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Original article

Seasonal variations of *Laurus nobilis* L. leaves volatile oil components in Isfahan, Iran

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Abstract

Laurus nobilis L. (sweet laurel) is one of the volatile oil bearing plants of Lauraceae family. It is cultivated in different parts of Iran and its leaves and fruits have been used in food, cosmetics and pharmaceutical industries. There are a few reports about the effects of some environmental conditions on the quality and quantity of laurel volatiles. The goal of our work was to search the seasonal variations on the *L. nobilis* leaves volatile composition. The volatiles of four samples of the dried leaves of *L. nobilis* collected in March, June, September and December 2009 in Isfahan, Iran were prepared by using a Clevenger type apparatus for 3 hours and were analyzed by gas chromatography-mass spectroscopy. Twenty-nine, thirty-one, thirty-three and thirty-four components consisting 96.91%, 97.66%, 97.46% and 95.44% of the total compounds were identified of the volatiles obtained with yields of 1.1%, 1.5%, 1.4% and 0.8% (w/w), subsequently. The main compound was found to be 1,8-cineole (30.80-40.25%). Although twenty-seven out of thirty-six volatile components were similar in different seasons, there were some differences between other compounds of our four samples. While the essential oil composition of the March and June plant samples were characterized by presence of 1,8-cineole, δ-3-carene and camphor, the volatiles of September and December plant samples contained 1,8-cineole, camphene and sabinene. Some compounds like eugenol, methyl eugenol and α-terpenyl acetate were not affected apparently by seasonal changes.

Keywords: 1,8-cineole, GC/MS, *Laurus nobilis*, seasonal variation, volatile oil

Introduction

Laurus nobilis L. is one of the evergreen trees of the world that belongs to the Laurel family, Lauraceae [1]. It is a primitive angiosperm family and consists of several medicinal and nutritive plants. *Cinnamomum*, *Persea*, *Laurus*, *Sassafras* and *Litsea* genera that are recognized by their aroma and volatiles in pharmaceutical, food and cosmeceutical industries belong to this economically important family [1-4]. *L. nobilis* was a recognized medicinal herb in Iranian Traditional Pharmacy and Medicine. Medicinal activities of its leaves and fruits were mentioned

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in different books of Persian outstanding scientists and hakims such as Rhazes and Avicenna. They had mentioned the name of this plant in their books as "Ghar". They considered it as a warm and dry remedy and introduced it for the treatment of several hepatic, neurological, dermatological and urological disorders [5,6]. The Persian people call this beautiful and aromatic tree as "Barg-e Boo" and "Rand" in Farsi [4,7,8]. There is a comprehensive monograph about this plant in the "Iranian Herbal Pharmacopoeia" [9]. Cultivation and propagation of this tree is popular in Iran. It was transported from Mediterranean region to Iran in Qajar dynasty and nowadays it is spread in different parts, especially Northern and central provinces [8]. The *L. nobilis* leaves have a strong, fragrant, balmy, redolent and sweet aromatic scent. Their essential oil is produced commercially and traditionally by several countries and used for different purposes from meat seasoning and soup flavoring to soap and toiletries production and also in pharmaceutical industries [4,7,9-12]. The leaves of *L. nobilis* have also several secondary metabolites such as flavonoids, tannins and alkaloids [9]. The most important medicinal effects of *L. nobilis* leaves volatile oil are antibacterial, antifungal, antioxidant, wound healing, food preservative, alpha glucosidase inhibitory and anti-inflammatory properties. Some parts of these pharmacological activities are related to the major compound of the oil, 1,8-cineole [4,9,11,13,14]. Therefore, investigating the variations of 1,8-cineole and some other volatile oil components in different seasons is important to check the oil quality and quantity.

There are a few reports about the effects of geographical, genetic and environmental variations on the quality of *L. nobilis* leaves volatile oil [7,15,16]. The goal of this study was to investigate the seasonal variations on *L. nobilis* leaves volatiles oil in Isfahan as one of the central cities of Iran.

Experimental

Plant material and volatile oil isolation

The leaves of *L. nobilis* var. *angustifolia* were collected from a same place in the campus of Isfahan University of Medical Sciences, Isfahan, Iran during the four periods of the year 2009 in March, June, September and December at an altitude of 1600 m. The place of plant gathering was located at the longitude of 51° 39' 35" and latitude 32° 36' 6". It was systematically authenticated by Department of Biology, Faculty of Sciences, Isfahan University, Isfahan, Iran. A voucher specimen (2659) was deposited at the Herbarium of Isfahan School of Pharmacy. The leaves were dried in the shade. The dried powders of plant material (100 g) were chopped in distilled water separately and their volatile fractions were isolated by hydro-distillation in a Clevenger type apparatus for 3 h according to the method recommended in British Pharmacopoeia [17,18]. Volatile oil extraction of each sample was carried out in triplicate. The oil samples were homogenized and dried over anhydrous sodium sulfate and stored in four dark glass bottles in a fridge.

Gas chromatography-mass spectroscopy

GC/MS analysis was carried out on a Hewlett Packard 5972A mass selective detector coupled with a Hewlett Packard 6890 gas chromatograph, equipped with a cross-linked 5% PH ME siloxane HP-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 μm).

The GC operating conditions were as follows: carrier gas, helium with a flow rate of 2 mL/min; column temperature, 60-275 ºC at 4 ºC/min; injector and detector temperatures, 275 ºC; volume injected, 0.1 μL of the oil; split ratio, 1:25.

The MS operating parameters were as follows: ionization potential, 70 ev; resolution, 1000; ion source temperature, 200 ºC.

Identification of compounds

Identification of components in our four volatile oil samples was based on GC retention indices

Table 1. Composition (%) and retention indices (RI) of the volatile oils of *Laurus nobilis* leaves in different seasons; I. March sample, II. June sample, III. September sample, IV. December sample

No.	Compound	RI[a]	percentage			
			I	II	III	IV
1	tricyclene	925	0.22	0.38	0.17	0.40
2	α-pinene	936	5.81	6.59	3.00	5.31
3	camphene	948	4.56	3.58	10.22	2.56
4	sabinene	971	5.60	2.33	5.87	9.16
5	1-octen-3-ol	974	-	0.21	0.19	-
6	ß-pinene	976	1.40	1.83	2.48	2.87
7	3-octanone	981	-	0.11	0.15	-
8	myrcene	988	0.17	1.28	0.95	0.10
9	δ-3-carene	1012	8.89	3.02	3.97	4.59
10	α-terpinene	1015	0.75	0.97	0.88	0.35
11	α-phellandrene	1020	0.14	1.51	0.87	0.57
12	ß-phellandrene	1030	-	-	0.54	0.27
13	1,8-cineole	1033	34.29	40.25	37.32	30.80
14	γ-terpinene	1057	4.80	1.02	0.96	6.13
15	cis-sabinene hydrate	1065	0.18	0.19	0.11	0.12
16	α-terpinolene	1087	0.27	0.12	0.24	0.15
17	linalool	1100	2.17	1.92	1.40	1.65
18	camphor	1145	2.16	7.80	1.18	5.69
19	borneol	1160	3.17	3.26	2.94	2.12
20	pinocarvone	1164	-	0.11	-	0.14
21	4-terpineol	1175	1.19	1.30	1.09	0.98
22	α-terpineol	1190	2.92	1.61	1.66	2.04
23	myrtenol	1192	0.14	0.26	2.24	1.04
24	ß-fenchyl alcohol	1245	1.09	0.22	1.25	1.02
25	trans-sabinene hydrate	1253	0.31	0.20	0.75	0.43
26	piperitone	1258	0.25	0.17	0.30	0.29
27	bornyl acetate	1284	0.10	1.09	0.26	0.54
28	eugenol	1354	2.88	2.25	2.74	2.03
29	α-terpenyl acetate	1356	6.14	7.08	6.93	5.85
30	neryl acetate	1368	0.18	-	0.30	0.32
31	ß-elemene	1389	-	-	0.79	0.49
32	methyl eugenol	1406	5.18	5.05	4.28	4.17
33	ß-caryophyllene	1415	1.17	1.46	0.92	1.25
34	α-humulene	1460	0.78	0.49	-	0.25
35	germacrene-D	1481	-	-	0.51	1.49
36	caryophyllene oxide	1580	-	-	-	0.47

[a] Retention indices on HP-5MS capillary column

relative to *n*-alkanes and computer matching with the Wiley 275.L library, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature [4,7,17-21]. *N*-alkanes were injected after the oil at the same chromatographic condition.

Results and Discussion

There are several reports about the volatile oil composition of *L. nobilis* leaves in the literature [3,10-12,14,22-24] and the subject of a few of them is about seasonal variations of the oil components [7,15,16].

According to the results of our study, we have found that plenty of different monoterpenes varied obviously in our oils collected through the year. The season of the leaves collection of this evergreen tree had affected the quality and quantity of the volatile oil.

The yields in essential oil ranged between 0.8% and 1.5% by weight. The higher value was obtained from the leaves collected in June, and the lower value was from oil samples coming from December. March and September samples had 1.1% and 1.4% (w/w) volatile oil. Twenty-nine, thirty-one, thirty-three and thirty-four components consisting 96.91%, 97.66%, 97.46% and 95.44% of the total compounds were identified in the volatiles, respectively. In all of our plant volatiles, the major component was found to be 1,8-cineole, which was in accordance with most of the previous reports about this plant [3,7,9-12,14-16,22-24]. All of our four volatile oil samples produced relatively matching GC profiles characterized by a relatively high total ratio of some ether compounds like 1,8-cineole, methyl eugenol and eugenol. Table 1 reports the results of the composition of our four volatile oil samples collectied in March, June, September and December 2009. 1,8-cineole content of our samples during the year 2009, ranged from 30.80% (December) to 40.25% (June).

The composition of the volatile oil from the June sample had higher content of 1,8-cineole (40.25%) than others. Interestingly δ-3-carene, camphor, camphene and sabinene were found as the second major compounds of the March, June, September and December samples, respectively. Some of the compounds like eugenol, methyl eugenol and α-terpenyl acetate were not affected apparently by seasonal changes. Other compounds present in considerable amount were δ-3-carene, camphor, camphene, sabinene, α-pinene, borneol, α-terpenyl acetate, methyl eugenol and eugenol.

Seasonal variations of the volatile oil of *L. nobilis* revealed that the harvesting time and season of collecting *L. nobilis* leaves are key factors on the sweet laurel volatiles characterizations in the yield and composition that may lead to difference in the pharmacological activities. In general, in both months of June and September, exactly from full flowering period of *L. nobilis* trees to their bearing ripe fruits, we have access to the higher contents of 1,8-cineole in the oil. Meanwhile, the quantities of June and September oils are higher than others. They are relatively in accordance with the results of one of the recently published data about harvesting time of *L. nobilis* leaves [7]. Due to these evidences, it seems that the best time for harvesting the leaves of this aromatic tree in Isfahan, Iran is from June till September.

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Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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