075	-	-	36.54
β-myrcene	1090	27.72	1.41	-
Limonene	1107	2.14	6.21	5.09
β-phellandrene	1128	2.55	0.72	4.13
α-terpinolene	1153	0.59	2.93	1.40
p-cymene	1179	-	-	18.03
Benzene, 1-methyl-2	1180	0.24	-	-
2,4,6-octatriene	1243	0.12	-	-
α-cubebene	1258	-	0.13	-
1,3,5-undecatriene	1273	0.08	-	-
Copaene	1287	0.09	0.25	0.13
Bicycloelemene	1297	0.73	1.53	-
Benzene, 1-methyl-4	1312	-	-	0.50
α-bourbonene	1324	0.06	-	-
1,3,6,10-dodecatetraene	1364	0.11	0.53	-
Bicyclo[4,4,0]dec-1-ene	1373	1.40	2.64	-
Limoneneoxide	1382	-	-	0.48
β-elemene	1396	1.04	1.95	0.31
Benzene, 2-methoxy-4-methyl-1	1433	-	-	3.06
Caryophyllene	1434	3.26	13.21	-
1,6,10-dodecatriene	1441	-	0.24	-
γ-cadinene	1460	1.06	1.94	0.18
Epi-bicyclosesquiphellandrene	1469	0.07	-	-
Bornylacetate	1475	0.15	4.25	0.63
3-cyclohexen-1-ol	1477	-	-	0.42
α-muurolene	1487	0.19	0.65	0.19
α-caryophyllene	1506	0.68	2.21	0.62
δ-cadinene	1511	1.31	3.17	1.11
α-amorphene	1521	0.81	2.15	-
Ethanol	1527	0.57	1.58	-
Germacrene D	1535	3.30	6.71	1.08
Estragole	1542	0.12	-	-
δ-cadinene	1546	0.08	-	0.91
α-terpineol	1555	-	-	2.19
2-dodecanone	1561	0.07	-	0.26
Benzoicacid	1564	0.60	-	-
Bicyclogermacrene	1569	0.52	1.09	-
Borneol	1576	0.11	1.73	0.42
Myrtenol	1588	-	-	0.34
1,3-cycloheptadiene	1598	-	-	0.32
Cycloheptane	1608	-	-	0.29
Butanoicacid	1632	-	-	0.14
Bicyclo[3,1,1]hept-2-ene-methanol	1637	-	-	0.29
Carveol	1680	-	-	0.41
2-cyclohexen-1-one	1688	-	-	0.07
Adamantane	1729	-	-	0.96
Benzenemethanol	1736	-	0.08	0.42
Verbenone	1742	-	0.15	0.56
Isoledene	1809	0.05	-	-
Naphthalene	1884	0.45	1.86	-
Caryophylleneoxide	1936	-	0.27	1.28

(Table 1 continued)

Constituents	RRI	*P. strobus*	*P. parviflora*	*P. mugo* subsp. *mugo*
Sapathulenol	1969	0.23	0.52	0.73
3-hexene-1-ol-benzoate	1975	0.60	-	-
α-cadinol	2028	-	0.12	-
Longipinane	2076	-	-	0.18
Phenanthrene	2171	-	-	0.84
Caprolactam	2252	0.24	0.12	-
Diethylphythalate	2296	-	0.08	-
1,3-benzenediamine	2564	0.95	3.52	0.49
	Total	95.90	95.07	95.79

Table 2 Chemical compositions of *P. resinosa, P. flexilis* and *P. nigra*.

Constituents	RRI	*P. resinosa*	*P. flexilis*	*P. nigra*
α-pinene	1022	12.96	33.29	2.68
Camphene	1049	1.47	-	4.79
β-pinene	1068	1.93	16.24	-
3-carene	1073	12.93	-	-
β-myrcene	1089	-	0.72	5.02
1,3,5-cycloheptatriene	1105	1.93	-	-
Limonene	1107	-	0.76	8.06
α-terpinolene	1152	-	-	0.19
Eucalyptol	1156	-	-	0.78
p-cymene	1179	0.85	0.46	0.07
α-cubebene	1258	1.87	-	0.06
Copaene	1288	2.92	-	0.09
Benzene, 1-methyl-4	1311	-	-	0.07
Sabinenehydrate	1350	-	-	0.21
Epi-bicyclosesquiphellandrene	1354	1.06	-	-
3-cyclopentene-1-acetaldehyde	1357	-	0.19	-
Fenchylacetate	1362	-	-	0.04
2,6-octadienal	1377	-	0.41	0.10
Limonene oxide	1382	1.00	2.09	-
β-linalool	1387	-	-	0.27
β-elemene	1397	-	-	0.36
6-octenal	1404	0.25	-	-
Caryophyllene	1434	27.60	1.52	0.73
Cyclopentene	1436	-	1.26	-
1.6.10-dodecatriene	1449	-	-	0.09
Thujol	1454	-	1.69	-
Bicyclo[2.2.1]heptan-2-ol	1455	-	-	0.09
γ-cadinene	1459	-	0.41	-
Bornyl acetate	1474	1.15	0.60	-
β-bisabolene	1482	1.08	1.00	-
Aceticacid	1486	0.96	-	31.12
α-muurolene	1492	-	-	0.38
Isobornylacetate	1494	-	-	0.48
Exo-methyl-camphenilol	1503	-	-	2.10
Camphor	1507	2.27	-	-
Trans-pinocarveol	1510	-	5.34	-
Bicyclo[2.2.1]heptan-2-one	1512	1.79	-	21.45
Cis-α-bisabolene	1514	0.58	-	-
Trans-pinocarvone	1516	-	3.71	-
α-amorphene	1521	0.97	-	1.52
Isoborneol	1527	0.71	-	0.30
Germacrene D	1535	-	6.13	0.27

(Table 2 continued)

Constituents	RRI	*P. resinosa*	*P. flexilis*	*P. nigra*
Estragole	1542	-	-	0.09
δ-cadinene	1547	0.80	-	0.26
3-cyclohexane-1-methanol	1561	-	0.15	-
β-fenchylalcool	1564	-	-	1.07
Borneol	1578	3.45	1.02	8.64
Citronellol	1587	-	2.95	0.85
Bicyclo[3,1,1]heptan-2-one	1597	-	0.81	-
Cyclopentene	1620	-	-	0.14
Bicyclo[3,1,1]hept-2-ene-methanol	1637	-	2.68	-
Calamenene	1648	0.71	-	0.05
Benzoicacid	1657	-	-	0.06
2,6-octadien-1-ol	1667	-	-	0.17
2-cyclohexen-1-ol	1680	-	0.87	-
2-cyclohexen-1-one	1714	-	-	0.52
Thymol	1729	0.52	0.26	-
Benzenemethanol	1735	-	0.21	0.16
Verbenone	1741	3.03	4.07	0.20
1-butanol	1794	-	-	0.05
Naphthalene	1884	9.37	0.22	2.01
Benzene, 1,2-dimethoxy-4	1923	-	-	0.08
Caryophyllene oxide	1936	-	2.85	-
3-cyclohexen-1-carboxyaldehyde	1988	-	0.22	-
Cyclohexanone	2051	-	-	0.10
Ethanone	2146	-	-	0.02
Diethylphythalate	2295	0.29	0.14	0.02
Phenol	2454	-	-	0.39
1,3-benzenediamine	2564	1.71	0.78	0.10
	Total	96.20	93.05	96.25

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. It is noteworthy that, except for *P. nigra*, α-pinene was detected as main compounds of all studied *Pinus* taxa (Tables 1 and 2). The main components of *P. nigra* from Turkey were α-pinene, β-pinene, β-caryophyllene and germacrene D [1]. α-pinene was the main constituent of *P. slyvestris* (14.76%), *P. nigra* (45.36%) and *P. halepensis* (47.09%), too [22]. Like these results α-pinene (12.96%, 33.29%, 32.96%, 25.56% and 9.00%) was among the major compound in *P. resinosa, P. flexilis*, *P. strobus*, *P. parviflora* and *P. mugo* subsp. *mugo*, respectively (Tables 1 and 2).

In *P. koraiensis* α-pinene (22.3%), bornyl acetate (8.3%) and camphene; in *P. merkusii* β-caryophyllene (43.1%), caryophyllene oxide (11.9%) and α-humulene (9.0%); in *P. palustris* α-terpineol (27.3%), β-pinene (25.2%) and α-pinene (11.6%); in *P. parviflora* α-pinene (20.2%), bornyl acetate (18.3%) and β-caryophyllene (8.7%); in *P. petula* caryophyllene oxide (14.8%), β-phellandrene (12.1%); in *P. ponderosa* β-pinene (38.2%), α-pinene (13.0%); in *P. pumila* α-pinene (18.3%), δ-3-carene (10.4%); in *P. rigida* β-pinene (15.2%), α-pinene (11.1%); in *P. rudis* β-pinene (21.4%), caryophyllene oxide (20.0%) were reported as main components. According to these results studied *Pinus* taxa can be separated into two groups: One group is the one which contains a large amount of α-pinene, and the other which contains little α-pinene. *P. koraiensis*, *P. parviflora*

and *P. pumila* belong to the first group and contain around 20% of α-pinene, and all the other species belong to the second group [3]. In this study, *P. resinosa*, *P. flexilix*, *P. strobus*, *P. parviflora* belong to the first group and contain around 22% of α-pinene, and *P. nigra* belong to the second group which contain little α-pinene (2.68) (Table 2). Tumen et al. [22] reported that, limonene (62.8%) in *P. pinea* and β-pinene (39.6%) in *P. brutia* were found in higher amounts. On the other hand, in this study limonene (8.06%, 6.21% and 5.09%) was found in *P. nigra*, *P. parviflora* and *P. mugo* subsp. *mugo*, respectively; whereas limonene was not detected in *P. resinosa*. β-pinene was not detected in *P. parviflora* and *P. nigra*; on the other hand β-pinene was found in higher amounts in *P. flexilis* (16.24%) and *P. strobus* (8.01%) (Tables 1 and 2). The main differences among the under studied samples are from chemical composition a high percentage of β-myrcene (27.72%) only in *P. strobus*; acetic acid (31.12%), bicyclo[2.2.1]heptan-2-one (21.45%) and borneol (8.64%) only in *P. nigra*; *p*-cymene (18.03%) only in *P. mugo* subsp. *mugo*; camphene (5.68%) only in *P. parviflora*. Moreover, α-pinene was detected at lower amount only in *P. nigra* (2.68%) among studied *Pinus* taxa (Tables 1 and 2).

A comparison of the data presented in this paper with those in the literature for other taxa of *Pinus* show that there are qualitative and quantitative differences in the levels of some of the compounds present. Moreover, constituents such as sesquiterpenes (δ-cadinene, γ-cadinene or oxygenated terpenes as α-cadinol and τ-cadinol/τ-muurol (not separated by GC)) were found in higher amounts (2.8%-7.7%) than in the previously studied *Pinus* species [23]. In *P. strobus* spathulenol (2.8%) and α-selinene/germacrene B (3.0%) were also identified in larger amounts in comparison to the essential oil from North American pine and other conifers oils [23]. For the essential oil of *P. parviflora*, 33 components were identified representing 95.07% of the oil (Table 2). α-pinene was the predominant compound (25.56%) followed by caryophyllene (13.21%), germacrene D (6.71%), limonene (6.21%), camphene (5.68%) and bornylacetate (4.25%). Above observations are in contrast to the data published by Lis-Balchin et al. (1998) [24], who correlated lack of the antifungal activity of the needle pine oil with the high content of α- and β-pinenes. On the other hand, these results are in good agreement with the data reported by Magiatis et al. (1999) [25]. For volatile constituents of *P. mugo* subsp. *mugo*, 39 components were identified representing 95.79% of the oil (Table 2). 3-carene was the predominant compound (36.54%) followed by *p*-cymene (18.03%), α-pinene (9.00%), limonene (5.09%) and β-phellandrene (4.13%). The results of these studies demonstrate that Δ-3-carene is present in needles as traces, what is in contrast to other reports. Dormont et al. (1998) [26] and Hanover (1975) [27] determined in the oleoresin as well as the foliage volatiles the levels of Δ-3-carene as high as 10%-40%. HS-SPME/GC-MS method was used in a previous study by Kilic (2013) [28].

It is possible to say that, *P. strobus*, *P. parviflora*, *P. mugo* subsp. *mugo*, *P. resinosa* and *P. flexilis* showed α-pinene, whereas *P. nigra* showed acetic acid chemo-type essential oils.

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