SHORT REPORT

Rec. Nat. Prod. 8:1 (2014) 56-60

records of natural products

Geographical Variation in the Composition of *Abies bornmuelleriana* Mattf. Needle Oils

Güneş Uçar* and Mualla Balaban Uçar

Department of Wood Chemistry, Faculty of Forestry, University of Istanbul, Istanbul, Türkiye

(Received April 05, 2012; Revised March 11, 2013; Accepted October 03, 2013)

Abstract: This study is to investigate the effect of the geographic variation on the composition of needle oils of *Abies bornmuelleriana* endemic to Turkey. Samples were taken from the stands distanced up to 500 km from each other. By means of GC-MS/FID analyses, it was found that main compounds making up about 90 percent of the needle essential oils exhibit notable systematic variation depending on the longitude where the trees grow. Among most occurring monoterpenes, the percentages of α-pinene and camphene tend to increase in west–east direction. To compensate these increments a decrease was observed in mainly the three monoterpenes, β-pinene, β-phellandrene and limonene.

Keywords: Needle oil; Terpenes; *Abies bornmuelleriana*; GC-MS.
© 2014 ACG Publications. All rights reserved.

1. Plant Source

Being endemic to Turkey, the Uludag or Turkish fir (*Abies bornmuelleriana* Mattf.) is one the four different fir species growing naturally. It ranges throughout the northwestern part of Anatolia, and mostly forms mixed stands. With elevation of some 2500 meters Uludag Mountain and its surroundings are the highest ecoregion of Northwest Anatolia. Uludag ecoregion is considered to be the western boundary of the Uludag fir while this tree species does spread easterly as far as the mouth of the Red River near the town of Bafra (Figure 1) [1,2].

Figure 1. Distribution of *Abies bornmuelleriana* in Northwest Anatolia [2]
■ Sampled stands; 1 Uludag, 2 Cicekli, 3 Ilgaz, 4 Dutman

2. Previous Studies

Although many investigations have been carried out on essential oils of the fir species, only a few works report on the essential oil composition of Anatolian counterparts *Abies nordmanniana*, *A. cilicica*, *A. equi-trojani*, and *A. bornmuelleriana* [3, 4, 5]. Bagcı and Babac [6] attempted to characterize *Abies* species of Turkey by means of morphometric and chemosystematic analyses. In the most detailed work Ucar et al [7] analyzed the oils isolated from needles of 60 trees of the natural fir

*Corresponding author: E-mail: gunucar@gmail.com

Thearticlewaspublishedby Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 11/18/2013 EISSN:1307-6167

species of Turkey by GC and GC-MS. The authors brought forward clear evidence that Turkish fir species *A. bornmuelleriana* and *A. nordmanniana* can be distinguished from each other based on the presence of the monoterpene δ-3-carene in needle oils. This is the only study on the composition of the needle essential oil of *Abies bornmuelleriana*. Yet some works dealing with antimicrobial activity of essential oil of *A. bornmuelleriana* have also appeared [8, 9, 10]. It was demonstrated that *A. bornmuelleriana* essential oil had the highest antimicrobial activity against certain bacteria and yeast species [8].

3. Present study

Since *A. bornmuelleriana* is endemic to Turkey, a special emphasis is given to the oil composition of this species and more additional specimens were collected from different stands distributed in west-east longitudinal direction. By means of GC-MS-FID analyses of isolated oils, it is aimed to assess particularly the variations of the oil composition caused by geographic locations.

For the present study, we used the analysis data of Uludag location for an earlier work [7]. Moreover three additional locations were chosen to collect needle specimens; Dutman being easternmost location about 500 km distant from Uludag, and Cicekli and Ilgaz lying between Uludag and Dutman (Fig.1). Due to the fact that the quantity and composition of needle essential oils can also vary considerably depending on the season [11, 12], the needles were collected in September and October 2008. Six sound representative trees of 30-40 cm diameter in each location were so selected that a minimum distance of 100 m between trees was observed. 3-5 cm thick branches from each tree were cut and their current year green shoots were collected. About 1 kg of needle material was packed into a plastic bag, and the bags were transported to the laboratory within the same day, stored at the refrigerator for few days until hydrodistillations were performed. 700-800 g of green shoots with needles were transferred in a wide-necked- 5-liter flask and rinsed once with cold distilled water in the flask to remove dust, and then 3 L of distilled water was added. The flask was placed in a heating mantle and connected to a proper apparatus for recovery of distillate. The heating rate was regulated at the appropriate level to enable the collection of about 350 mL of distillate in 35–40 minutes. One liter of distillate was considered to contain representative amount of volatile isolates.

The oily layer on the distillate was isolated by solvent extraction with petroleum ether (3x50 mL). The volume of solvent was reduced to 10-15 mL under light vacuum by removing the excess solvent. The oils in the tightly closed volumetric flasks were kept at 0 °C in the refrigerator until they were analyzed.

Analyses were made in the GC/MS (Shimadzu, QP 5050A) and in GC/FID (Thermo Finnigan Trace DSQ) instruments to identify and quantitate (FID quantification in Table I) the compounds in the volatiles. Identification of most compounds was based on the libraries NIST 21, NIST 107 and WILEY 229. A private MS-data (Ucar library) library and Adam's library [13] was also used to identify some compounds. Analyses were carried out on a 30 m nonpolar fused silica DB-1 column (0.25 mm, 0.25 µm film thickness), with a helium flow rate of 1.0 mL/min and split ratio of 10:1. The following temperature program was maintained:

5 min at 60 °C, 3 °C/min to 120 °C, 5 °C/min to 200 °C, 10 °C/min to 260 °C and 8 min at 260 °C.

The results of GC analyses of needle oils were shown in Table 1. Value for each compound is the average of six trees. Compounds exceeding 0.5% in average at least for one location were taken in the list. There were some unidentified compounds. These compounds were either left out or *m/z* values of main mass fragments were included below the Table1.

No qualitative differences were observed in the composition of the essential oils obtained from different regions but quantitative differences were important mostly in respect of monoterpene hydrocarbons.

A total of 38 constituents were identified, representing 96.6 - 96.9% of the oils. The oils were characterized by very high amount of monoterpene hydrocarbons (about 80%) accompanied by smaller amounts of oxygenated monoterpenes (between 4,84-7,7%) and sesquiterpenes (3,03-9,05%) as well as traces of diterpenes.

Table 1. The composition of needle oils from four *Abies bornmuelleriana* stands

No	Compound	RI	Uludag[7]	Cicekli	Ilgaz	Dutman
1	santene	882	0,42	1,08	1,05	1,25
2	tricyclene	922	0,31	0,40	0,46	0,47
3	α-pinene	936	25,3	29,8	37,0	40,6
4	camphene	947	2,70	3,94	4,55	4,54
5	β-pinene	973	34,8	32,1	26,7	21,0
6	myrcene	988	2,29	2,04	1,80	1,63
7	α-phellandrene	997	0,65	0,86	0,98	0,86
8	α-terpinene	1012	0,13	0,14	0,15	0,15
9	p-cymene	1014	0,06	0,06	0,08	0,08
10	β-phellandrene	1023	10,0	8,21	7,65	5,77
11	limonene	1025	11,0	5,54	1,87	1,79
12	γ-terpinene	1052	0,15	0,14	0,16	0,15
13	2-nonanone	1075	0,07	0,13	0,06	0,09
14	terpinolene	1081	0,89	1,06	1,27	1,26
15	nonanal	1085	0,03	0,10	0,09	0,10
16	linalool	1087	0,17	0,10	0,16	0,43
17	α-fenchol	1095	0,19	0,23	0,25	0,29
18	α-campholenal	1100	0,06	0,10	0,11	0,14
19	trans-pinocarveol	1120	0,04	0,07	0,09	0,12
20	camphenehydrate	1128	0,14	0,16	0,18	0,24
21	α-phellandrenol	1146	0,05	0,05	0,06	0,09
22	borneol	1147	0,17	0,22	0,25	0,26
23	terpinen-4-ol	1161	0,25	0,29	0,36	0,35
24	α-terpineol	1172	3,08	3,59	4,25	4,96
25	linalyl acetate	1244	0,11	0,02	0,04	0,06
26	bornyl acetate	1268	0,41	0,42	0,51	0,49
27	α-terpinyl acetate	1332	0,14	0,03	0,11	0,07
28	β-caryophyllene	1407	0,43	1,07	0,80	1,68
29	α-humulene	1442	0,24	0,58	0,46	0,87
30	germacrene D	1469	0,27	0,06	0,04	0,27
31	δ-cadinene	1507	0,03	0,05	0,01	0,07
32	caryophyllene oxide	1562	0,03	0,06	0,04	0,06
33	longiborneol	1577	0,07	0,04	0,03	0,02
34	selinen-4-ol isomer†	1597	0,16	0,05	0,26	0,28
35	eudesmol<10-epi-γ->	1601	0,05	0,13	0,17	0,15
36	selin-11-en-4-α-ol	1635	1,75	3,69	4,68	5,65
37	abieta-7,13-diene	2078	0,07	0,04	0,08	0,15
38	labdadienol†	2125	0,06	0,09	0,05	0,20
	Total identified		**96,8**	**96,7**	**96,9**	**96,6**

RI retention indices on nonpolar DB-1 column calculated against n alkanes (C9-C22), (bold: main compounds, i.e. ≥1 %)

†Relevant mass fragments of unidentified compounds:

RI	***m/z* (rel. int.)**
1597	222(4), 204(56), 189(40), 161(62), 147(16), 135(29), 121(34), 105(43), 93(32), 91(27), 81(83), 79(21), 71 (19), 55 (24), 43(100), 41 (41)
2125	290(15), 272(17), 257(6), 195(29), 177(100), 148(33), 135(33), 133(44), 121(38), 119(33), 107(40) 105(29), 95(53), 93(29), 91(34), 81(42), 79(28), 71(41), 69(58) 55(57), 43(99), 41(71)

The main components of the oils were α-pinene, β-pinene, β-phellandrene and limonene with different concentrations. α-terpineol was the major oxygenated component (3,08-4,96%). The oil contained as sesquiterpene hydrocarbons β-caryophyllene (0,43-1,68%) and α-humulene (0,24-0,87) and as oxygenated sesquiterpenes mainly selin-11-en-4-α-ol (1,75-5,65%). Selin-11-en-4-α-ol was isolated from the needle oil of *A. bornmuelleriana* and identified by means of NMR analysis [14].

The limonene percentage of the needle oils increased drastically to 25 % (average) in *A. equi-trojani* [7]. Among Anatolian firs, the next highest limonene percentage with 11 % was found in the *A. bornmuelleriana* grown in Uludag region. This is a distinct feature for *A. equi-trojani* which forms stands in the Kazdagi region lying nearby to the west of Uludag region. The absence of δ-3-carene in needle oils of *A. bornmuelleriana* distinguishes this species from *A. nordmanniana* and *A. cilicica* subsp. *cilicica* having large amounts of δ-3-carene [7].

The essential oils of *A. cephalonica* which grows to the southwest of *A. bornmuelleriana* in Greece contained 21% β-pinene > 16% α-pinene > 14% limonene [15]. The percentages of these three monoterpene hydrocarbons from Uludag and Cicekli show the same chemical profile as *A. cephalonica*.

Figure 2 visualizes these systematic variations in the percentages of main compounds of *A. bornmuelleriana* attributable to longitudinally different locations of the trees. The percentage of α-pinene was the lowest in northwest stand of *A. bornmuelleriana* Uludag (25,3%), and this percentage increased steadily up to 40,6% toward to the northeast stand Dutman. This remarkable increment of α-pinene content was balanced by the decreased amount of β-pinene, myrcene, β-phellandrene and limonene.

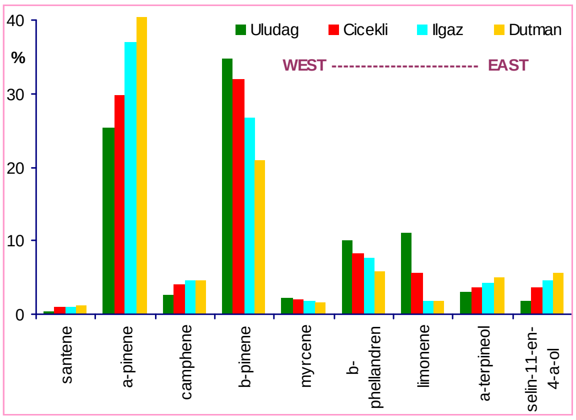

Figure 2. Systematic variation of main compounds of *Abies bornmuelleriana* needle oils from longitudinally different locations

As a result, considering the percentages of the nine main monoterpenoids and one sesquiterpene which make up about 90 % percent of the oils, a systematic variation in west-east direction is notable. As we move from western boundary Uludag to Dutman easternmost boundary of *A. bornmuelleriana*, santene, α-pinene, camphene, α-terpineol and selin-11-en-4-α-ol exhibit an increment whereas the percentages of β-pinene, myrcene, β-phellandrene and limonene dropped to some extent.

Cluster analysis: Also, a cluster analysis was conducted on the distribution of 38 compounds of Table 1 by means of commercial SPSS software. As shown in Figure 3, the four locations of *A. bornmuelleriana* divided in two groups according to this statistical evaluation. The western stands Uludag and Cicekli with larger amounts of β-pinene and β-phellandrene formed the first group, while the remaining east stands Ilgaz and Dutman exhibiting higher percentages of α-pinene represented the other group.

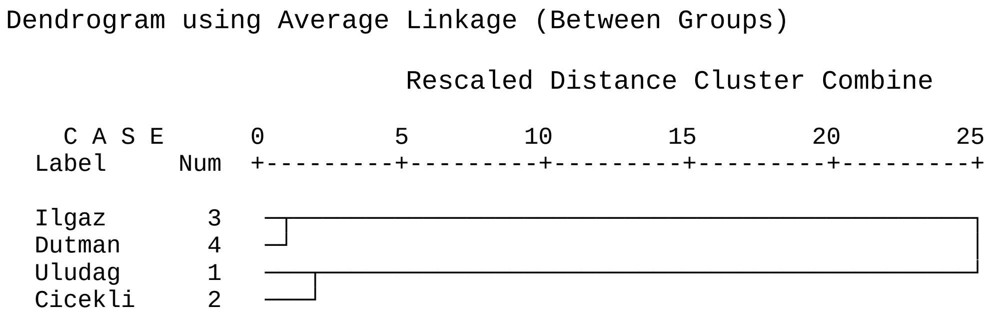

Figure 3. Hierarchical cluster analysis of Turkish fir locations based on average percentage composition of needle essential oils

References

[1] H. Kayacık (1959). Orman ve Park Ağaçlarının Özel Sistematiği I. Cilt Gymnospermae (Açık Tohumlular). İstanbul Üniversitesi Orman Fakültesi Yayınları, İ.Ü. Yayın No: 813, O.F. Yayın No: 60, İstanbul.

[2] H. Kayacık (1965). Orman ve Park Ağaçlarının Özel Sistematiği. I. Cilt Gymnospermae (Açık Tohumlular). 2.Baskı, İstanbul Üniversitesi Orman Fakültesi Yayınları, İ.Ü. Yayın No: 1105, O.F. Yayın No: 98, İstanbul.

[3] T.G. Sagarevishvili (1999). Components of essential oil *Abies nordmanniana*, *Chem. Nat. Compd.*, **35**, 586.

[4] E. Bagcı, K.H.C. Baser, M. Kurkcuoglu, T. Babac and S. Celik (1999). Study of essential oil composition of two species of *Abies cilicica* (Ant. et Kotschy.) Carr. From Turkey, *Flav. And Fragr. J*. **14**, 47-49.

[5] B. Fady, M. Arbezand A. Marpeau (1992). Geographic variability of terpene composition in *Abies cephalonica* Loudon and *Abies* species around the Aegean: hypothesis for their possible phylogeny from the Miocene, *Trees*, **6**, 162-171.

[6] E. Bagcı and M. T. Babac, (2003). A morphometric and chemosystematic study on the *Abies* Miller (fir) species in Turkey, *Acta Bot.Gall.*, **150**, 355-367.

[7] G. Uçar, M. Balaban Uçar, H. Özdemir and E. Atıcı (2010). Chemical Characterization of Volatile Needle Oils from Anatolian Fir Species: *Abies nordmanniana* (Stev.) Mattf.,*A. bornmuelleriana* Mattf., *A. equitrojani* Aschers et Sint. and *A. cilicica* Carr, *J. Essent. Oil Res*., **22**, 548-554.

[8] E. Bagcı and M. Dıgrak (1996). Antimicrobial Activity of Essential Oils of some *Abies* (Fir) Species from Turkey, *Flav. Fragr. J*. **11**, 251-256.

[9] E. Bagcı and M. Dıgrak (1997). In vitro antimicrobial activities of some fir essential oils, *Turk.J. Biol.* **21**, 273-281.

[10] M. Benli, U. Bingol, F. Geven, K. Guney and N. Yigit (2008). An investigation on antimicrobial activity of endemic plan species from Turkey, *Afr. J. of Biotech*., 7, 1-5.

[11] E. V. Rudloff and M. Granat (1982). Seasonal variation of the terpenes of the leaves, buds and twigs of balsam fir *(Abies balsamea), Can. J. Bot.* **60**, 2682-2685.

[12] M. R. Wagner, K. M. Clancey and R. W. Tinus (1989). Maturational variation in needle essential oils from *Pseudotsuga menziesii*, *Abies concolor* and *Picea engelmannii*, *Phytochemistry*, **28**, 765-770.

[13] R.P. Adams (2007). Identification of Essential Oil Components by Gas Chromatograpy/Mass Spectrometry,4th Ed. Allured Pub. Corp.Carol Stream, IL.

[14] M. K. Sakar, D. Ercil, E. Del Olmo and A. San Feliciano(1999). Constituents of *Abies nordmanniana* ssp. *Bornmuellerian*, *Fitoterapia*, **70**, 103-105.

[15] V. Roussis, M. Couladis, O. Tzakou, A. Loukis, P.V. Petrakis, N.M. Dukic and R. Jancic (2000). A comparative study on the needle volatile constituents of three *Abies* species grown in South Balkans, *J. Essent. Oil Res.* **12**, 41-46.

© 2014 ACG Publications.