UNIVERSITY OF NIŠ
The scientific journal "FACTA UNIVERSITATIS"
Series: "Physics, Chemistry and Technology" Vol.1, N° 2, 1995. 188-192
Editor of Series: Predrag Dimitrijević
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ESSENTIAL OIL OF *PINUS PEUCE* NEEDLES

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Abstract: Needle oil of Pinus peuce Griseb. produced from five different growth stages at the location Gine vode, Šar planina mountain, Serbia, was examined for yield and composition during 1994. Although only twelve components were identified in the oil, the main constituents were α-pinene (18.64%), γ-muurolene (17.93%), δ-3-carene (13.82%), sabinene (13.23%) and bornylacetate (12.23%). The prevailing amount of monoterpenes was found in every stage of growth. The richness of the oil in α-pinene and γ-muurolene has chemotaxonomic implications.

Key Word Index: *Pinus peuce* Griseb.; *Pinaceae*; essential oil composition; oil yield; chemotype; α-pinene; γ-muurolene.

Introduction

Pinus peuce Griseb, a tertiary relic and endemic of the Balkan peninsula, was found on Perister, Macedonia, from A. Grisebach 1839, who described it as *Pinus peuce* 1844. Its Eastern frontiers are the Bulgarian mountains Rila, Pirin, Slavjanka, Western Rodhops, Vitosha and Stara planina [1]. It spreads in the Central parts of the Balkan peninsula on Perister, Nidže, Kožuf, Korab, Rudoka and Šar planina in Macedonia, Koprivnik, Prokletije, Zeletin, Šar planina and the mountains near the source of Ibar river in Yugoslavia, Perim Dagh and Rila Dagh in Albania and in the Northern Greece. *P. peuce* is the mountain species (600-2200 m altitude), and it grows on silicate soil [2].

The needle oil of *P. peuce* Griseb. has not been the subject of much studies [3-8]. Tsankova et all. examined an industrially obtained oil in Bulgaria by GLC, CC and fractional distillation. IR and GLC co-injection of pure compounds were used for identification [4]. Jankov et all. studied the essential oils of many conifers, as well as of *P. peuce*, using the same methods of analysis as Tsankova, including TLC [5]. Kolesnikova et all. examined trees grown in botanical gardens using GLC and co-injection of pure compounds, IR and UV spectra for the identification of the oil constituents [6,7]. The most recent study

UDK: 543:547.21; Received 22.July 1995.

about the chemical composition of the essential oil of naturally grown *P. peuce* was carried out by Gorunović et all. [8] using GC/MS method.

The aim of this paper was to provide more information on the composition of the needle oil obtained from naturally grown trees of P. peuce, and to compare them with the literature data.

Experimental

Needles (bulk samples) of *P. peuce* were collected during the active vegetative period in 1994, (from April until the end of August) [9] from the location Gine vode (Šar planina mount, Serbia). The plant material was collected at different stages of growth as follows:

1st stage - the initial vegetation stage (4.4.1994)
2nd stage - the budding stage (1.5.1994)
3rd stage - the blooming stage (28.5.1994)
4th stage - the fading stage (2.7.1994)
5th stage - the seed forming stage (3.8.1994).

After collection of the plant material, the needles were separated from the branches and cut up to a length of 1.5-2 cm. A mixture of 600 g of the cut needles and 4.5 dm^3 water was hydrodistilled for 2.5 h using a Clevenger-type procedure [10]. The oil was extracted from the distillate with Et_2O and then dried overnight with anh. Na_2SO_4. After filtration of the Na_2SO_4, the solvent was removed by distillation at atmosphere pressure and the pure oil kept at ca. 4°C until analysis.

Identification Procedure. The oil was analyzed on an analytical GC and GC/MS and most constituents were identified by comparison of their mass spectra to those from the MS library [11].

Analytical GC. A Varian model 3700 Gas Chromatograph, equipped with a 2.5 m x 1/8" column packed with 10% OV-101, and FID was used for GC measurements.. The operating conditions were: temperature program 70°-200°C at 3°C/min. and an injector and detector temperature of 220°C; carrier gas N2 (20 mL/min.). Peak areas were calculated electronically by a Varian CDS-111 data calculator.

GC/MS analyses were performed on a H.P. 5890 Gas Chromatograph equipped with a fused silica 25 m x 0.25 mm, SE-30 capillary column, and a Hewlett-Packard 5970 series Mass Selective Detector. The column was temperature programmed from 100°-290°C at 3°/min. with a carrier gas (He) at a flow rate 1 mL/min. and a 40:1 split.

Results and discussion

Table 1 shows the obtained results. The amount of oil is expressed in % (calculated per weight of the plant material dried during 2.5 h at 120°C).

Table 1. Needle oil content (%, v/w) of *P. peuce* Griseb. during the 1994 vegetation period.

stages of growth				
1st	2nd	3rd	4rd	5th
0.82	0.67	0.64	0.70	0.68

The observed decrease in the oil content during the 1st to 3rd stage of development of the trees is a phenomenon that has also been observed in other pine species by Chernodubov and Deryuzhkin [9]. We believe that the phenomenon is the result of the metabolism of essential oil components during the early stages of plant growth, when intensive physiological processes are taking place. Furthermore, we believe that the minimum oil content found at the 3rd stage is related to a large increase of a tissue mass; however there is no significant increase in the oil. In the 4th and 5th stages young tissue growth usually terminates, while the oil content increases because of intensive photosynthesis. It is believed that the oil yield probably would show an increase until the end of the vegetation period. An increase of the oil content and other organic substances during this period is interpreted as a preparation of plant for winter [9].

The results of the analysis of the *P. peuce* oil can be seen in Table 2.

Table 2. Chemical composition (%) of *P. heldreichii* oil

compound	growth stages					average
	1st	2nd	3rd	4rd	5th	
α-pinene	13.82	20.99	19.71	19.26	19.41	18.64
δ-3-carene	13.22	13.73	14.14	13.54	14.48	13.82
sabinene	13.61	13.28	11.93	9.26	18.06	13.23
limonene	8.78	11.11	10.68	2.39	2.60	7.11
borneol	1.61	1.82	2.63	0.35	0.92	1.47
α-terpineol	0.66	0.28	0.54	0.25	0.16	0.48
bornylacetate	8.88	10.28	11.60	15.93	14.44	12.23
α-terpineolacetate	1.79	1.03	0.93	0.88	1.32	1.19
β-caryophyllene	2.74	1.46	1.39	1.35	1.73	1.73
α-humulene	0.93	0.50	0.42	0.49	0.54	0.58
γ-muurlene	23.17	18.97	20.18	15.96	11.39	17.93
cardinene	5.28	0.63	0.62	6.36	0.62	2.70

It can be seen from this data that the identified components amounted 91% of the whole oil mass. A prevailing amount of monoterpene hydrocarbons and their oxygenated derivatives was found, the content of which depended on the growth stage and amounted 61-72%. The sesquiterpenes content was 14-32%. A ratio of mono- and sesquiterpenes is in good correlation with the literature [4-7]. Some differences between our results and the recent study [8] of *P. peuce* needle oil could be influenced by different ecological factors.

The main components of the oils were α-pinene (18.64%) and γ-muurolene (17.93%). A survey of the literature reveals that the richness of the oil in α-pinene and γ-muurolene makes this pine a unique species, because of the more than 60 pine species, studied [3-7, 9, 12-25] none has such a high percentage of these components in their oil..

The fact that α-pinene and γ-muurolene together reached 30.80-39.96% of the oil mass (depending of the growth stage and location), undoubtedly points out the chemotaxonomic importance of these compounds. Therefore it seems to us that these components represent a possible chemotype for *P. peuce*, and as such they could be used as specific compounds for to determine characteristics of *P. peuce*.

Acknowledgments: We thank The Government of Republic of Serbia for the financial support of this project. We also thank Prof. D. Pejčinović and Prof. S. Gligorijević, The University of Priština, Yugoslavia, for the botanical identification of *Pinus peuce* Griseb.

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ETARSKO ULJE IZ IGLICA *PINUS PEUCE*

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Sadržaj: Ispitivan je sadržaj i sastav etarskog ulja *Pinus peuce* Griseb. populacije sa staništa Gine vode na Šar planini tokom pet različitih faza razvića 1994. godine. Iako je identifikovano samo dvanaest komponenata etarskog ulja, utvrđeno je da su glavni sastojci ulja α-pinen (18,64%), γ-muurolen (17,93%), δ-3-karen (13,82%), sabinen (13,23%) i bornilacetat (12,23%). Monoterpeni su u odnosu na seskviterpene dominirajuća grupa jedinjenja u ulju i to u svakoj fazi razvića ponaosob. Količinska zastupljenost α-pinena i γ-muurolena u ulju ima hemotaksonomski značaj.