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Closely eluting bornyl and isobornyl acetates are chemotaxonomic markers in the Pinaceae by virtue of their unique mass spectra

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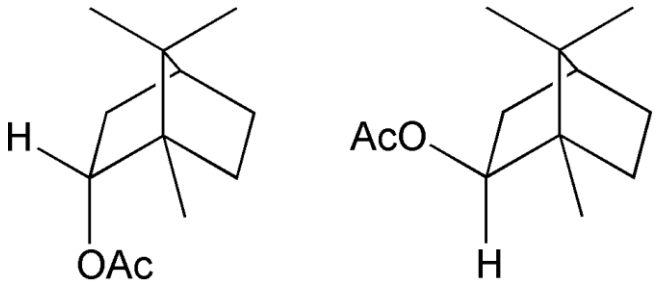
Abstract
Gas chromatography / mass spectrometry (GC/MS) of *Abies balsamea*, *Picea mariana* and *Tsuga canadensis* leaf essential oils assigned bornyl acetate as a major peak in *A. balsamea* and *P. mariana*, while isobornyl acetate was identified as the major peak in *T. canadensis*. Though these two isomers elute closely on GC, their characteristic mass spectra allow unequivocal structural assignment. Under electron impact (EI) conditions, bornyl acetate, the *endo*- isomer, displays a significant molecular ion *m/z* 196, while isobornyl acetate, the *exo*- form, displays a marked loss of acetic acid, resulting in a molecular ion an order of magnitude less intense than that of bornyl acetate. Thus GC/MS analyses with authentic standards may be prerequisite for chemotaxonomic studies on Pinaceae essential oils. A re-examination of reports of bornyl acetate in *T. canadensis* and of isobornyl acetate in *P. abies* is suggested.

Keywords: bornyl acetate; isobornyl acetate; *Abies*; *Picea*; *Tsuga*; GC/MS

1. Introduction
During studies on commercially available essential oils of the family Pinaceae, gas chromatography / mass spectrometry (GC/MS) analyses indicated that the monoterpene constituent profile of *Picea mariana* (Mill.) Britton (black spruce) was quite similar to *Tsuga canadensis* (L.) Carriére (eastern or Canadian hemlock). The essential oils of both genera are typified by substantial amounts of α- and β-pinene, camphene, and Δ-3 carene along with other monoterpenes [1]. The total ion chromatograms (TIC) of *P. mariana* and *T. canadensis* oils each displayed a very large peak at about 20 minutes retention time. GC mass spectra (70 eV) of the predominant peak in each oil predicted bicyclic monoterpene monoacetate (*m/z* 196, M^+, $C_{12}H_{20}O_2$). Database searches [2, 3] of these peaks' electron impact [EI] fragmentation patterns identified them as either bornyl acetate (1) or isobornyl acetate (2) (Kovats retention Indices = 1285 for both isomers [2]).



1 **2**

Fig 1: Chemical structures of bornyl acetate [**1**] and isobornyl acetate [**2**]

Furthermore, interpretation of the Adams GC/MS database established the major peak in *P. mariana* essential oil as bornyl acetate, while the corresponding peak in *T. canadensis* oil was identified as the stereoisomer of 1, isobornyl acetate (**2**). Similar analyses of balsam fir *Abies balsamea* (L.) Mill. essential oil confirmed bornyl acetate as a major constituent in its leaf oil as well. The literature shows that bornyl acetate, the *endo*- isomer, is a chemotaxonomic marker for *Picea* [1, 4-20] as well as for *Abies* [1, 4, 19-26], while isobornyl acetate, the *exo*- variant,

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is characteristic of *Tsuga* [1, 27-28]. Because a number of papers have been published with the opposite isomeric assignment; that is, bornyl acetate from *Tsuga* [9, 20, 26, 29-31], as well as isobornyl acetate from *Picea* [32], the mass spectra of compounds 1 and 2, obtained with authentic chemical standards, are reproduced herein. Differences in the relative intensities of diagnostic ions enable unequivocal isomer assignment, and may obviate any need for fractionation and further characterization by optical rotation or other spectrochemical means. Thus, a structural reassessment of the outlier *Tsuga* and *Picea* collections may be warranted.

2. Experimental

2.1 Essential oil samples

A. balsamea needle essential oil of Canadian origin was obtained from Sigma-Aldrich, Milwaukee, WI, product # W523518, CAS # 8021-28-1, lot # 08203kov. *P. mariana* organic oil was provided by the Lebermuth Company, South Bend, IN, CAS # 91722-19-9, lot #1410001758. *T. canadensis* essential oil was purchased from Spectrum Chemical, New Brunswick, NJ, item # S1803, CAS # 8008-18-8, lot # XG0316. The essential oils were diluted 1:10 with a 1:1 mixture of GC-grade isopropyl alcohol and hexane (diluent) prior to analysis, except for *T. canadensis*, which was diluted 1:20.

2.2 Chemical standards

Analytical standard (-)-bornyl acetate was purchased from Sigma-Aldrich, product # 45855, CAS # 5655-61-8, batch # BCBM5398v, 99.3% purity by GC. ($\pm$)- Isobornyl acetate was also from Sigma-Aldrich, product # W216003, CAS # 125-12-2, lot # mkbj9565v, 97.5% purity by GC. Both standards were diluted 1:20 with diluent to acquire the maximum signal, and were further diluted with a calibrated micropipette and class A volumetric glassware in order to generate a dilution series for each.

2.3 GC/MS analysis

Minor modifications to Adams' GC/MS method [2] are described in the following. Essential oils and chemical standards were analyzed on an Agilent 7890B gas chromatograph equipped with an Agilent 7693 autosampler and coupled to an Agilent 5977A Mass Selective Detector operating in the EI mode at 70 eV. The column was an Agilent 122-5532 Ultra Inert DB-5ms, [(5%-Phenyl)-methylpolysiloxane, equivalent to USP Phase G27], 30 m x 0.25 mm ID x 0.25 µm film thickness. The column temperature was programmed from 60° to 246 °C (62 min) using He as carrier gas. The injector temperature was 220 °C. The injection volume was 0.5 µL with a 50:1 split injection mode. Analyses were run using constant He pressure mode: 108 kPa, retention-time locked [33] to *n*-hexadecane at 32.91 min. The MS interface temperature was 250 °C. The detector voltage was 0.9kV and the mass range was 41-415 u. The scan speed was 1562 u/s; interval: 0.50 s (2 Hz). Data handling and processing were controlled by Agilent MSD Productivity Chem Station for GC and GC/MS Systems Data Analysis Application.

3. Results and Discussion

The TICs for *A. balsamea*, *P. mariana* and *T. canadensis* oils are presented in Figures 2, 3, and 4, respectively, with assignments for some major monoterpenes based on application of retention and spectral data from the Adams database.

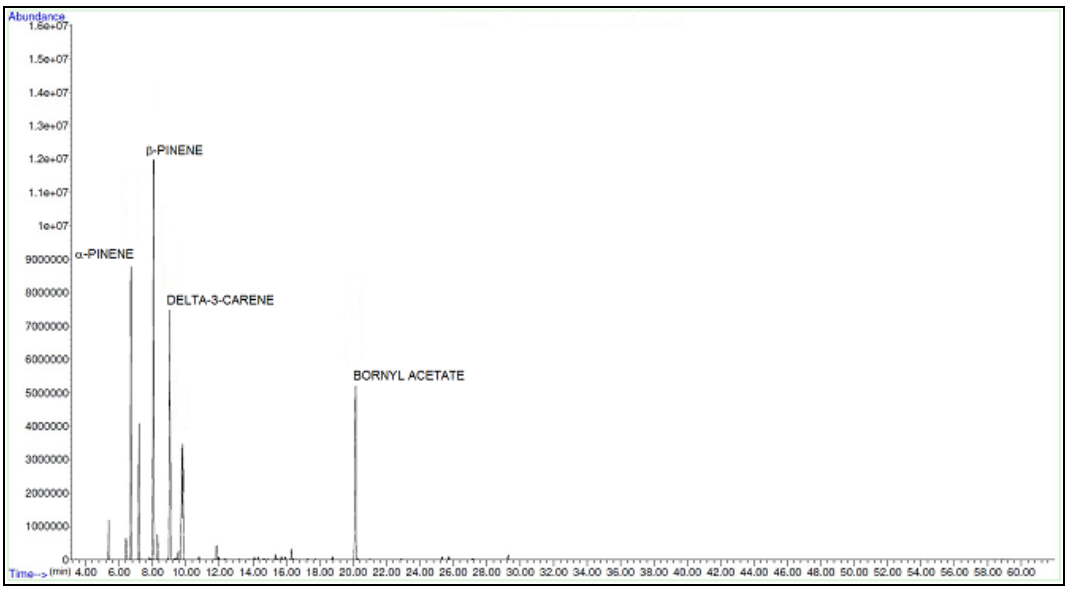

Fig 2: Mass spectrometry total ion chromatogram for *Abies balsamea* leaf oil

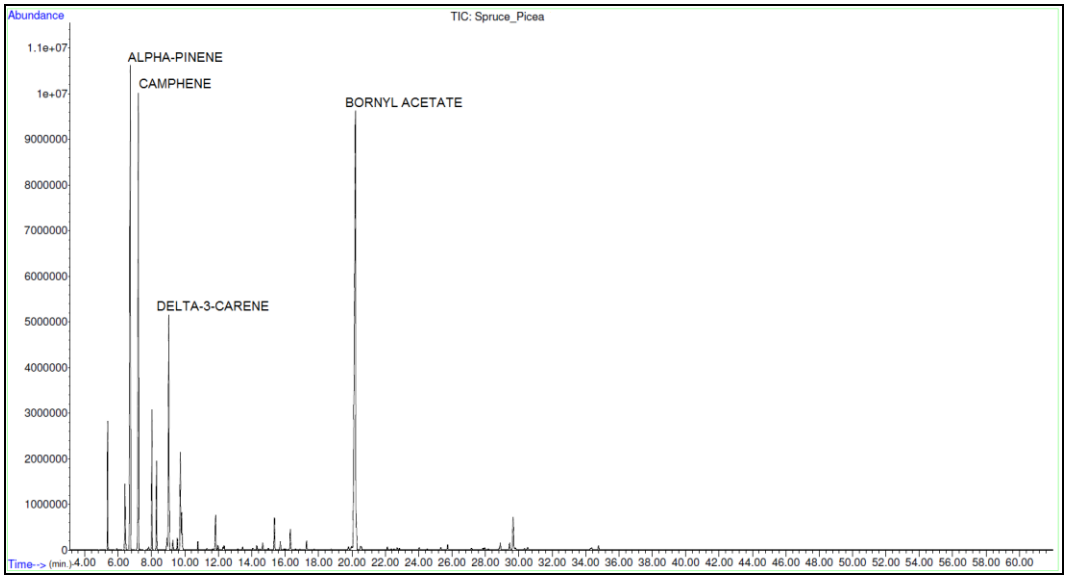

Fig 3: Mass spectrometry total ion chromatogram for *Picea mariana* leaf oil

Bornyl and isobornyl acetate appear to elute at nearly the same retention time. Both isomers from *Chrysanthemum* flower have previously proven separable on a nonpolar GC column, with isobornyl acetate eluting five seconds after bornyl acetate [34]. In practice, identification of the correct isomer is more difficult when their true and experimentally verifiable elution order on a

Fig 4: Mass spectrometry total ion chromatogram for *Tsuga canadensis* leaf oil

nonpolar column, whether denoted by Retention Index (RI) or Time, Arithmetic Index, or User Index, is incorrect in the popular Adams database, both paper and digital versions [2]. The database author recently communicated that re-analysis on back-to-back runs generated retention times of 19.42 (RI 1282) and 19.48 (RI 1283) minutes, respectively, for bornyl and isobornyl acetate (Adams, RP, personal communication, 24 Aug 2016). Undeniably, peak assignment exclusively by retention data is common in the literature, but may be prone to error because of the close elution of two isomers unless at least one, but preferably both chemical standards are available for co-injection. Bornyl and isobornyl acetate are not enantiomeric, though they share the same connectivity between atoms in the molecules. They are not mirror images, but exhibit *endo/exo* isomerism. Chromatographic performance parameters calculated by the software for naturally occurring 1 and 2 in three essential oil matrices are given in Table 1 below:

Table 1: Chromatographic performance parameters for bornyl and isobornyl acetate in commercial essential oils.

	bornyl acetate (1) in *A. balsamea*	**bornyl acetate (1) in *P. mariana***	***iso*-bornyl acetate (2) in *T. canadensis***
Peak width (min.)	0.24	0.39	0.45
Resolution	11	6.3	3.2
Tailing	0.78	0.62	0.59
RMS signal to noise	18265	54814	102045
Plates	40200	14580	11494

Note: Noise was calculated between 38 and 48 minutes.

Suboptimal performance for isomer 2 in *T. canadensis* may be due to a small amount of bornyl acetate 1, not split by the software integration, but verified by MS analysis, fronting on the major peak of compound 2 in the *T. canadensis* oil sample (see Figure 4).

In the 1950s, Shaw reported *l*-bornyl acetate from *T. canadensis* [29], however the optical rotation (α_D -20°) is half that expected, compared to pure (-)-bornyl acetate (Sigma-Aldrich analytical standard certificate of analysis, α_D -44°). Thus, Shaw may have been characterizing an impure compound. In similar fashion, von Rudloff, who in the 1960s pioneered preparative GC on the Pinaceae terpenes, reported low magnitude optical rotations (α_D +7°, +20°) for *d*-bornyl acetate from *P. sitchensis* and *P. engelmannii*, respectively, and established bornyl acetate as a taxonomic and phylogenetic marker for *Picea* [5]. Interestingly, von Rudloff's subsequent examination of *P. rubens* [6] yielded the antipode (α_D -21°) of bornyl acetate in contrast to his previous work. In 1975, von Rudloff [9] predicted the ascendancy of GC/MS for identifying compounds of diagnostic and chemosystematic value, including bornyl acetate in *Picea*, *Abies* and other conifers, as well as, strikingly, *Tsuga*. Although equipped with GC/MS, Kubeczka and Shultze [20] assigned bornyl acetate as a marker for *T. canadensis* as well. In 2004, analyses with a chiral GC column established that large amounts of (-)-bornyl acetate were present in *Pseudotsuga menziesii*, *Picea engelmannii* x *glauca* and *Abies lasiocarpa* x *bifolia* [14]. We sourced a virtually optically inactive (α_D +1° per certificate of analysis) standard for isobornyl acetate. As a chiral column was unnecessary for at least an incomplete separation of the *endo-/exo-* isomers in question, it was observed that the differential response of the mass spectrometer to *endo-/exo-*isomerism in bornyl and isobornyl acetates might be exploited for unequivocal isomer assignment, thus aiding species identification. Considering the apparent confusion in the literature, Figures 5 and 6 present the mass spectra of the commercially sourced standards of bornyl and isobornyl acetate, introduced into the mass spectrometer by the described GC method.

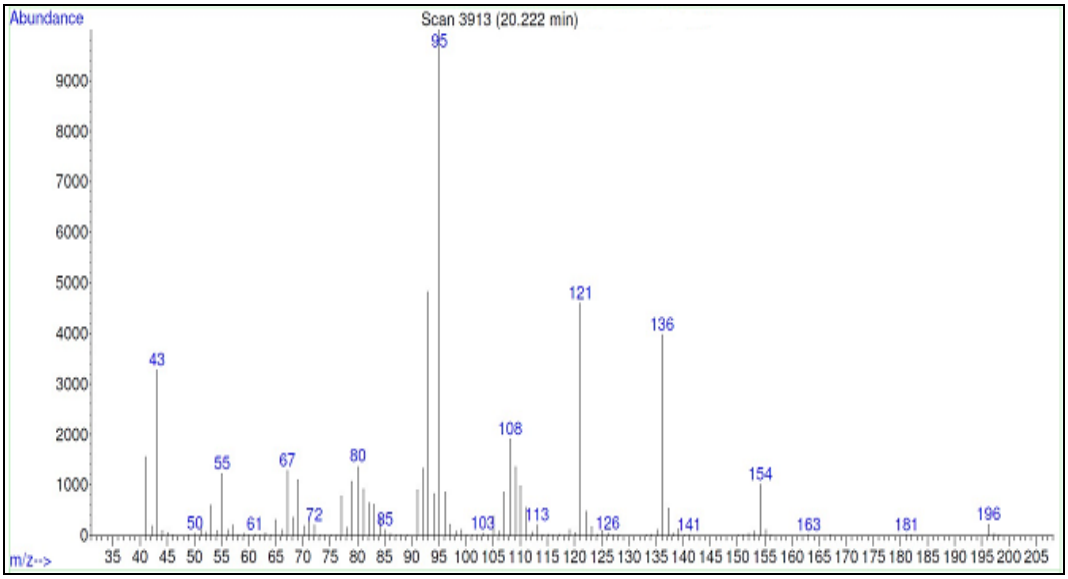

Fig 5: Mass spectrum of bornyl acetate (1) reference standard.

Pure reference compounds were analyzed to preclude any doubt regarding identification, i.e. which isomer yields which spectral features, and also to rule out any contribution of the essential oil matrix to the spectra. However, the spectra of the naturally occurring analytes found in these essential oils of commerce appear virtually indistinguishable from those obtained using the pure chemical standards.

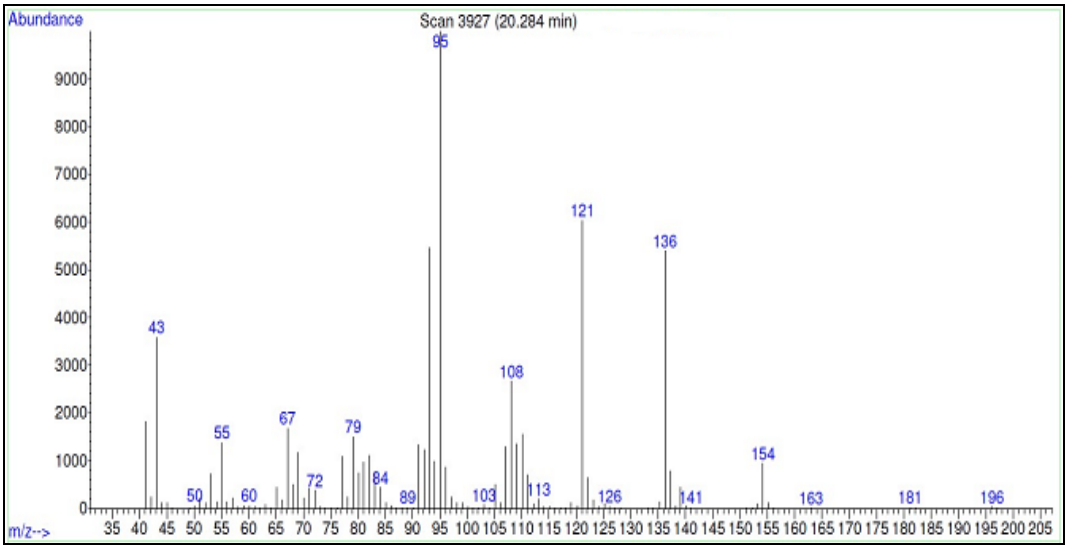

Fig 6: Mass spectrum of isobornyl acetate (2) reference standard.

An anonymous reviewer kindly suggested that a dilution series of each standard be injected under the same conditions in order to explore how the fragmentations of the molecules might vary according to the mass of standard compound introduced into the mass spectrometer. Approximate masses were calculated volumetrically from the dilution series and take into account the 50:1 split setting of the GC method. The ranges of the dilution series were chosen to cover analyte amounts that would be encountered working with essential oils. The results of these experiments are presented in Table 2:

Table 2: % Intensities of ions relative to base peak *m/z* 95 vary with approx. mass introduced to column (N.D. = none detected).

	$[M]^+$ (*m/z* 196)	**$[M\text{-ketene}]^+$ (*m/z* 154)**	**$[M\text{-HOAc}]^+$ (*m/z* 136)**
bornyl acetate (**1**) 500 ng	2.2%	10.1%	39.6%
200 ng	2.1%	9.9%	39.7%
20 ng	1.9%	9.7%	39.5%
4 ng	1.6%	9.6%	38.3%
1 ng	N.D.	6.1%	37.2%
isobornyl acetate (**2**) 500 ng	0.24%	9.4%	54.1%
200 ng	0.23%	8.9%	55.8%
20 ng	0.12%	8.0%	56.9%
4 ng	N.D.	6.2%	56.1%
1 ng	N.D.	N.D.	53.6%

One notable difference between the spectra of bornyl and isobornyl acetate is the relative size of M^+ (*m/z* 196) compared to the base peak *m/z* 95. The molecular ion is an order of magnitude more intense in bornyl than isobornyl acetate. Concomitantly, the loss of acetic acid (*m/z* 136) is significantly greater for isobornyl acetate (relative intensity 56%) than for bornyl acetate (relative intensity 39%). It was also noted that the relative ion heights for bornyl acetate presented as *m/z* 79 < 80 and *m/z* 109 > 110; while for isobornyl acetate the opposite pattern, *m/z* 79 > 80 and *m/z* 109 < 110, was observed. Other than these features, the spectra of the two isomers are quite similar, which combined with close elution, explains why misidentifications may have occurred. It was noted that as the amount of isobornyl acetate decreased, the most intense peak in the mass spectrum actually shifted from *m/z* 95 to *m/z* 93. However, for consistency, the percentages in Table 2 are all based on the base peak *m/z* 95. In general, the results show that the relative intensities of the ion fragments do not vary much as a function of mass. Therefore it would be unlikely to transpose the identifications of bornyl and isobornyl acetate as long as high quality mass spectra were available, regardless of how much material was injected. At the lowest amounts injected, the molecular ions were indistinguishable from the noise. Though the method was not applied quantitatively to the essential oils, it is apparent that limits of detection and of quantification would depend on which ion(s) were monitored. As a cautionary note, it was previously demonstrated that instrument design and configuration may influence mass spectra observed for a particular compound [35].

Regarding the spectra observed for bornyl and isobornyl acetates using the present equipment, it is observed that both isomers, when exposed to the beam of ionizing electrons, undergo facile losses of ketene $[M - 42]^+$ and especially of acetic acid $[M - 60]^+$, yielding *m/z* 154 and 136, respectively. Isomer **2**, the *endo*-form, has molecular geometry that can be envisioned to position the acetate moiety "out" into the electron beam for a significant time, exposing the acetate group to high energy electrons that give rise to a large $[M\text{-HOAc}]^+$ fragment and a diminished molecular ion. On the other hand, the *endo*-acetate (1), with the acetate group under the molecule as drawn in Figure 1, tumbles in the vacuum with the acetate shielded from electron impacts, at least partially, by the hydrocarbon scaffold of the bicyclic portion of the molecule, thus yielding a more intense molecular ion and a smaller loss of acetic acid than isobornyl acetate **2**. Adams' MS database reveals similar behavior for the molecular ions of borneol compared to isoborneol. Though this might be of spectrochemical interest, borneol and isoborneol are well separated chromatographically on nonpolar GC columns, so further pursuit on these lines is beyond the scope of the research.

Identification of the correct isomers of enzymatically produced secondary metabolites is ecologically and economically important; for instance, isobornyl acetate, correctly identified in *T. canadensis* and six other *Tsuga* species by GC/MS [27], may be an attractant to a major forest pest, the parasitic hemlock woolly adelgid *Adelges tsugae* [28]. Conversely, bornyl acetate produced by Douglas fir (*Pseudotsuga menziesii*) was shown to be a feeding deterrent to another significant forest pest, the western spruce budworm, *Choristoneura occidentalis*. [36] The questionable report by McClure and Hare of bornyl acetate in *Tsuga* [30] could be overlooked considering they were restricted to a flame ionization detector only and thus had no MS data. More puzzling are relatively recent studies in which investigators had access to GC/MS or authentic standards and yet made an incorrect structural assignment [31-32]. The apparently erroneous report of isobornyl acetate in *P. abies* [32] is surprising because it references work describing the correct isomer, bornyl acetate, in that same species [13].

4. Conclusions

As analytical technology evolves, details of the natural world emerge that require retroactive corrections to the common body of knowledge. Positive outcomes resulting from a better understanding of chemical signalling between predator and prey organisms might include design of more selective pesticides or of pheromone baits to help preserve endangered forests. Because this investigation was restricted to only three species of the diverse Pinaceae, further research is encouraged to determine if misidentification of bornyl and isobornyl acetates is more widespread than reported here.

5. Acknowledgments

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6. References

1. Tisserand R, Young R. Essential Oil Safety: A Guide for Health Care Professionals, 2nd ed. Churchill Livingstone Elsevier, Edinburgh, UK, 2014.
2. Adams RP. Identification of Essential Oil Components by Gas Chromatography / Mass Spectrometry, 4th ed. Allured Publ. Corp., Carol Stream, IL, USA, 2007.
3. NIST14 Website. [http://www.nist.gov/srd/nist1a.cfm]. Visited on 20 June, 2016.
4. Wagner H, Bladt S, Zgainski EM. Plant Drug Analysis: A Thin Layer Chromatography Atlas, Springer-Verlag, Berlin, DE, 1984.

5. Von Rudloff E. The volatile oils of the leaves of Sitka and Engelmann spruce. Can J. Chem. 1964; 42:1057-1062.
6. Von Rudloff E. The chemical composition of the volatile oil of the leaves of *Picea rubens* Sarg. and chemotaxonomic correlations with other North American spruce species. Phytochemistry 1966; 5:33-341.
7. Von Rudloff E. Seasonal variation in the composition of the volatile oil of the leaves, buds, and twigs of white spruce (*Picea glauca*). Can. J Bot. 1972; 50:1595-1603.
8. Hrutfiord BF, Hopley SM, Gara RI. Monoterpenes in Sitka spruce: within tree and seasonal variation. Phytochemistry 1974; 13:2167-2170.
9. Von Rudloff E. Volatile leaf oil analysis in chemosystematic studies of North American conifers. Biochem. Syst. Ecol. 1975; 2:131-167.
10. Von Rudloff E. Seasonal variation in the terpenes of the foliage of black spruce. Phytochemistry 1975; 14:1695-1699.
11. Schönwitz R, Merk L, Ziegler H. Naturally occurring monoterpenoids in needles of *Picea abies* (L.) Karst. Trees 1987; 1:88-93.
12. Schönwitz R, Kloos M, Merk L, Ziegler H. Patterns of monoterpenes stored in the needles of *Picea abies* (L.) Karst. from several locations in mountainous regions of southern Germany. Trees 1990; 4:27-33.
13. Martin DM, Gershenzon J, Bohlmann J. Induction of volatile terpene biosynthesis and diurnal emission by methyl jasmonate in foliage of Norway spruce. Plant Physiol. 2003; 132:1586-1599.
14. Pureswaran DS, Gries R, Borden JH. Quantitative variation in monoterpenes in four species of conifers. Biochem. Syst. Ecol. 2004; 32:1109-1136.
15. Nicolić B, Tesević V, Ethordević I, Marin PD, Bojović S. Essential oil variability in natural populations of *Picea omorika*, a rare European Conifer. Chem. Biodivers. 2009; 6:193-203.
16. Adams RP, Callahan F. The leaf essential oils of *Picea chihuahuana* Martinez and *P. martinezii* Patterson (Pinaceae). Phytologia 2014; 96:260-263.
17. Poaty B, Lahlah J, Porqueres F, Bouafif H. Composition, antimicrobial and antioxidant activities of seven essential oils from the North American boreal forest. World J. Microbiol. Biotechnol. 2015; 31:907-919.
18. Hachey JM, Simard S. Extraction and analysis of the essential oil of the needles and twigs of white spruce *Picea glauca* (Moench) Voss. J Wood Chem. Technol. 1987; 7:333-341.
19. Garneau FX, Collin G, Gagnon H, Pichette A. Chemical composition of the hydrosol and the essential oil of three different species of the Pinaceae family: *Picea glauca* (Moench) Voss., *Picea mariana* (Mill.) B.S.P., and *Abies balsamea* (L.) Mill. J. Essent. Oil Bear. Pl. 2012; 15:227-236.
20. Kubeczka KH, Schultze W. Biology and Chemistry of Conifer Oils. Flavour Frag. J 1987; 2:137-148.
21. Morozova SS, Katyuzhanskaya AN, Kekhov AV, Koshevoi EP, Mikhailova NS. Development of a method for determining bornyl acetate in the products of the processing of *Abies siberica*. Chem. Nat. Compd. 1976; 12:269-272.
22. Régimbal JM, Collin G. Essential Oil Analysis of Balsam Fir *Abies balsamea* (L.) Mill. J Essent. Oil Res. 1994; 6:229-238.
23. Wang W, Yan Xy, Shi Hb, Niu Zd, Sun Yl, Zhang Xp. Determination of bornyl acetate in volatile oil of *Abies nephrolepis* (Trautv.) Maxim. leaves by GC. Chin. J Pharm. Anal. (Yao Wu Fen Xi Za Zhi). 2004; 24:244-246.
24. Satou T, Matsuura M, Murakami S, Hayashi S, Koike K. Composition and seasonal variation of the essential oil from *Abies sachalinensis* from Hokkaido, Japan. Nat. Prod. Commun. 2009; 4:645-648.
25. Satou T, Matsuura M, Takahashi M, Murakami S, Hayashi S, Sadamoto K *et al*. Components of essential oils extracted from leaves and shoots of *Abies* species in Japan. Chem. Biodivers. 2011; 8:1132-1138.
26. Adams RP, Kauffmann M, Callahan F. The leaf essential oil of *Abies grandis* (Doug. ex D. Don) Lindl. (Pinaceae): revisited 38 years later. Phytologia 2015; 97:1-5.
27. Lagalante AF, Montgomery ME. Analysis of terpenoids from hemlock (*Tsuga*) species by solid-phase microextraction / gas chromatography / ion-trap mass spectrometry. J Agric. Food Chem. 2003; 51:2115-2120.
28. Lagalante AF, Lewis N, Montgomery ME, Shields KS. Temporal and spatial variation of terpenoids in eastern hemlock (*Tsuga canadensis*) in relation to feeding by *Adelges tsugae*. J. Chem. Ecol. 2006; 32:2389-2403.
29. Shaw AC. The essential oil of *Tsuga canadensis* (L.) Carr. J Am. Chem. Soc. 1951; 73:2859-2861.
30. McClure MS, Hare JD. Foliar terpenoids in *Tsuga* species and the fecundity of scale insects. Oecologia 1984; 63:185-193.
31. Broeckling CD, Salom SM. Volatile emissions of eastern hemlock, *Tsuga canadensis*, and the influence of hemlock woolly adelgid. Phytochemistry 2003; 62:175-180.
32. Vidrich V, Fusi P, Franci M, Michelozzi M. Characterisation of terpene profile in essential oil of two provenances of *Picea abies*. Ital. For. Mont. 2005; 60:287-292.
33. David F, Scanlan F, Sandra P, Szelewski M. Analysis of essential oil compounds using retention time locked methods and retention time databases. Agilent Application Notes 5988-6530EN and 5989-8574EN.
34. Wang G, Dong C, Sun Ya, Xie K, Zheng H. Characterization of volatile components in dry chrysanthemum flowers using headspace-liquid-phase microextraction-gas chromatography. J Chrom. Sci 2008; 46:127-132.
35. Adams RP, Weyerstahl P. cis- and trans-Sabinene hydrate: comparisons of quadrupole and ion trap mass spectra. J Essent. Oil Res. 1992; 4:197-200.
36. Cates RG, Henderson CB, Redak RA. Responses of the western spruce budworm to varying levels of nitrogen and terpenes. Oecologia 1987; 73:312-316.