

## ESSENTIAL OIL OF *Laurus nobilis* FROM MONTENEGRO

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UDC 547.913

Steam distilled oil from the shoots, separated leaves, and stem, as well as from the flower of laurel (*Laurus nobilis*), grown in Montenegro, were analyzed by GC and GC/MS. The yield of essential oil was as follow: 1.4% in young shoots, 1.5% in the separated leaves, and 0.7% in separated stems. The main constituents of all investigated oils were 1,8-cineole, methyleugenol, and  $\alpha$ -terpinyl acetate. Besides,  $\alpha$ -pinene,  $\beta$ -pinene, sabinene, and linalool were also present. It was interesting and important for commercial samples of laurel essential oil that there was no significant difference among the essential oil obtained from young shoots and those obtained from leaves and stem. The main constituents of the flower oil were 1,8-cineole (15.7%),  $\beta$ -caryophyllene (9.5%),  $\gamma$ -muurolene (7.1%),  $\alpha$ -terpinyl acetate (6.5%), and methyleugenol (3.9%).

**Key words:** *Laurus nobilis*, shoot, leaves, stem, essential oil composition, sabinene, 1,8-cineole, alpha-terpinyl acetate, methyleugenol.

Laurel, bay laurel, *Laurus nobilis* L. (Lauraceae), is an ornamental tree, indigenous to the western part as well as the southeast part of Europe [1]. The main commercial products are the leaves of laurel and the essential oil obtained from the leaves by steam distillation. The leaves are chiefly used as spice in cooking and the essential oil in food technology [2]. Rarely, leaves of laurel are used as a traditional drug for digestive disorders [3]. The leaves of laurel contain approximately 1–3% of essential oil, sesquiterpenoid lactones, and isoquinoline alkaloids. The main constituents of this oil are 1,8-cineole (30–70%), linalool (3–17%), and methyleugenol (2–8%) [4–6].

The essential oil of laurel is one of the main export products of former Yugoslavia. The main area for collected leaves of wild growing laurel is the southeast part of the Adriatic coast in Montenegro. The essential oil is produced by many little, private distilleries without standardized technology of distillation. Mainly, the essential oil is obtained by steam distillation of young shoots not separated from leaves. Besides this, the quality of this essential oil has still not been established as ISO or AFNOR standards. The Yugoslavian standard (JUS) dates back to 1968 [7]. All this constitutes a major problem for laurel essential oil producers.

This work represents our contribution to a better definition of the quality of *L. nobilis* leaves and essential oil, which are the most exported drugs from Serbia and Montenegro. Besides, we made a comparison of the composition of essential oils obtained from different parts of the laurel tree.

### EXPERIMENTAL

**Plant Material.** During the autumn of 2000, daily temperature was unexpectedly high (up to 20°C) for that part of the year. The laurel trees were in the second flowering phase in period from October to November. That is the usual time for collecting leaves for commercial purpose. Samples of young shoots were collected from the same tree, at two different localities (Budva and Bar) in the Adriatic coast of Montenegro. After drying at room temperature, the leaves were separated from the twigs.

**Oil Isolation.** Air-dried aerial parts were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus. Chromatographic analyses were done with essential oil solutions in ethanol (1%).

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TABLE 1. Percentage Composition of Essential Oil Obtained from Different Parts of the Laurel Tree, *Laurus Nobilis*, % m/m

Constituents	RIE	Locality Budva			Locality Bar			
		stem	shoots	leaves	stems	shoots	leaves	flowers
$\alpha$ -Thujene	926	0.23	0.43	0.51	0.19	0.44	0.51	0.07
$\alpha$ -Pinene	932	2.53	5.56	6.22	1.86	4.80	5.20	1.50
Camphene	946	0.38	0.51	0.53	0.13	0.22	0.20	0.32
Sabinene	972	4.21	9.58	10.02	4.08	10.02	9.83	3.14
$\beta$ -Pinene	974	2.50	4.33	4.70	1.99	4.03	4.09	1.60
Myrcene	992	0.52	0.66	0.54	0.56	0.98	1.07	0.36
$\alpha$ -Phellandrene	1004	0.18	0.18	0.21	0.18	0.27	0.29	-
$\alpha$ -Terpinene	1016	0.13	0.18	0.26	0.24	0.32	0.28	-
<i>p</i> -Cymene	1024	0.95	0.38	0.48	0.47	0.37	0.34	0.09
Limonene	1028	1.45	1.86	2.02	1.09	1.62	1.70	0.66
1,8-Cineole	1030	18.80	36.19	38.96	28.27	46.00	40.93	15.70
$\beta$ -Ocimene Z	1036							5.92
$\gamma$ -Terpinene	1058	0.40	0.49	0.62	0.36	0.56	0.62	0.16
<i>cis</i> -Sabinene hydrate	1068	0.23	0.37	0.31	0.43	0.38	0.28	0.22
<i>p</i> -Mentha-2,4(8)-diene	1087	0.14	0.17	0.21	0.22	0.24	0.27	0.09
Linalool	1101	5.77	4.25	4.06	7.65	6.67	6.05	1.37
Borneol	1165	0.18	0.12	-	0.12	0.08	0.09	0.21
Terpineol-4	1177	1.69	1.41	1.71	1.74	1.76	1.75	0.47
$\alpha$ -Terpineol	1191	1.36	1.58	1.60	2.86	2.28	2.34	0.79
Bornyl acetate	1286	0.12	0.08	-	0.26	0.11	0.10	0.33
Carvacrol	1294	-	-	-	0.23	0.18	0.17	0.08
Ocimenyl acetate	1318	-	-	-	0.26	0.17	-	0.06
<i>cis</i> -Isosafrole	1322	0.16	0.13	0.14	0.24	0.10	-	0.74
$\delta$ -Elemene		0.73	0.07	0.29	0.39	0.19	-	-
N. I.		0.61	0.47	0.46	0.55	0.56	-	0.41
$\alpha$ -Terpinyl acetate	1351	12.46	9.57	9.33	10.33	9.79	9.71	6.54
$\alpha$ -Cubebene		-	-	-	0.21	0.68	-	0.18
Eugenol	1358	1.35	1.51	1.28	3.61	-	1.10	1.16
$\alpha$ -Ylangene	1371							
$\alpha$ -Copaene	1376	0.20	0.11	0.84	-	-	-	
$\beta$ -Cubebene	1390	-	-	-	0.20	-	0.05	0.32
$\beta$ -Elemene	1392	-	0.63	-	1.16	-	0.50	2.62
Methyleugenol	1407	10.60	5.69	5.08	7.28	2.43	3.04	3.88
$\alpha$ -Gurjunene	1409	0.26	0.32	0.38	0.13	0.09	0.15	0.28
$\beta$ -Caryophyllene	1419	1.78	1.54	1.45	2.48	1.11	1.03	9.51
$\alpha$ -Guaiene	1439	0.17	0.11		0.28	0.19	0.30	0.32
N. I.	1449	0.72	0.57	0.53	0.68	0.31	0.33	-
N. I.	1450	0.30	0.27	0.26	0.37	0.08	0.12	-
$\alpha$ -Humulene	1453	-	-	-	0.19	-	0.05	0.15
$\gamma$ -Muurolene	1496	0.67	0.57	0.45	0.78	0.23	0.30	7.13
$\alpha$ -Muurolene	1500	0.50	0.27	0.27	0.47	0.17	0.32	0.38
N. I.	1502	-	0.75	-	0.73	0.36	-	-
$\alpha$ -Bulnesene	1505	1.01	-	0.73	1.01	0.19	0.58	3.44
<i>trans,trans</i> - $\alpha$ -Farnesene	1507	0.16	0.21	-	0.28	0.09	0.34	0.51
$\gamma$ -Cadinene	1516	0.93	0.25	-	0.99	0.15	0.15	1.13
$\delta$ -Cadinene	1524	0.35	0.27	0.34	0.54	-	0.39	0.84
<i>trans</i> -Cadina-1(2),4-diene	1531	0.91	-	0.54	0.19	-	-	1.18
N. I.	1537	-	0.79	-	-	-	-	-

TABLE 1. (continued)

Constituents	RIE	Locality Budva			Locality Bar			
		stem	shoots	leaves	stems	shoots	leaves	flowers
$\alpha$ -Copaen-8-ol	1541	0.43	0.25	0.15	0.44	0.05	0.12	0.49
Elemicin	1558	-	-	0.88	0.42	0.10	0.29	0.29
Germecrene D-4-ol	1575	-	-	0.62	-	0.09	0.15	-
Spathulenol	1577	4.21	1.36	-	1.64	0.08	0.17	4.13
Caryophyllene oxide	1582	4.83	1.05	-	2.67	0.26	0.29	5.56
Globulol	1587	1.54	0.42	0.36	0.18	0.08	0.08	0.81
Viridiflorol	1590	1.12	0.28	0.27	0.20	-	-	0.89
Humulene epoxide II	1605	1.08	0.10	-	0.40	-	-	0.57
10-epi-Eudesmol	1617	0.27	0.27	0.24	0.96	-	0.16	3.87
$\gamma$ -Eudesmol	1630	-	0.27	0.24	0.96	-	0.16	3.87
$\tau$ -Muurolol	1642	0.78	-	-	-	-	-	-
$\alpha$ -Muurolol	-	2.59	0.54	0.48	1.60	0.09	0.25	2.05
$\alpha$ -Cadinol	1654	0.40	0.15	-	0.35	-	-	0.99
Khusinol	-	0.93	0.26	-	0.47	-	-	0.97
<i>trans</i> -Nerolidol acetate	-	0.15	0.10	-	-	-	-	0.19
$\alpha$ -Bisabolol	1690	0.84	0.25	-	0.22	-	-	0.35
N. I.	1742	1.00	0.29	-	0.28	-	-	0.35
Total		96.23	98.16	98.74	96.63	98.98	96.52	95.58

RIE - Retention index (experimental data); N. I. = not indentified.

**GC.** A Hewlett Packard, HP-5890 gas chromatograph, equipped with a split-splitless injector, fused silica capillary column HP-5 (25 m  $\times$  0.32 mm; 0.5  $\mu$ m film thickness), and FID was employed. Oil solutions in ethanol (~ 1%) were injected in the split mode (1:30). The injector was heated at 250°C, the detector (FID) at 300°C, while the column temperature was linearly programmed from 40–240°C (4°C/min).

**GC/MS.** Analyses were carried out on a Hewlett Packard, HP G1800C GCD Series II analytical system equipped with split-splitless injector and fitted with an HP-5MS capillary column (30 m  $\times$  0.25 mm; 0.25  $\mu$ m film thickness). The chromatographic conditions were as above. The injector was heated at 250°C, the transfer line (MSD) at 280°C, while the column temperature was linearly programmed from 40–240°C (4°C/min.). EIMS spectra (70 eV) were obtained in the scan mode in the m/e range 40–450.

**Component Identification and Quantification.** Identification of individual constituents was made by comparison of their retention times with those of analytical standards of available terpenoids, and by computer searching, matching mass spectra with those in the Wiley 275 library of mass spectra [8]. Confirmation was done using the AMDIS program for determination of experimental values for retention indices of recorded constituents and comparing them with those from the literature. For quantification purposes relative area percentages obtained by FID were used.

The air-dried plant material was pulverized and the essential oil was obtained by hydrodistillation using a Clevenger-type apparatus. The content of essential oil was as follows: 1.4% in young shoots, 1.5% in the separated leaves, and 0.7% in separated stems (locality Budva).

The chemical composition of these oils was determined by GC (FID) and GC/MS techniques. The main constituents of all the investigated oils were as is usual for laurel, 1,8-cineole, methyleugenol, and  $\alpha$ -terpinyl acetate. Besides,  $\alpha$ -pinene,  $\beta$ -pinene, sabinene, and linalool were also present. It was interesting and important for commercial samples of laurel essential oil that there was no significant difference among the essential oils obtained from young shoots and those obtained from leaves and stem.

The bark peeled from the trunk and branches, collected in Budva, contained 0.75% of essential oil. The main constituents of the essential oil obtained from the bark were 1,8-cineole (26%), methyleugenol (10%), and  $\alpha$ -terpinyl acetate

(9%) (unpublished data). By comparison with the essential oil obtained from the leaves of laurel, the bark oil contained more methyleugenol, the same level of  $\alpha$ -terpinyl acetate, but less 1,8-cineole, as well as  $\alpha$ -pinene,  $\beta$ -pinene, sabinene, and linalool.

The flowers collected from laurel trees in Bar contained just a trace of essential oil. The same components characteristic for the laurel essential oil were detected in the flower's oil but in lower concentration: 1,8-cineole (15.7%),  $\alpha$ -terpinyl acetate (6.5%), methyleugenol (3.9%), sabinene (1.6%), linalool (1.4%),  $\beta$ -pinene (0.4%), and  $\alpha$ -pinene (0.3%) (Table 1). Besides, the flower oil contained a higher concentration of  $\beta$ -caryophyllene (9.5%) and  $\gamma$ -muurolene (7.1%). These results are only partly similar to those reported before for the composition of *L. nobilis* flower essential oil, mainly in the absence of high amounts of  $\beta$ -ocimene in the samples from Montenegro [9, 10].

## ACKNOWLEDGMENT

This study was supported by the Ministry for Science and Environmental Protection of Serbia through project 143012.

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