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Changes in the essential oil of Laurel (*Laurus nobilis* L.) during its vegetation

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Abstract:

The oil of laurel (*Laurus nobilis* L.) harvested during various growth stages of the plant (winter, dormancy; spring, flowering; summer, vegetative activity and autumn, fruit production) was investigated by GC and GC/MS. In all samples, 1,8-cineole was, the main compound. Oxygenated monoterpenes (61.46-77.59) and monoterpene hydrocarbons (7.88-22.08%) were the dominant groups in the oil from the leaves; oxygenated monoterpenes (46.68%), monoterpene hydrocarbons (13.79%), hydrocarbons (14.43%) and sesquiterpene hydrocarbons (15.69%) were the dominant groups in the oil from flowers; monoterpene hydrocarbons (23.93-32.05%), oxygenated monoterpenes (24.91-34.03%), sesquiterpene hydrocarbons (15.85%) and oxygenated sesquiterpenes (21.77-22.77%) were the dominant groups in the oil from the fruits.

Key words: *Laurus nobilis*, essential oil composition, vegetation.

INTRODUCTION

Laurel essential oil is obtained from leaves of *Laurus nobilis* L. (Lauraceae), an evergreen tree cultivated primary in the Mediterranean countries. The oil from laurel leaves is a light yellow to yellow liquid with an aromatic, spicy odor [1, 2].

The investigations on the oil composition from laurel leaves show that the main components vary depending on the origin, the collection period, the grown stage of the plant, and the method of obtaining the oil [3-8]. The leaf oil was found to be rich in 1,8-cineole (30-70%), α-terpinyl acetate (5.85-25.70%), linalool (3.66-16.4%), sabinene (2.30-14.05%), methyl eugenol (3.08-12.54%), β-ocimene, linalool, etc. [1-11]. Leaf oil is produced commercially and used extensively in the food industry [1, 2], perfumery and cosmetics [2] and pharmaceutical industries [12].

The most important medicinal effects of laurel leaf oil are due to its antioxidant [8, 13] and pharmacological [14] properties. Some of these activities are related to the major compound of the oil, 1,8-cineole.

The essential oils obtained from flowers [4, 9, 10, 11] and from fruits [4, 6, 11, 15, 16] contain volatile compounds such as 1,8-cineole, α-terpinyl acetate, linalool, sabinene, α-pinene, β-pinene, etc., in different quantities. The laurel fruits are used in traditional medicine for the treatment of different problems [11], and the essential oil is used in the food industry and perfumery [2].

The aim of this study was to investigate the seasonal changes in the yield and chemical composition of laurel essential oils.

MATERIALS AND METHODS

Plant material

The leaves, flowers and fruits of laurel (*Laurus nobilis* L.) were harvested from the Athos peninsula (North Greece) at four different phases of vegetation, respectively, in the middle of winter (dormancy), spring (flowering), summer (vegetative activity) and autumn (fruit production) of 2017:

The plant species was identified as *Laurus nobilis* L. by the Department of Botany, Paisii Hilendarski University of Plovdiv, Bulgaria.

The moisture content of the fresh raw materials was determined by drying to constant weight at 105 ^{o}C [17].

Essential oil isolation.

The fresh leaves were cut in pieces (1 cm long) and the fruits were cut in half. The oil content in the plant parts (75 g) was determined for 3 h in laboratory glass apparatus according to the British Pharmacopoeia, modified by Balinova and Diakov [18]. The oil obtained was dried over anhydrous sodium sulfate and stored in tightly closed dark vials at 4^{o}C until analysis.

Chemical composition of the essential oil.

Gas chromatography (GC) analysis was performed using an Agilent 7890A gas chromatograph, an HP-5 ms (30 m x 250 μm x 0.25 μm) column, a temperature of 35 ^{o}C/3 min, 5 ^{o}C/min to 250 ^{o}C for 3 min, 49 min in total; helium gas as carrier at 1mL/min constant speed; split ratio 30:1.

GC/MS analysis was carried out on an Agilent 5975C mass spectrometer, with helium gas as a carrier, the column and temperature being the same as the GC analysis.

The identification of chemical compounds was made by comparison to their relative retention time and library data. The components identified were arranged according to the retention time and their quantity was given in percentage.

All experiments were carried out in threefold repetition and the mean values with the respective error have been presented in the tables and figures below.

RESULTS AND DISCUSSIONS

The moisture of the plant materials and the essential oil yield has been shown in Table 1. All essential oils were light yellow liquids and had a specific odor.

The oil content in the leaves increased until the vegetative activity phase (3.3%), then, in the fruit production phase, it decreased. The differences in the oil quantities compared with those reported in literature, i.e. for leaves: 0.90% [12], 0.7-0.8% [4], 1.2% [9] and 0.77-1.43% [6]; for flowers: 1.1% [4] and 1.8% [9]; for fruits: 0.6% [4] and 0.63-0.95 % [6], were probably due to the climatic conditions in the respective place where the plant grows and the part of the plant processed.

The changes in the oil quality have been shown in Table 2. The essential oil from the different plant organs contained the same compounds, but the quantitative differences between all main compounds were quite large. The main component of all oils, regardless of the growth phase and the plant parts, was 1,8-cineole.

The data showed that the leaf oils obtained from the different phenological stages had nearly similar composition:

- In the dormancy phase (winter), 32 constituents accounting for 97.53% the total content were identified. As seen, the major constituents (over 3%) of the oil were as follows: 1,8-cineole (31.53%), α-terpinyl acetate (18.43%), sabinene (7.39%), α-pinene (4.00%), terpinene-4-ol (3.92%), β-pinene (3.68%), methyl eugenol (3.40%) and linalool (3.03%);

- In the flowering phase (spring), 44 components representing 98.13 % the total content were identified. As seen the major constituents (over 3%) of the oil were as follows: 1,8-cineole (27.69%), *α*-terpinyl acetate (21.98%), %), linalool (6.80%), methyl eugenol (6.41%). sabinene (5.39%), *α*-pinene (3.40 %), *β*-pinene (3.31%) and α-terpineol (3.12%).
- In the vegetative activity phase (summer), 47 components representing 97.80% the total content were identified. As seen, the major constituents (over 3%) of the oil were as follows: 1,8-cineole (39.32%), *α*-terpinyl acetate (18.82%), sabinene (9.85%), *α*-pinene (4.26%) and *α*-terpineol (3.89%).
- In the fruit production phase (autumn), 44 components representing 98.84% and of the total content were identified, respectively. As seen, the major constituents (over 3 %) of the oils were as follows: 1,8-cineole (35.02% and), *α*-terpinyl acetate (26.96%), *α*-terpineol (6.32%), methyl eugenol (4.71%), terpinene-4-ol (4.57%) and sabinene (3.69%).

The 1,8-cineole content (39.32 %) in the leaf oil composition from the summer sample was higher than the other leaf oils. The content of monoterpene hydrocarbons (*α*-pinene, *β*-pinene, *γ*-terpinene) and oxygenated monoterpene (*α*-terpineol) increased in the vegetation stages. The second compounds such as *δ*-2-carene, *δ*-3-carene, verbenol and trans-pinocarveol were found in the oils from the spring, summer and autumn samples, respectively. The difference in the chemical composition in our investigations and the reported data [3-8] may be due to the environmental conditions under which the plant had grown as well as the variation in the conditions of analysis.

The distribution of the major groups of aroma substances in the leaf oils has been shown in Figure 1. Oxygenated monoterpenes (61.46-77.59%) and monoterpene hydrocarbons (7.88-22.08%) were the dominant groups in the oils, followed by hydrocarbons (3.26-8.32%) and phenyl propanoids (5.88-7.90%).

A total of 45 components representing 97.68% of the total content were identified in the flower oil. The major constituents (up 3%) of the oil were as follows: 1,8-cineole (20.67%), bornyl acetate (9.91%), *α*-terpinyl acetate (7.84%), *β*-elemene (5.43%), *α*-humulene (4.15%), *β*-pinene (4.10%) and *α*-pinene (4.07%). As can be seen, some of this oil was rather atypical for leaf oils because of their low 1,8-cineole and high bornyl acetate content. The difference in the chemical composition in our investigations (higher bornyl acetate content and lower content of eugenol and methyl eugenol) and the reported data [4, 9, 10, 11] may be due to the environmental conditions under which the plant had grown. The distribution of the major groups of aroma substances in the flower oil has been shown in Figure 2. Oxygenated monoterpenes (46.6 %), monoterpene hydrocarbons (13.79%), hydrocarbons (14.43%), and sesquiterpene hydrocarbons (15.69%) were the dominant groups in the oil.

The results presented in Table 2 reveal a clear difference in the chemical composition of the fruit oils obtained during its vegetation:

- In the vegetative phase (summer), 44 components representing 96.26% of the total content were identified in the oil from unripe fruits. The major constituents (over 3%) of the oil were as follows: *β*-ocimene (28.57%), 1,8-cineole (20.23%), *β*-elemene (10.04%), *α*-cadinol (4.95%/nd), germacrene D-4-ol (4.02%), dehydrosaussurea lactone (3.83%), aromadendrene oxide-(2) (3.48%), *τ*-muurolol (3.20%) and *α*-farnesene (3.01%).
- In the fruit production phase (autumn), 44 and components representing 98.61% of the total content were identified in the oil from ripe fruits. As seen, the major constituents (over 3 %) of the oil were as follows: 1,8-cineole (29.73% and), *β*-elemene (10.29%), germacrene D-4-ol (4.12%), *α*-cadinol (4.08%), dehydrosaussurea lactone (3.93%), aromadendrene oxide-(2) (3.56%), *τ*-muurolol (3.28%) and *α*-farnesene (3.08%).

These data are nearly similar to the qualitative results obtained in other investigations [3, 5, 10, 14, 15], which may be attributed to the different climatic conditions at the place of harvesting.

The distribution of the major groups of aroma substances in the oils from unripe and ripe fruits has been shown in Figure 3. Monoterpene hydrocarbons (23.93%), oxygenated monoterpenes (34.03%), oxygenated sesquiterpenes (21.77%), and sesquiterpene hydrocarbons (15.86%) were the dominant groups in the fruit oils. Monoterpene hydrocarbons (32.05%), oxygenated monoterpenes (24.91%), oxygenated sesquiterpenes (22.77%) and sesquiterpene hydrocarbons (15.85%) were the dominant groups in the oil from unripe fruits. The total sum of monoterpenes decreased and the oxygenated monoterpenes increased during the maturation of fruits.

The seasonal variations of the essential oils obtained from different plant parts of laurel revealed that the harvesting time and the region of plant cultivation were key factors in laurel characterizations in terms of yield and chemical composition.

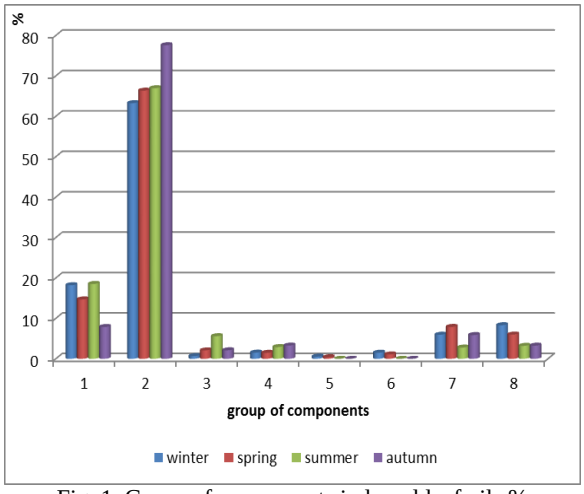

Fig. 1. Group of components in laurel leaf oils,%
1 – monoterpene hydrocarbons; 2 – oxygenated monoterpenes; 3 – sesquiterpene hydrocarbons; 4 – oxygenated sesquiterpenes; 5 – diterpenes;
6 – triterpenes; 7 – phenyl propanoids; 8 – hydrocarbons.

Table 1. Moisture of plant parts and essential oil yield.

Properties	Winter	Spring		Summer		Autumn	
	leaves	leaves	flowers	leaves	unripe fruits	leaves	ripe fruits
Moisture, %	66.3±0.55	20.2±0.20	10.9±0.10	42.8±0.40	72.8±0.69	44.7±0.41	50.9±0.48
Yield, % (v/w), in abs. dry mass	0.2±0.01	0.6±0.01	0.5±0.01	3.3±0.07	1.7±0.03	2.2±0.04	1.8±0.03

Table 2. Percent composition of laurel essential oils.

	Compounds	RI	Winter	Spring		Summer		Autumn	
			leaves	leaves	flowers	leaves	unripe fruits	leaves	ripe fruits
1.	*α*-Thujene	931	0.39	0.27	0.12	0.13	0.09	0.47	0.09
2.	*α*-Pinene	939	4.00	3.40	4.07	4.26	0.57	0.79	0.58
3.	Camphene	954	0.83	0.93	2.08	0.61	0.10	0.34	0.10
4.	Sabinene	971	7.39	5.39	1.25	9.85	0.73	3.69	0.74
5.	*β*-Pinene	979	3.68	3.31	4.10	1.79	0.38	0.93	0.39
6.	*β*-Myrcene	991	0.48	0.16	0.20	0.23	0.16	0.22	0.17
7.	*δ*-2-Carene	1001	-	0.29	0.34	0.11	-	0.16	-
8.	*α*-Phellandrene	1003	0.20	-	-	-	-	-	-
9.	*δ*-3-Carene	1007	-	0.18	0.20	0.17	-	0.27	-
10.	*p*-Cymene	1025	-	0.22	0.43	0.12	0.15	0.21	0.15
11.	1,8-Cineole	1032	31.53	27.69	20.67	39.32	20.23	35.02	29.73
12.	*β*-Ocimene	1040	-	0.55	2.35	0.29	28.57	0.13	21.28
13.	*γ*-Terpinene	1055	0.79	0.22	0.36	0.42	0.12	0.60	0.12
14.	Terpinolene	1087	-	0.28	0.41	0.26	0.13	0.19	0.13
15.	*β*-Linalool	1096	3.03	6.80	0.84	0.16	0.15	2.74	0.15
16.	n-Nonanal	1128	-	-	0.37	-	-	-	-
17.	Verbenol	1131	-	0.09	0.39	0.22	0.16	0.12	0.16
18.	L-*trans*-Pinocarveol	1137	-	0.20	0.32	0.34	0.11	0.23	0.11
19.	*cis*-*β*-Terpineol	1143	-	0.20	0.44	0.44	0.71	0.44	0.73
20.	Pinocarvone	1152	-	0.27	0.37	0.26	0.22	0.10	0.22
21.	Terpinen-4-ol	1179	3.92	2.32	1.09	1.81	0.43	4.57	0.44
22.	*α*-Terpineol	1189	2.89	3.12	1.38	3.89	0.58	6.32	0.59
23.	Nerol	1229	-	0.29	-	-	-	-	-
24.	Bornyl acetate	1269	1.90	1.60	9.91	0.22	0.20	0.19	0.20
25.	*α*-Terpinyl acetate	1333	18.43	21.98	7.84	18.82	1.19	26.96	1.22
26.	Eugenol	1363	2.21	1.12	0.51	0.56	0.23	0.71	0.24
27.	*β*-Elemene	1368	-	1.07	5.43	2.58	10.04	0.90	10.29
28.	Methyleugenol	1371	3.40	6.41	1.77	1.50	0.11	4.71	0.11
29.	Ilangene	1387	-	-	0.68	0.18	-	0.16	-
30.	Cinnamyl acetate	1407	0.20	-	-	-	-	-	-
31.	*β*-Caryophyllene	1429	0.38	0.70	1.68	0.48	0.88	0.35	0.90
32.	Isoeugenol	1438	-	-	-	0.55	-	0.18	-
33.	*α*-Humulene	1453	-	0.31	4.15	0.12	0.09	0.09	0.10
34.	*γ*-Elemene	1467	-	-	-	0.92	0.22	0.42	0.22
35.	Germacrene D	1484	0.27	0.26	1.73	0.33	1.02	0.17	1.05
36.	*α*-Farnesene	1488	-	-	-	-	3.01	-	3.08
37.	*γ*-Cadinene	1508	-	0.18	1.33	0.26	-	-	-
38.	*δ*-Cadinene	1514	-	0.22	2.01	0.61	-	-	-
39.	Germacrene D-4-ol	1568	-	-	-	0.19	4.02	0.11	4.12
40.	Caryophyllene oxide	1574	0.91	0.30	0.65	0.53	2.02	1.91	2.07
41.	(-)-Spathulenol	1619	0.58	0.45	0.75	0.27	0.42	1.01	0.43
42.	*τ*-Muurolol	1628	-	-	-	-	3.20	-	3.28
43.	*α*-Cadinol	1643	-	-	-	1.68	4.95	-	4.08
44.	Aromadendrene oxide-(2)	1678	-	-	-	-	3.48	-	3.56
45.	n-Heptadecane	1700	0.35	0.25	0.59	0.42	0.51	0.16	0.52
46.	Dehydrosaussurea lactone	1726	-	-	-	0.18	3.83	0.21	3.93
47.	n-Heneicosane	2100	1.11	0.81	1.89	0.35	0.42	0.39	0.43
48.	Phytol	2105	0.53	0.39	0.90	-	-	-	-
49.	n-Docosane	2200	1.06	0.77	1.81	0.20	0.23	0.22	0.24
50.	n-Tricosane	2300	0.70	0.51	1.19	0.39	0.47	0.44	0.48
51.	n-Tetracosane	2400	0.67	0.49	1.15	0.18	0.21	0.20	0.22
52.	n-Pentacosane	2500	0.99	0.72	1.68	0.16	0.19	0.18	0.19
53.	n-Hexacosane	2600	1.27	0.92	2.15	0.31	0.38	0.35	0.38
54.	n-Heptacosane	2700	1.45	1.05	2.46	0.63	0.75	0.71	0.77
55.	Octacosane	2800	0.51	0.37	0.80	0.50	0.60	0.57	0.62
56.	Squalene	2817	1.47	1.07	2.50	-	-	-	-
	Total,%		**97.53**	**98.13**	**97.68**	**97.80**	**96.26**	**98.84**	**98.61**

* nd - not determined

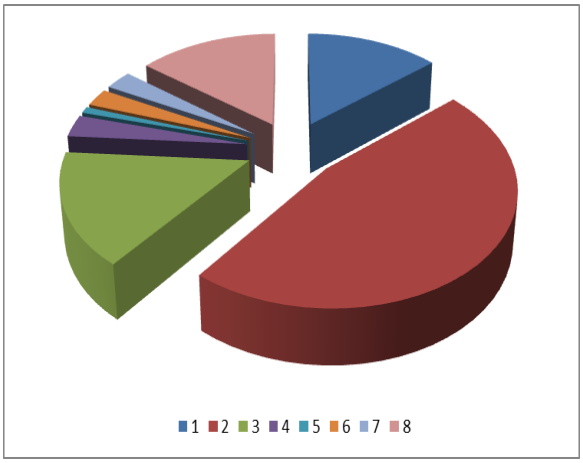

Fig. 2. Group of components in laurel flower oil,%
1 – monoterpene hydrocarbons; 2 – oxygenated monoterpenes; 3 – sesquiterpene hydrocarbons; 4 – oxygenated sesquiterpenes; 5 – diterpenes;
6 – triterpenes; 7 – phenyl propanoids; 8 – hydrocarbons.

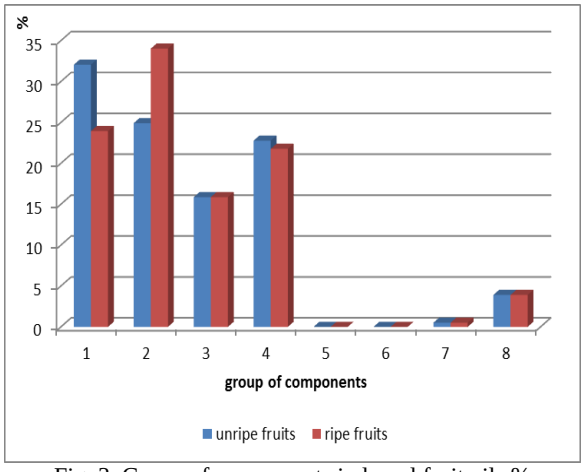

Fig. 3. Group of components in laurel fruit oils,%
1 – monoterpene hydrocarbons; 2 – oxygenated monoterpenes; 3 – sesquiterpene hydrocarbons; 4 – oxygenated sesquiterpenes; 5 – diterpenes;
6 – triterpenes; 7 – phenyl propanoids; 8 – hydrocarbons.

CONCLUSION

In conclusion, the most suitable time for the harvesting of leaves and fruits of *Laurus nobilis* L. grown on the Athos peninsula (North Greece) is the vegetative activity phase (summer) and the fruit production phase (autumn), respectively, when the 1,8-cineole and oil content are high.

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