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Seasonal Depending Variations of the Composition and Biological Activities of Douglas Fir (*Pseudotsuga menziesii*) Essential Oils from Bulgaria

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The seasonal depending variations of the essential oils of the needles with twigs of Bulgarian Douglas fir (*Pseudotsuga menziesii*, Pinaceae) were analyzed by GC-FID, GC-MS and olfactoric evaluation. In totally 8 investigated essential *P. menziesii* oils about 60 compounds could be identified with monoterpenes (especially sabinene and β-pinene) as main constituents. The antimicrobial effects against bacteria, fungi and worms of these volatiles as well as their olfactoric properties will be discussed.

Keywords: *Pseudotsuga menziesii*, Pinaceae, essential oil of the needles with twigs, GC-FID, GC-MS, antimicrobial activity

Introduction

Douglas fir (*Pseudotsuga menziesii* Mirb. Franco) is a well-known and wide-spread Pinaceae species in Europe and America. Beside the use of the essential *P. menziesii* oils of the needles, the twigs and the bark in various fields of cosmetics and perfumery also some studies [1-5] of antimicrobial activities against bacteria, fungi and worms have been published.
In our investigation the seasonal depending variation of the essential oil composition using GC-FID, GC-MS and olfactometry as well as of the biological effects using antimicrobial testings will be discussed.

Results and discussion

From June 1999 to February 2000 in total 8 samples of Douglas fir (*Pseudotsuga menziesii* Mirb. Franco, Pinaceae) essential oils of needles with twigs from Bulgaria were obtained by steam distillation and the volatiles investigated by GC-FID, GC-MS, olfactometry and antimicrobial testings to get information about the qualitative and quantitative composition as well as the biological activities of the single oils.

The olfactoric evaluation of the samples showed that the significant fresh fir odor with pinenes, citronellal, citronellol and teatree-oil notes has been found in all oils with only lightly different intensities.
Using gas chromatographic-spectroscopic methods (GC-FID and GC-MS) with columns of different polarities more than 80 volatiles could be detected and about 60 of them identified as Douglas fir essential oil constituents (see Table 1).

Table 1. Composition of the Douglas fir essential oils of fresh needles and twigs from Bulgaria (Identification by mass spectra, retention-index and olfactoric correlations in accordance to [6-9]).

Compound[a]	1	2	3	4	5	6	7	8	RI[b]
cis-3-hexen-1-ol	2.7	1.3	0.9	0.7	0.6	0.6	0.3	0.5	841
hexanol	1.5	1.5	1.3	1.4	1.2	0.9	0.5	0.4	849
α-thujene	0.7	0.1	0.3	0.8	0.5	0.3	0.4	0.7	935
α-pinene	8.9	6.5	6.1	6.8	7.9	5.1	5.3	4.4	944
heptanol	0.4	0.2	0.1	0.4	0.5	0.2	0.4	0.5	952
camphene	0.3	0.4	0.6	0.9	1.1	1.2	1.4	1.3	954
cis-3-hexenyl acetate	1.3	0.7	0.6	0.4	0.2	0.3	0.1	0.3	972
sabinene	16.7	14.9	16.9	13.9	13.2	14.7	14.4	15.4	974
β-pinene	22.0	20.8	14.7	19.8	25.9	16.3	15.9	14.2	978
β-myrcene	2.3	1.7	1.2	1.5	1.2	0.9	1.6	1.2	991
α-phellandrene	0.8	1.3	0.8	1.6	0.8	2.1	1.8	1.3	1007
1,4-cineole	tr[c]	tr	0.1	0.1	0.2	0.3	0.2	0.1	1009
α-terpinene	2.1	4.4	3.7	1.8	2.1	2.2	1.9	2.1	1016
p-cymene	0.6	0.4	0.9	1.4	0.9	1.3	1.4	0.9	1019
1,8-cineole	0.2	0.5	0.7	0.9	1.0	0.7	0.9	0.4	1021
limonene	1.2	1.1	1.6	1.4	0.7	0.3	0.5	0.2	1025
cis-β-ocimene	0.1	0.3	1.0	1.2	1.1	1.7	1.0	1.8	1028
β-phellandrene	2.5	0.6	3.7	3.2	4.2	3.2	3.0	3.1	1031
trans-β-ocimene	0.2	0.4	0.6	0.5	0.7	1.1	1.3	1.5	1040
γ-terpinene	2.3	4.4	2.8	3.2	4.1	5.3	5.2	5.7	1057
dihydro-myrcenol	nd[d]	tr	tr	0.1	0.1	tr	nd	tr	1064
α-terpinolene	6.4	11.8	13.1	11.3	11.7	15.0	16.2	16.1	1077
linalool	0.5	0.6	0.7	0.7	0.6	1.1	1.3	1.6	1096
myrcenol	tr	tr	0.1	0.2	tr	tr	tr	nd	1103
phenylethanol	nd	tr	0.2	0.3	nd	0.2	0.6	0.5	1105
β-pinene oxide	tr	0.1	0.4	0.8	1.2	1.4	1.5	1.7	1109
fenchol	nd	tr	0.1	0.2	tr	0.2	tr	nd	1116
isopulegol	nd	nd	tr	0.1	0.1	tr	nd	0.1	1129
camphor	0.1	0.2	0.3	0.5	0.2	0.3	0.4	0.3	1132
trans-pinocarveol	*tr*	*0.1*	0.2	0.3	tr	tr	0.1	nd	1135
citronellal	3.0	0.4	1.2	0.9	1.6	1.2	1.9	1.8	1139
trans-verbenol	nd	tr	0.1	0.2	tr	0.1	nd	0.1	1141
borneol	tr	tr	0.1	0.1	0.2	tr	nd	0.2	1163
p-cymen-8-ol	nd	tr	tr	0.1	0.1	nd	nd	nd	1168
terpinen-4-ol	6.9	9.6	5.2	3.8	3.1	7.3	7.4	8.7	1182
α-terpineol	5.1	2.5	2.4	1.5	1.0	1.3	0.9	1.5	1186
dihydrocarveol	tr	tr	0.1	0.2	tr	nd	tr	nd	1190
octyl acetate	tr	tr	tr	0.1	nd	nd	nd	nd	1194
β-cyclocitral	0.4	0.3	0.4	0.6	0.2	tr	0.3	0.2	1201
citronellol	1.3	1.8	3.2	2.8	2.3	3.9	3.4	3.3	1217
carvone	tr	tr	0.1	0.1	nd	tr	tr	nd	1227
geraniol	0.1	0.4	0.9	1.1	0.3	0.5	0.6	0.5	1238
isopulegyl acetate	tr	tr	0.1	0.1	nd	tr	nd	nd	1259
thymol	nd	tr	tr	0.1	nd	0.1	tr	nd	1271
bornyl acetate	nd	tr	tr	0.1	nd	tr	0.2	nd	1278
citronellyl acetate	3.4	1.5	3.5	3.9	3.6	4.8	3.3	4.2	1334

α-longipinene	nd	tr	0.1	0.2	nd	tr	tr	0.1	1360
geranyl acetate	tr	nd	0.2	0.1	0.2	0.2	0.1	0.1	1364
α-cubebene	nd	tr	0.2	tr	nd	tr	0.2	tr	1370
α-copaene	nd	nd	0.1	0.2	nd	tr	0.1	nd	1374
decyl acetate	nd	nd	tr	tr	nd	nd	0.1	tr	1392
β-elemene	tr	0.1	0.5	0.2	nd	0.2	0.3	0.2	1400
β-caryophyllene	0.5	0.7	0.9	0.7	0.2	0.1	0.3	0.5	1430
trans-β-farnesene	0.2	0.5	0.7	0.4	0.6	tr	0.2	0.3	1436
α-humulene	tr	0.1	0.2	0.1	nd	tr	0.1	0.2	1446
germacrene D	tr	tr	tr	0.1	0.6	0.2	0.3	0.4	1471
α-muurolene	tr	tr	tr	0.2	0.1	tr	nd	tr	1495
δ-cadinene	0.2	0.4	0.7	0.8	0.5	0.2	0.4	nd	1516
caryophyllene oxide I	tr	tr	0.1	0.2	tr	tr	0.1	0.2	1577
δ-cadinol	0.1	0.4	0.3	0.2	0.4	0.1	0.1	nd	1675

[a]in order of their retention indices using a non-polar column
[b]retention indices (non-polar column)
[c]trace compound
[d]not detected

As main compounds of the 8 *P.menziesii* essential oil samples the monoterpenes β-pinene, sabinene, α-pinene, α-terpinolene, terpinen-4-ol, citronellal, citronellol, citronellyl acetate, β-phellandrene and γ-terpinene, only with changes in their concentration of each sample, were identified.
The correlation of the olfactoric data with gas chromatographic spectroscopic data ones allow the conclusion that the significant Douglas fir odor impressions is especially determined by β-pinene, sabinene and α-pinene, the citronellal and citronellol notes by these monoterpenes and the teatree oil notes by terpinen-4-ol, α-terpineol, α-terpinolene and linalool. The change of the odor notes intensities are directly depending on the variation of the concentrations of the above mentioned monoterpenes.
Using biological testings on different microorganisms (see experimental part) the antimicrobial activity of the 8 *P.menziesii* essential oil samples was investigated. Significant differences in the inhibition of bacteria growth by some Douglas fir essential oils were found (see Table 2).

Table 2: *Antimicrobial activieties of Pseudotsuga menziesii essential oils (needles with twigs) from Bulgaria (+++ strong effects, >25mm; ++ medium effects, to 25mm; + weak effects, to 15mm; 0 no effects)*

Microorganism	1*	2	3	4	5	6	7	8
Pseudomonas aeruginosa	+	+	+	+	+	++	+	+
Proteus vulgaris	++	++	++	++	0	0	+++	+++
Serratia marcescens	+	+	+	+	0	+	++	+
Bacillus subtilis	0	++	++	0	++	+++	+++	++
Bacillus cereus	0	++	+++	+	+	++	+++	+
Staphyllococcus aureus	+	+	+	+	+	+	+	+

*number of essential oil sample (see experimental part)

In general it was found that the used Douglas fir essential oils have shown the highest activity against *Proteus vulgaris*, *Bacillus cereus* and *Bacillus subtilis*.
By correlation of the microbiological data with the identified main constituents of the single oil samples it is significant that terpinen-4-ol, α-terpinolene, γ-terpinene and α-terpinene rich Douglas fir samples (samples 2, 3, 6, 7 and 8) have stronger effects against the most tested microorganisms.
In conclusion we can report, that the seasonal depending variation of the composition of *Pseudotsuga menziesii* essential oils (needles and twigs) from Bulgaria has significant influences on the odor impression as well as the antimicrobial activity. The strongest effects of Douglas fir oil against microorganisms have been found in testings with *Proteus vulgaris*, *Bacillus subtilis* and *Bacillus cereus*. The *P. menziesii* essential oils with high concentrations of the monoterpenes terpinen-4-ol, α-terpinolene, γ-terpinene and α-terpinene show more antimicrobial activities.

Experimental

Plant material and isolation of the essential oil:

The raw material was gathered in June 1999 (sample 1), August 1999 (2), September 1999 (3), October 1999 (4), November 1999 (5), December 1999 (6), January 2000 (7) and February 2000 (8) in the region of Kazanlik at the Balkan Mountains. The essential oils were obtained by steam distillation of the fresh plant parts in a tinned copper laboratory apparatus of 5 dm^3 for 2 hours. The steam distillation was carried out at the rate of 10% and atmospheric pressure of the steam (0.1 Mpa). The dynamic of the primary oil distillation was determined by measuring the oil yield every 30 min. The procedure was stopped when no more oil could be collected in the receiver in a 30 min time period. The yields of essential oils are as follows: Sample 1: 0.65%, 2: 0.80%, 3: 0.64%, 4: 0.73%, 5: 0.82%, 6: 0.74%, 7: 0.77% and 8: 0.79%.

Olfactoric evaluation of the essential oils:

200 microlitre of the essential oil (diluted with dichloromethane) was placed on a commercial odor strip (Dragoco Co.) and the odor evaluated after 10 seconds (solvent evaporation) by professional perfumers. Only minor variations of the odor impressions were found.

Instrumentation:

The volatiles of the totally 8 essential oil samples of Bulgarian Douglas fir were analyzed first by GC (Shimadzu 14A resp. Varian-3700) with FID and integrator

systems (Shimadzu C-R6A-Chromatopac resp. Shimadzu C-R1B-Chromatopac). Carrier gas: hydrogen; injector-temperature: 250°C; detector-temperature: 320°C; temp.-program: 40°C/ 5min. to 280°C/ 10min. with heating-rate of 8°C/min.; columns: 30m x 0.32mm bonded FSOT-RSL-200 fused silica (film thickness: 0.25 micron; Biorad Co.); quantification by %-peak-area-calculation; compound identification partly by co-injection of pure compounds and retention-time correlations in accordance with [6-9].
The second used hyphenated system was GC-MS (Shimadzu GC-17A with QP5000 and datasystem Compaq-ProLinea with Class5k-software resp. Hewlett-Packard GC-HP5890 with HP5970-MSD and datasystem PC-Pentium (Böhm Co.) with HPCHEM-software). Carrier gas: helium; injector-temperature: 250°C; interface-heating: 300°C; ion-source-heating: 200°C; EI-mode; 70 eV; scan-range: 41-450 amu; temp.-program and column see GC-FID part. Mass spectra correlations with Wiley-, NBS- and NIST-library spectra on-line resp. in accordance to [9] off-line.

Microbiological testings:

The antibacterial activity was studied using the Agar-Diffusion-Cup-Method: cups [3,4]: 0.6cm and volume: 0.01ml for each product. The following microorganisms were used: *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Serratia marcescens*, *Bacillus subtilis*, *Bacillus cereus* and *Staphylococcus aureus*.

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REFERENCES

[1] Buchbauer, G., Jirovetz, L., Wasicky, M., Nikiforov, A. (1994), J.Agric. Food Chem. **42**:2852.
[2] Chalchat, J.C., Garry, R.Ph., Bastide, P., Fabre, F., Malhuret, R. (1991), Plant.Med.Phytother. **25**:184.
[3] Stojanova, A., Metodiev, S., Karova, E., Posterpresentation (no. MM67) at the First Balcan Conference of Microbiology „Microbiologia Balkanica '99), Plovdiv (Bulgaria), October 5-9 , 1999.
[4] Zaika, L. (1988), Food Safety **9**:97.
[5] Yonov, N. (1992), Sci.Woods **4**:69.
[6] Davies, N.W. (1990), J.Chromatogr. **503**:1.
[7] Jennings, W., Shibamoto, T. (1980). Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatography, Academic Press, New York.

[8] Private Retention-time tables of flavor and fragrance compounds.
[9] Schmaus, G. (1988). Thesis: Untersuchungen über die Zusammensetzung der ätherischen Wurzelöle verschiedener mittel- und westeuropäischer Peucedanum-Arten (Apiaceae) unter besonderer Berücksichtigung von *Peucedanum palustre* (L.) Moench und *Peucedanum lancifolium* Lange, University of Würzburg, Germany.

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