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YIELDS AND COMPOSITION OF SAGE OILS FROM DIFFERENT REGIONS OF THE YUGOSLAVIAN ADRIATIC COAST

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Yugoslavia is one of the leading exporters of sage (*Salvia officinalis* L.) and Dalmatian sage oil. The value of the Dalmatian sage is based on the high content of thujone (α- and β-) in the leaves and oil. The price of Dalmatian sage on the market depends on the thujone content, but the content of 1,8-cineole is also commercially important. Thujone is, furthermore, responsible for the strong antimicrobial activity of sage oil (1-3). Analyses of this material by gc revealed a high content of 1,8-cineole and camphor, and it seems that some kind of interdependence exists between the contents of thujone, 1,8-cinole, and camphor (4-9). Most authors attribute the differences in yield and chemical composition of sage oil to various geographic origins (10-12). The existence of infraspecific taxa leads to infraspecific chemical differences in the Lamiaceae family (12). However, the differences in chemical composition of the essential oils could stem from the maturity of the plant (6). Although official sage, which grows in the wild along the Adriatic coast, was thoroughly investigated [physical and chemical properties, thujone content, tlc (13-18)], data about other components of the oil are scarce (19-23). Therefore, we decided to collect samples of wild plants growing in different Yugoslavian pedoclimatic and geographical localities and to determine the yield and composition of essential oil obtained from such samples.

MATERIALS AND METHODS

Leaves from wild plants were collected at three different localities during the summers of 1979-1982, and were dried. The essential oil was obtained by steam distillation for 3 h in an apparatus according to the Yugoslavian Pharmacopoeia (24). Based on the growth location, we divided our samples into two groups: those from the mainland and those from islands (coastal and inland regions). We have determined the yield in essential oil (Table 1) for each sample and the contents of particular oil components (Table 2) using gc/ms, as well as the refractive index. Gc analyses were performed using a gas chromatograph Pye Unicam 104 (Cambridge, England) and two columns: one 2 m long, 3 mm internal diameter glass column packed with 10% Reoplex 400 on Chromosorb W 80/100 mesh, and another one of the same dimensions packed with 15% SP 1000 on Chromosorb PHP 80/100 mesh. The temperatures were: injection port, 240°; detector, 260°; column, 140°, and 160°. The quantification of chromatograms was done by Spectra Physics SP 4000 computor (Darmstadt, W. Germany). Mass spectra of individual components were recorded by a gc/ms apparatus Kratos MS 25 coupled with a Data System DS 50S (Manchester, England). Glass column 2 m long, 3 mm internal diameter packed with 15% SP 1000 on Chromosorb PHP 80/100 mesh was used. The operating temperatures were: injection port, 220°; column, 160°; separator, 230°; and ion source, 200°. The spectra were recorded in the EI mode, at 70 eV, scanning 1 sec/decade, and compared with those of standard samples and those in the Data System library.

RESULTS AND DISCUSSION

The harvesting of sage leaves for commercial purposes begins in mid-July and extends until December, weather conditions permitting. Leaves might be harvested even earlier, but in the experience of pluckers, the harvesting should take place after blossoming to allow complete maturity. Devetak (25) reported that sage leaves should be collected from July through September because during this interval the sage possesses the highest

TABLE 1. Comparative Analysis of the Essential Oil and Thujone from Leaves of *Salvia officinalis* Collected from Different Areas of Yugoslavia

Sample No.	Locality	Date of harvest	Esential oil % (v/w)	Refractive index at 20°	Total thujone (%)
1	Velebit[a]	15 August 1980	2.6	1.4688	30.08
2	Velebit	15 August 1981	2.6	1.4696	29.01
3	Velebit	15 August 1982	2.7	1.4689	29.97
4	Povljana	27 August 1980	1.4	1.4682	34.29
5	Pag	15 August 1981	1.9	1.4695	37.00
6	Pag	15 August 1982	1.8	1.4652	42.25
7	Hvar	26 July 1979	2.0	1.4663	41.36
8	Hvar	27 July 1980	3.2	1.4670	39.06
9	Hvar	15 August 1981	3.1	1.4674	37.39
10	Hvar	15 August 1982	3.5	1.4692	33.54
11	Velo Grablje	26 July 1979	2.6	1.4658	39.44
12	Velo Grablje	27 July 1980	3.4	1.4638	51.62
13	Velo Grablje	15 August 1982	2.9	1.4631	55.13

[a]Harvested at 1000 m above sea level (near Starigrad-Paklenica).

quality as a raw material. Nine of our samples were collected during August and four at the end of July, i.e., after blossoming.

Vernazza and Nadali (13) report that the highest percentage of oil can be obtained in July; the yield in oil decreases afterwards, up to about 25% in October. Our samples from Mt. Velebit Nos. 1-3, were collected at the same time (15 August) and at the same place. The samples from the island Pag, Nos. 4-6, were collected on 15 August and 27 August. The samples from the island Hvar—four from the coastal region near Hvar city, Nos. 7-10, and three more from the inland at Velo Grablje, Nos. 11-13—were collected four years in succession in the same period, even from the same plants.

The biosynthesis of oil depends on the length of the daylight period and temperature (26). It is well known that the average temperature away from the islands' coasts is a few degrees lower than that in the coastal region (the magnitude of the difference depending on the altitude of the island's terrain), and this may explain the differences in essential oil composition. The influence of elevation above sea level is obvious in samples 1-3 from Mt. Velebit collected at 1000 m. A comparatively high oil yield was obtained from these samples: 2.6-2.7%. The largest difference in oil yields was found between samples from the islands Pag [sample 4 (1.4%)] and Hvar [sample 10 (3.5%)]. The low percentage of oil in the former sample might be explained by geographic factors. Pag belongs to the Mediterranean region of the submediterranean climatic zone where dry and hot weather predominates during the summer, while Hvar belongs to the Mediterranean region of eumediterranean zone where the average temperature is lower and the average amount of rain is higher (27).

In the early stage of ripening, the essential oil contains mostly monoterpene hydrocarbon, including 1,8-cineole (6). The analysis of oils showed a low content of 1,8-cineole in samples 3, 9, and 13. The content of 1,8-cineole was high—22% in sample 4 from the island Pag (Table 2).

As far as α- and β-thujone content is concerned, it is interesting that some of the oil samples had a high percentage, whereas some others had an unusually low percentage. The samples from island Hvar (7-10, 12) contained more β-thujone than α-thujone, which influences the optical rotation of these oils.

High contents of camphor were found in samples 1-3 having a low content of α- and β-thujone (29.01-30.08%).

TABLE 2. Comparative Analyses of Essential Oils of *Salvia officinalis* from Yugoslavia by Gc-Ms

Compound	Mainland			Islands									
	Velebit[a]			Pag /Coastal region/			Hvar /Coastal region/				Hvar /Inland region/		
	1[b]	2	3	Povljana	Pag	Pag	Hvar	Hvar	Hvar	Hvar	Velo Grablje		
				4	5	6	7	8	9	10	11	12	13
α-thujene	0.87[c]	—	0.36	1.38	0.29	0.16	—	—	0.72	0.27	—	0.45	0.18
α-pinene	5.73	5.94	6.64	3.11	2.92	3.27	4.22	3.53	3.62	4.13	2.68	2.65	4.90
camphene	6.49	6.16	8.13	4.46	4.89	4.89	6.55	6.90	7.16	8.48	5.48	3.54	2.40
β-pinene	2.68	1.42	2.07	3.38	1.31	1.23	2.01	2.59	1.07	1.60	2.92	2.96	1.08
myrcene	—	1.01	1.27	—	1.24	0.27	—	—	1.21	1.19	—	0.46	1.57
limonene	2.63	1.82	2.42	3.61	2.09	2.34	1.03	1.44	2.43	2.39	3.64	2.16	2.56
1,8-cineole	10.43	10.33	8.95	22.46	16.63	13.28	12.27	9.98	7.84	12.08	11.27	13.11	9.19
p-cymene	0.80	0.93	0.73	0.77	0.87	1.12	1.77	1.39	1.05	1.20	1.74	1.66	1.42
unidentified	0.09	—	0.29	0.16	0.31	0.42	0.10	0.12	0.32	0.25	0.15	—	0.25
α-thujone	26.17	23.61	25.59	30.35	28.91	36.33	14.80	7.17	12.29	10.42	24.25	22.29	29.38
β-thujone	3.91	5.40	4.38	3.94	8.09	5.92	26.56	31.89	25.10	23.12	15.19	29.33	25.75
linalyl acet.	0.78	—	—	—	—	—	—	—	—	—	3.49	0.51	—
camphor	23.14	20.50	21.64	10.71	19.61	16.64	12.81	12.89	14.86	14.43	15.18	8.49	6.99
linalool	2.40	0.41	0.37	1.64	0.62	0.72	4.66	—	0.53	0.92	2.65	2.03	0.32
bornyl acet.	2.77	2.12	3.52	2.12	4.16	3.42	0.59	6.35	4.32	5.32	1.55	1.49	2.51
isoborneol	0.04	2.80	2.42	0.44	0.74	0.93	—	—	2.12	1.79	0.27	0.14	1.45
borneol	10.27	9.99	11.03	7.55	8.43	12.42	15.54	12.43	11.42	9.01		7.94	6.45
unidentified	0.60	—	—	0.21	—	—	—	—	0.121	—	0.15	—	0.14
unidentified	—	0.22	—	—	0.16	0.17	—	—	0.31	—	—	0.18	0.22

[a]Harvested at 1000 m above sea level (near Starigrad-Paklenica).
[b]Sample no.
[c]Percent of total oil.

The samples from the coastal region of the island Hvar (7-10) have a high percentage of borneol.

Vernazza (14) emphasized that the refractive index of the oil is a parameter which, despite some exceptions, highly depends on its thujone content: the higher the percentage of thujone, the lower the refractive index. We found this to be correct (Table 1).

Before the appearance of gc, the quality of Dalmatian sage oil was evaluated on the content of ketones calculated as thujone. According to the Pharmacopoea Jugoslavica (24), Dalmation sage oil must contain a minimum of 40% of ketone calculated as thujone (hydroxylamine method). According to specifications and standards of the Essential Oil Association (USA) Dalmatian sage oil should contain a minimum of 50% of ketones calculated as thujone (28).

In analyzing the results obtained for our samples (Table 2), one can see that all samples meet the standards of the Pharmacopoea Jugoslavica and the Essential Oil Association except samples 2 (49.51%), 4 (45.00%), and 10 (47.97%). The general characteristic of our samples of sage leaves is that all samples contain oil with a high percentage of ketones.

If the quality of leaves and oil of sage are estimated in relation to the geographic region considered, we could conclude the following.

In the samples from the mainland area, Nos. 1-3, the percentages of oil and camphor are relatively high, while the percentage of thujone is low. However, these three values do not differ considerably in the samples mentioned, and we have the impression of one homogenous sample.

Comparing the percentage of oil and thujone in the samples from the island of Pag, Nos. 4-6, it is remarkable that the percentage of oil is very low and the average content of thujone is under 40%.

The samples originating from the island of Hvar have the highest differences in content of oil and thujone; therefore, it is very difficult to obtain a homogenous sample. The content of oil is high on the average, and the content of thujone in samples from the inland region, Nos. 11-13, is also high.

In our future work, we plan to study the amount and composition of essential oil of Dalmatian sage with special attention given to the stage of vegetation of the plant.

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