

The Effects of Cultivation Area and Altitude Variation on the Composition of Essential Oil of *Laurus nobilis* l. Grown in Eastern, Western and Central Karadeniz Region

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Abstract: *Laurus nobilis* L. is one of the most valuable non-wood forest products on world export market and Turkey. Turkey is the biggest provider country for *Laurus nobilis* in the world. Therefore, laurel is an important commercial product for our country. In this study, the effects of cultivation area and altitude variation on essential oil content and quantity of laurel leaves were examined which grown in Trabzon, Bartın and Samsun. It was aimed to determine chemical composition of laurel's leaves grown in Karadeniz region. *Laurus nobilis* L. leaves were collected in three different height ranges. These were 0-100 m, 100-300 m, 300-600 m. Leaves were shade-dried and crushed. A device called "Clevenger" was used for getting volatile oil and their yields were calculated according to dry weight. The yields of essential oils ranged between 0.91% to 1.66 %. These essential oils were obtained from Bartın (B₂) (100-300 m) and Artvin (A₁) (0-100 m) respectively. The major components of these essential oils were 1,8- cineole (19.71%-35.63%), α -terpinyl acetate (12.86%-21.24%), sabinene (5.98%-9.40%), α - pinene (3.67%-8.45%) and β - pinene (2.91%-5.87%) were the most abundant volatile compounds in the leaves of bay.

Keywords: Laurel, essential oils, GC-MS

1. INTRODUCTION

Laurel has an important product in the trade of non-wood forest products in terms of Turkey and this respect laurel plant is one of the high value-added products for our country. According to foreign trade statistics of Turkish Statistical Institute (TSI) the year of 2015, 2,207,550 kg of bay leaf were exported from Turkey and the financial provision corresponds to 6,365,257 \$.

The bay, known as *Laurus nobilis* L., is a peculiar plant of Mediterranean Region. It belongs to Magnoliophyta (closed seeds) branch and it is in the Magnoliopsida class. *Laurus nobilis* which is a member of the Lauraceae family of Laurales, is a genus of *Laurus* [1]. The leaves are short and thick. The fresh leaves are thin and they have light green vein and they have red tinged yellow color, then their color turns into light green, with little aromatic odor. The fresh shoots are green, the next is red, black and hairless. Their maximum length is 2 cm [2]. The place of use of *Laurus nobilis* L. is, thanks to its phenolic compounds which are taking

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place in substances of laurel leaves, in food industry as natural antioxidants with antioxidant properties [3]. In particular, thanks to its volatile compounds in dried leaves, they are used in meat, soup, candy and sauce making as a flavoring [4-7]. Bay leaves and volatile oils of leaves have an effects of antiepileptic, anticonvulsive [8-10], antimicrobial [10], antibacterial [11,12].

The antibacterial effect which are found in bay leaf essential oil, thanks to the high ratio of methyl eugenol and 1,8-cineole in the bay volatile oil. Thus, they have antibacterial properties against bacteria that cause foodborne diseases such as, *Staphylococcus aureus*, *Staphylococcus intermedius*, *Klebsiella pneumoniae*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium* and *Staphylococcus aureus* [12, 13].

The antimicrobial effect is due to the presence of compounds such as 1,8-cineole, camphor, myrcene, β -caryophyllene, eugenol, α -pinene, β -pinene and p-cymene in the content of volatile oil in bay leaf [2, 14]. Apart from beneficial properties, there have lots of unique properties of bay leaf volatile oil, such as having thermal stability, does not showing phototoxic effect, positive effects against migraine, headache, high blood sugar, bacterial and fungal infections [9, 15]. Leaf volatile oil is advised to use as a mucolytic agent (antipyretic) in advanced asthmatic disorders, upper and lower respiratory tract disorders since it has a 1,8-cineole (volatile compound) in it. So it has an anti-inflammatory effect (pain, fever cutter) which is from oxygenated monoterpenes and also it is a high concentration in bay volatile oil [16]. Recent studies have shown that bay leaf and its' essential oil have a positive effect against colitis which causes flatulence in stomach [17].

In recent years, it is indicated that the high rate of volatile oil of the leaves in the southern regions of our country makes bitter taste in the bay leaves. Thus, tendency towards the bay leaves in the EU countries is shifted progress to the laurel leaves grown in Russia. These leaves have less essential oils than Mediterranean Regions. Russia is on the shore to the black sea. Therefore, the Karadeniz Region may also be a suitable region for bay exports. Based on this, Laurel leaves were collected from Artvin, Trabzon, Samsun and Bartın from Karadeniz Region and then essential oils were obtained and they were compared in oil yields and essential oil compositions.

2. MATERIAL and METHODS

2.1. Plant Material

The areas where the bay leaves were collected are given in Table 1. Laurel leaves were collected at three altitudes. These were 0-100 m, 100-300 m, 300-600 m but in Artvin (A₃) (300-600m) plant material were not collected because this plant were not encountered.

Dried bay leaves (11 samples of 75 gr each) were subjected to the hydro distillation for 3 hours on a Clevenger-type apparatus. The essential oils were removed from the water and stored at 4°C until gas chromatography-mass spectrometry (GC-MS) analysis.

Table 1. Locations and altitudes of collected laurel leaves.

Location	Altitude (m)		
	0-100	100-300	300-600
Artvin	90	230	-
Trabzon	20	200	600
Samsun	77	186	380
Bartın	10	200	400

2.2. GC-MS Analysis

GC-MS analysis was carried out with a 5975 Agilent apparatus equipped with Inowax FSC column (60 m long x 0.25 mm i.d. x 0.25 µm film thickness). The column temperature program was 60 °C during 10 min, with 4 °C/min increases to 220 °C, then wait 10 min in 220 °C and 1 °C/min increases to 240 °C. The carrier gas was helium at a flow-rate of 0.8 ml/min. Split mode injection (ratio 1:50) was employed. Injector temperature was 250 °C. Mass spectra were taken over the m/z 35–450 range with an ionizing voltage of 70 eV. The relative delay times for defining the essential oil components were determined by comparing the mass spectrum profiles of the materials using Wiley GC/MS Library, Adams Library, and Mass Finder 2.1 Library mass spectrum libraries on the computer to which the device was connected [18].

3. RESULTS and DISCUSSIONS

3.1. Extraction Yield

In our study, volatile oil yield which was obtained by Hydro distillation method are shown in Table 2. The highest yield of volatile oil in our work was 1.66 % Artvin (0-100 m) and the lowest amount was 0,91% Bartın (100-300 m) was obtained. Our volatile oil yield results vary between 0.91% and 1.66%.

Fiorini et al., investigated laurel leaf which were collected from France. They reported that the yield of laurel leaf essential was 0.57% [19]. Our results are much higher than this research.

Table 2. Avarage yield of *Laurus nobilis* L. essential oil.

<i>Location</i>	<i>Avarage Yield of Essential Oil (ml/100gr)</i>		
	<i>0-100 m</i>	<i>100-300 m</i>	<i>300-600 m</i>
Trabzon, (T₁,T₂,T₃)	1,33±0,0070	1,58±0,0141	0,99±0,1272
Bartın, (B₁,B₂,B₃)	1,47±0,0170	0,91±0,1484	1,44±0,1767
Samsun, (S₁,S₂,S₃)	0,92±0,0212	1,17±0,0848	1,33±0,6997
Artvin, (A₁,A₂)	1,66±0,0212	1,60±0,0001	-

3.2. GC-MS Analysis

Fifty-six compounds accounting for 78.07%–98.78% of the essential oils were identified by capillary GC-MS. Components are listed in Table 3 according to their retention times. There are lots of factors affecting the production of secondary metabolites in plants. It can be explained as an environmental, geographical, physiological, genetic, political and social factors [20]. The percentage of volatile compounds also depends on climate conditions and edaphic factors.

Table 3. The chemical composition of essential oils of leaves from laurel (*Laurus nobilis* L.).

C. N.*	Compound	R.T.**	Peak Area (%)										
			Ar ₁	Ar ₂	T ₁	T ₂	T ₃	S ₁	S ₂	S ₃	B ₁	B ₂	B ₃
1	α - Pinene	8.861	3.68	4.05	5.96	5.75	3.67	8.02	5.98	8.45	6.0	7.33	6.15
2	α -Thujene	8.950	-	-	-	-	0.37	-	0.76	-	-	-	-
3	Camphene	10.568	-	-	-	-	-	1.31	1.97	1.17	0.98	1.72	0.97
4	β - Pinene	12.463	3.18	3.65	4.28	4.01	2.91	5.87	5.60	4.98	4.52	5.27	4.68
5	Sabinene	13.138	8.0	9.40	8.53	7.52	7.44	6.10	5.98	6.40	7.31	7.67	8.56
6	Myrcene	15.109	-	-	0.96	1.11	0.72	-	-	0.59	0.97	1.04	1.21
7	Phellandrene	15.156	2.8	2.73	-	-	-	-	-	-	-	-	-
8	α -Terpinene	15.867	-	-	-	0.82	0.61	1.05	0.97	1.14	1.02	0.78	1.03
9	Cineol (dehydro-1,8)	16.288	-	-	-	-	-	-	0.54	0.75	-	-	-
10	Limonene	16.873	-	-	2.33	2.37	2.03	2.44	-	-	2.25	2.62	-
11	1,8- Cineole	17.387	26.54	34.78	26.42	19.71	25.92	35.63	30.90	28.53	28.67	24.62	28.71
12	γ -Terpinene	18.968	0.99	1.32	1.44	1.45	1.09	1.93	1.77	1.79	1.64	1.37	1.69
13	O-Cymene	20.100	-	-	0.83	0.98	1.62	2.55	3.11	1.50	0.84	0.85	0.59
14	P-Cymene	20.109	1.19	1.06	-	-	-	-	-	-	-	-	-
15	Cis-Sabinene Hydrate	27.602	0.32	0.45	-	-	-	-	-	0.48	-	-	-
16	α - Terpinolene	20.608	0.40	0.50	-	0.53	-	-	0.54	0.57	0.58	0.51	0.66
17	Linalool	30.430	0.99	2.66	1.45	2.38	2.98	-	1.35	1.38	3.06	3.83	3.38
18	Pinocarvone	31.439	-	-	-	-	-	-	0.84	-	-	-	-
19	Bornyl Acetate	31.744	0.50	0.47	-	0.52	0.44	2.38	3.44	2.07	1.24	1.97	1.77
20	β -Elemene	32.106	1.89	0.45	1.56	1.11	0.73	-	-	0.36	0.47	0.73	0.58
21	2-Undecacone	32.220	-	-	-	-	-	-	-	0.43	-	-	-
22	Terpinene-4-ol	32.389	4.83	5.27	5.33	5.84	4.54	5.03	3.74	3.46	4.18	3.29	3.65
23	Myrtenal	33.374	-	-	-	-	-	-	0.92	0.63	-	-	-
24	δ -Patchhoulene	33.754	-	-	0.76	-	-	-	-	-	-	-	-
25	α -Gurjunene	33.765	-	-	-	-	0.58	-	-	-	-	-	-
26	δ -Terpinyl Acetate	33.917	2.49	2.52	1.52	2.53	1.84	1.56	1.70	1.57	1.44	1.45	1.87
27	Trans-Pinocarveol	34.060	-	-	-	-	-	-	0.87	0.65	-	-	-
28	α - Humulene	34.728	0.52	-	-	0.58	-	-	-	-	-	-	-
29	α -Terpinyl Acetate	35.440	20.73	20.36	21.24	18.1	20.86	20.26	12.86	17.20	18.28	18.72	18.03
30	Germacrene-D	35.886	-	-	1.03	-	0.49	-	-	-	-	0.46	-
31	Neryl Acetate	36.043	-	1.06	-	-	-	-	-	-	-	-	-
32	Lavanduly 2 Methyl Butanoate	36.046	1.97	1.06	-	-	-	-	-	-	0.85	-	-
33	Neryl Propanote	36.050	-	-	0.9	1.51	0.84	-	-	-	-	-	1.14

C.N.*:Compound number; RT.**:Retention time.

Tablo 3. (Continued).

C. N.*	Compound	R.T.**	Peak Area (%)										
			Ar ₁	Ar ₂	T ₁	T ₂	T ₃	S ₁	S ₂	S ₃	B ₁	B ₂	B ₃
34	β- Selinene	36.210	1.12	-	1.34	0.79	0.80	-	1.04	-	-	-	0.93
35	Bicyclogermacrene	36.553	0.79	-	-	-	-	-	-	-	0.96	1.24	1.00
36	δ- Cadinene	37.159	0.33	-	-	-	-	-	-	-	0.40	0.72	0.63
37	Naphthalene	37.256	0.54	-	0.66	-	0.88	-	-	-	-	-	-
38	γ - Cadinene	37.299	0.54	-	-	-	-	-	-	-	-	-	-
39	Cis-α- Bisabolene	37.504	0.51	-	1.13	1.42	0.94	-	-	-	-	-	-
40	Myrtenol	38.007	-	-	-	-	-	-	0.68	0.69	-	-	-
41	Geraniol	38.073	0.54	0.39	-	-	-	-	-	-	-	-	-
41	Nerol	38.114	-	-	-	-	-	-	0.68	0.65	-	-	0.73
42	Muuroladien-8 Beta-ol		-	-	-	-	0.90	-	-	-	-	-	-
43	Caryophyllene Oxide	43.369	0.84	0.64	1.86	2.63	1.88	-	1.45	0.74	0.53	0.73	0.46
44	Methyl Eugenol	43.832	0.49	2.73	6.00	3.06	2.58	2.96	4.55	2.88	4.72	4.54	3.39
45	Juneol	45.238	-	-	-	0.69	-	-	-	-	-	-	-
46	Spathulenol	46.600	0.81	-	-	0.34	0.58	-	-	-	1.09	2.89	1.07
47	Trans-Cinnamyl Acetate	47.292	-	-	-	0.50	-	-	-	-	-	-	-
48	Cinnamyl Acetate	47.309	-	-	-	-	-	-	-	2,47	-	-	-
49	Eugenol	47.668	5.27	3.38	1.94	2.47	1.02	1.42	2.31	1.38	2.54	2.84	2.69
50	Methyl Isoeugenol	47.904	-	-	-	0.92	1.46	-	-	-	-	-	-
51	Elemicin	48.886	-	-	0.80	-	-	-	-	-	-	-	-
52	α- Eudesmol	48.941	0.47	-	-	0.73	0.62	-	-	0.32	-	-	0.37
53	β- Eudesmol	49.131	0.64	-	1.17	1.19	1.91	0.83	1.03	0.92	0.62	0.63	0.58
54	Intermedeol	49.255	-	-	-	-	0.50	-	-	-	-	-	-
55	Caryophylla- 4(12),8(13)-dien- 5.beta.-ol	50.635	-	-	-	1.16	0.91	-	0.75	0.50	-	-	-
56	Chavibetol Acetate	51.864	-	-	-	0.64	-	-	-	-	-	-	-
	Total		93.98	98.64	98.78	91.60	92.11	96.79	96.33	92.46	78.07	97.82	96.15

C.N.*: Compound number; RT.**: Retention time.

Our results show high similarity in Karadeniz region. The highest volatile compounds were 1,8-cineole (19.71%-35.63%), α -terpinyl acetate (12.86%-21.24%), sabinene (5.98%-9.40%). Yilmaz et al., reported that 1,8-cineole (51.8 %), α -terpinyl acetate (11.2 %) and sabinene (10.1 %) as the major compounds in their research which is similar with the results of our study but results is much higher than our results [21].

GC-MS analysis of volatile oils shows that the majority of volatile oil compounds come from monoterpenes, and the majority of these monoterpenes are oxygenated monoterpenes and also Peris and Blazquez reported that oxygenated monoterpenes represented quantitatively the highest concentration of bay leaf volatile oils [22]. 1,8 cineole (19.71% -35.63%), α -terpinyl acetate (12.86% -21.24%), terpinene-4-ol (3.29% -5.84%), δ -terpinyl acetate (1.44%-2.53%) and linalool (0.99%-3.83%) were the most abundant volatile compounds from oxygenated monoterpenes of our research. 1,8-cineole and α -terpinyl acetate were found to be the most abundant volatile compounds among the oxygenated monoterpenes. The results obtained in this respect are similar to the work done by Yalçın et al., but the amount of 1,8-cineol (58.59%) was found to be higher in the relevant study [23].

The other main compounds α -terpinyl acetate (12.86%-21.24%) is a monocyclic monoterpene. It has an ester in its structure [23]. It was reported by Chericoni et al., as a (6.0%), Bouzouita et al., as a (11.20%), Dadalioglu and Evrendilek, as a (16.87%) and Ozcan and Chalcat, as a (4.04-9.87%) [13, 24-26]. On the other hand, the second most common compound among bicyclic monoterpenes are sabinene (5.98%-9.40%), α - pinene (3.67%-8.45%) and β - pinene (2.91%-5.87%). These were the most abundant compound as well for our research. These compounds were reported in these articles as well: Derwich et al., sabinene (6.13%), α - pinene (3.72%) and β -pinene (3.14%) [12], Verdian-rizi, sabinene (5.8% -6.5%), α -pinene (2.6%-3.2%), β -pinene (2.4%-2.9%) [27], Chalchat et al., sabinene (7.07%), β -pinene (2.84%), α -pinene (3.17%) [28] and Moghtader and Salari, α -pinene (%5.25) and β -pinene (%3.99) [29] and also linalool is a bicyclic monoterpene, was found abundant in Bartın province (3.06%-3.83%). It has a significant effects against several symptoms especially in convulsions [30].

Therefore, we detected some sesquiterpenes in lower amounts of our research. These are β -elemene (0.36%-1.89%), caryophyllene oxide (%0.46-2.63%), β - eudesmol (0.58%-1.91%). These compounds have been detected in several essential oils and the other oils have not been contained this compounds. These compounds have inhibitory effect on ethanol absorption [31]. Methyl eugenol (0.49% -6.00%) from the phenylpropanoids was a compound that we have detected in our analysis as a high amounts. Methyl eugenol has an anesthetic and relaxing effect on muscles [23].

4. CONCLUSION

In this study, volatile compounds which were in the content of volatile oil in the leaves of the *Laurus nobilis* L. that grow in Karadeniz Region (Eastern, Western and Central), were revealed and we also aimed to determine if there is any similarity in the essential oils of laurel leaves which are grown in Karadeniz Region. We determined similarities in substances of essential oils. 1,8 cineole is the major compound but the amount of 1,8 cineole is considerably less than most of the previous studies. Since 1,8-cineole is a flavoring compound, if the compound is found in a small amount in volatile oil, it promotes that the taste of the oil can be much softer. The results of our research support, exporting laurel leaf to the EU countries from Karadeniz Region.

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Conflict of Interests

Authors declare that there is no conflict of interests.

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