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# STUDY OF THE VOLATILE OIL IN LAURUS NOBILIS L. WIDE SPREAD IN SYRIA

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## ABSTRACT

The volatile oil was extracted from samples taken from three regions of Syria with different heights, by distillation Method. Gas Chromatography was used in order to determine the analytical different of the chemical components of these oils, and then a comparative study was done between the analytic results of extracted essential oil of the Syrian *Laurus* and other studies. The analytic chemical study of the volatile oils of *Laurus* nobilis L. showed the richness with 1.8 Cineol,  $\alpha$ -Pinene,  $\beta$ -Pinene,  $\alpha$ -Humulene and Sabinen, which are consistent with the reference studies.

KEYWORDS: Laurus nobilis, GC, Volatile oil, 1.8 Cineol.

## INTRODUCTION

Laurus nobilis L. is an aromatic, evergreen (Vardapetyan et al. 2013) and perennial (Zeković et al. 2009), shrub or small tree (Nurba et al. 2005), usually growing up to height of from 3 to 15 m high (9 to 50 feet) at a slow rate (Moghtader et al. 2013). It belongs to Lauraceae family, which comprises numerous aromatic and medicinal plants (Hogg et al. 1974). The genus Laurus is consists of two species Laurus azorica and Laurus nobilis (Basak et al. 2013). Laurus nobilis is a very important medicinal plant and there is a comprehensive monograph about this plant in many Herbal Pharmacopoeia (Ghannadi et al. 2002), and it has a long history of folk use in the treatment of many ailments (Moghtader et al. 2013). Laurus Leaves appear in January (Moghtader et al. 2013), alternate, they are 5-12 cm long and 1.8-5 cm wide, oblong, leathery, on both surfaces, lateral veins 10-12 pairs, margin slightly undulate, apex acute or acuminate, with a darkgreen color (Peter et al. 2010). The leaves do not fall during winter (Basak et al. 2013), they have a strong pungent odor because they contain essential oil up to 3%. The leaves can be used either dried or fresh but the flavour is stronger in dried and grinded leaves, but the leaves which are stored longer than one year, or in bade situation, they will lose their flavour (Bown, 2001). Laurus leaves essential oil is used in the flavourings industry (Bauer et al. 1985), as food preservative, in the preparation of hair lotion due to its antidandruff activity and for the external treatment of psoriasis, and also, it has antibacterial properties (Dadalioglu et al. 2004; Seyed et al. 1991). There are many studies on the chemical composition of the essential oil obtained from Laurus grown

Mediterranean and Europe, showed that the geographical, genetic and environmental factors, are the key factors on the *Laurus* volatiles specifications in the yield and composition (Amin et al. 2007; Marzouki et al. 2009; Verdian-rizi., 2008). However, remains 1-8 cineole the predominant compound (Zeković et al. 2009). Beside 1-8 cineole, *Laurus* essential oil contains many components such as sabinene, linalool, alpha-terpinyl acetate, alpha-pinene and terpinen-4-ol (Table1).

Chmit et al.

European Journal of Pharmaceutical and Medical Research

	Tunisia	Algeria	Morocco	Turkey	Iran	France	Italy	Russia	Colombia	Croatia	Armenia	Georgia	Serbia
<b>Country Reference</b>	Ben	Ben	Ben	Kosar	Maghtadan	Dovelogeonee	Loizzo	Michanina	Quiiana	Politeo	Vardanatyan	Vardanatvan	Politeo
Materials	Jemâa et	Jemâa et	Jemâa et	et al.	2013	2013	et al.	ot al 2011	2007	et al.	et al 2013	et al 2013	et al.
	al. 2011	al. 2011	al. 2011	2005	2013	2013	2007	ct al. 2011	2007	2006	ct al. 2015	ct al. 2015	2007
α-Thujene	0.22	0.44	0.33		0.38	0.62	0.44		0.2		19.18	12.23	
α-Pinene	2.52	4.58	4.31	4.7	5.25	5.66	5.72	5.8	2.9	1.9	7.44	6.41	2.1
Sabinene	0.41	0.25	0.42	8.1	8.7	8.67	6.17	9.5	1.5	1.2			5.7
Trans-sabinylacetate									0.1				
β-Pinene	1.39	1.95	1.92	4.0	3.99	4.30	3.46	4.6	6.1		6.90	5.55	
Camphene	7.21	8.91		0.5	3.86	0.45	0.14		0.1		0.39	0.17	
Myrcene	0.30	0.87	0.80	0.6	1.86	1.01	0.45		1.3		0.74	0.32	
α-Phellandrene					0.73	0.39	0.12				0.69	0.53	
α-Terpinene	0.11	0.28	0.32	0.3	2.12	0.63	0.68			0.3			0.3
ρ-Cymene					0.31	0.83	2.23			0.2			0.2
Limonene				1.3	3.47		1.10			0.9	3.21	1.17	0.9
β-Phallandrene	3.85	5.71										0.31	
1,8 Cineole	24.55	34.62	38.86	46.8	25.7	39.81	35.15	49.8	22.0	34.9	59.96	72.06	45.5
β-Ocimene	0.04	0.28				0.21	0.08		0.3		1.35	0.92	0.2
γ-Terpinene	0.26	0.22	0.62	0.6	3.48	1.05	1.50		0.6	0.8	0.14	0.10	0.7
Terpinolene		0.15	0.20	0.2	0.22	0.31	0.49		0.1	0.2			0.3
ρ-Cymenene				0.6									
Linalool	17.67	12.57	9.45	2.1	1.65	6.00	7.08	4.4	16.4	13.5			8.5
Sabinol					2.45								
Thujone													
carveol									0.1				
Carvone				0.2									
Terpineol	1.29	0.90	5.83	4.6	3.79	2.63	2.42	2.3	4.9	0.3		0.16	1.5
Borneol					2.37	0.16							
Terpinen-4-ol				1.8	1.24	3.10	4.42	1.7	1.6	2.4			2.1
Camphor	2.66												2.1
Elemene					2.30								
Elemicin						0.08			0.9				
Eugenol	2.18		1.42	1.3	1.69	1.50	3.73	7.1	2.0	3.4			2.5
Methyl Eugenol	12.40	2.84	3.93	1.3		5.71	2.52		2.9	13.5			10.0
α-Ylangene							0.17						
β-Bourbonnene													

Table 1. Comparison between the chemical compositions of the components of volatile oil extracted from *Laurus* leaves that grow in some countries

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Chmit et al.

# European Journal of Pharmaceutical and Medical Research

β-Elemene	0.08	0.31	0.16			0.16			0.3		 	
β-Caryophyllene	0.27	0.74	0.05		0.87	0.29	0.38		9.0	2.1	 	
CaryophylleneOxide				0.4	0.58	0.11			0.3	2.3	 	1.7
Elemene							0.10				 	
α-Guaiene	0.10	0.8					0.22		0.1		 	
Selinene		0.04	0.18						0.1		 	
α-Humulene					2.19						 	0.2
Germacrene			0.1		1.53	0.06			0.1		 	
Bicyclo Germacrene									0.9		 	
Cadinene					2.68	0.10			0.1	0.2	 	0.2
α-Cadinol									0.5		 	
Bicyclogermacrene											 	
Linalyl acetate	0.69	0.41				0.14			0.3		 	
Bornyl acetate		0.15	0.52	0.4	1.79	0.24			0.6	0.3	 	0.3
Cinnamyl acetate			0.06			0.04			2.2		 	
Terpinyl acetate				7.9	0.24	11.79	4.43	9.6	11.1	12.2	 	9.1
Neryl acetate			0.13						0.2	0.3	 	
Spathulenol				0.5	3.38		0.31		0.7		 	
α-Bulnesene									0.4		 	
Viridiflorol				0.1					0.2		 	
Geranyl acetate	0.08								0.1		 	
α-Guaiene											 	
Cubebol						0.06					 	
Elemol									0.1		 	
Nerol						0.22					 	
Nerolidol									0.1		 	
$\Delta^3$ -Carene	0.11	0.42	0.19			0.22				0.4	 	0.4
α-Farnesene										0.6	 	0.6
β-Eudesmol									0.6		 	

*Laurus* nobilis L. fruits are small and olive-like, fleshy, dark purple when mature, ovoid, thin, brittle, wrinkled pericarp, which when broken discloses the seed kernel, the seed-coats adhering to the inner surface of the pericarp. The fruits contain both fixed and volatile oils. The essential oil is existing up to 1% (Baytop, 2000), while *Laurus* fruits contain around 26% fixed oil (İlisulu, 1992), this oil is a green, granular, melting at 40°C, to a dark green aromatic fluid, and consisting of a semi-solid fat.

The need of the medicinal plants to use in treatment and food industries is increasing, due to increasing of population and to spread new types of diseases, also due to the resistance caused by pathogens such as bacteria which become stubborn against some types of antibiotics. The increasing demand for plants caused to wanton harvesting of plants, which led to decrease the enumeration of these plants and the spread of commercial cheat, and this led to a sharp decline in the population and natural spread, which led to the spread of fraud in the trade of these herbs, which called for the need to develop analytical methods to ensure the purity of these samples and free of fraud. The methods Chromatography techniques are the most important to determine the purity of the essential oil, which are extracted from Laurus leaves. Given the scarcity of the chemical studies on Laurus in Syria, which has not been reported to date. The aim of the current study was to determine the consistence the extracted Laurus volatile oils and to compare the results of these study with others.

## MATERIALS AND METHODS

#### **Plant samples**

The samples of *Laurus* leaves were collected from three deferent areas in Syria, the first one sample was from the city Kesab, which is located at 800 m above the sea level with the Coordinates  $(35^{\circ}55'30''N 35^{\circ}59'19''E)$ , the second one from the city Slinfah, which is located at 1130 m above the sea level with the Coordinates  $(35^{\circ}36'1''N 36^{\circ}10'43''E)$ , and the last one from Mount Nabi Yunis area, which is located at 1562 m above the sea level with the Coordinates  $(35^{\circ}38'29''N Startes)$ 

#### 36°13′0.9″E).

The Samples were air-dried at room temperature in the shade (final moisture content about 10.0%). Before using them, the dried samples were grinded. At the end of the milling process, the particle sizes were in the range of 0.8-0.9 mm.

### Volatile oil extraction

The volatile oils of *Laurus* leaves were obtained by the process of hydro distillation in the Clevenger apparatus. (100g) of grounded *Laurus* dried leaves, were placed in a flask (2.5L) and hydro distilled for 2.5h. The oil samples were dried using anhydrous Sodium Sulfate and stored at  $4^{\circ}$ C in the darkness.

#### Volatile oil analysis

50 microns of the volatile oil sample was taken and extended to 250 microns by hexane, it mixed then 1 micron from it was injected in Gas chromatography (GC) for analysis. Gas chromatography (GC) analysis was carried out on a Shimadzu GC 2010 with FID detector and a DB23 capillary column (60 m×0.25 mm; film thickness 0.25  $\mu$ m). The carrier gas was helium with a flow rate of 0.72 ml/min., the oven temperature for first 4 min. was kept at 60 °C and then increased at a rate of 4 °C/min. until reached to the temperature of 250 °C and keep on for 5 mints, injector and detector temperature were set at 250°C.

Standards of each comparative compound of volatile oil are also used to determine the amount of each component in the injected volatile oil in the GC under the same used parameters.

#### **RESULTS AND DISCUSSION** Volatile oil extraction

As seen in the (Table 2), *Laurus nobilis*, which is grow in the Mount Nabi Yunis gives the high rate of leaves volatile oil, while *Laurus* which is grow in the city Kesab gave the double amount of volatile oil compared with *Laurus* which is grow in the city Slinfah:

Table 2: The Volatile Oil amount of Lauri	us nobilis leaves.
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Region	Kesab	Slinfah	Mount Nabi Yunis	
Coordinatos	35°55′30″N	35°36′1″N	35°38′29″N	
Coordinates	35°59′19″E	36°10′43″E	36°13′0.9″E	
Height above sea level	880 m	1130 m	1562 m	
V.O amount mL/100g	1.45	0.79	2.1	

#### Volatile oil analysis

As seen in (Table 3), 18 different compounds were determined. Although there was no marked difference in the composition of leaves volatile oils:

Region		Kesab	Slinfah	Mount Nabi Yunis					
Coordinates		35°55′30″N	35°36′1″N	35°38′29″N					
Coordinates		35°59′19″Е	36°10′43″E	36°13′0.9″E					
Height above sea	level	800 m	1130 m	1562 m					
Compound	Compound Structure		Concentration %						
α-Pinene		3.386	2.618	3.850					
Camphene		0.091	0.054	0.274					
β-pinene		3.179	2.656	3.246					
Sabinen		8.897	7.089	3.560					
Myrcene		0.419	0.380						
α-Phellandrene		0.069	0.083	0.076					
Limonen		1.934	1.485	0.883					
γ-terpinene		0.670	0.643	0.809					
Trans $\beta$ ocymene		0.713	0.632						
1.8 Cineol		58.660	62.059	73.702					
P-Cymene		0.610	0.902	2.236					
Linalool		0.971	0.323	0.356					
Terpinen-4-Ol		2.965	3.841	2.581					
β-Caryophllen		0.657	0.493	1.177					
α-Terpineol		3.499	2.868	1.118					
α-humulene		10.906	10.097	3.369					

Table 3: The essential oil composition of Laurus leaves.

### CONCLUSION AND DISCUSSION

In this study, the essential oils of leaves of Laurus nobilis L. collected from three deferent areas in Syria, were obtained by hydro distillation and analyzed by gas chromatography with flame ionization detection (GC-FID) to determine their chemical composition and identification of their chemo types. The yield of essential oil extracted from the leaves varied from 0.79% (Slinfah) to 2.1% (Mount Nabi Yunis). These differences could be attributed to the geographical characteristics of the ecological zone of the samples, as the leaves of Laurus nobilis L., which is grew in the mountains, gave the highest rate of volatile oil than other areas. The comparison of the samples did not reveal any big difference in their qualitative composition, but the collection of the samples from different areas in Syria allowed observing the change of the volatile oil quantities. Eighteen components were identified, and the volatile compounds in the leaves of Laurus mainly consisted of 1.8 Cineole (58.66%-73.70%), followed by α-humulene (3.37%-10.91%), Sabinen (3.56%-8.89%), α-Pinene (2.62%-3.850%), and  $\beta$ -pinene (2.66%-3.25%). In the present study, the chemical composition of the essential oils is comparable to that of previous reports with some variation in the constituents (Table 1).

This could be due to different chemo types or may result of different environmental conditions. Previous studies reported that geographical origin, seasonal and maturity variation, genetic variation, vegetation stages, part of plant utilized and postharvest drying and storage may influence the essential oil composition (Marotti et al. 1994; Hussain et al. 2008; Anwar et al. 2009). Moreover, there are many reports showing the variation in chemical composition of the essential oil

with respect to geographical regions (Uribe et al. 1992; Souto et al. 1997; Celiktas et al. 2006; Van Vuuren et al. 2007). Uribe et al. 1992 also reported that the essential oil composition varied significantly depending on the locations where the plants grew. Furthermore, climatic factors such as heat and drought were also related to the essential oil profiles (Milos et al. 2001). In addition to, Vokou et al. 1993 pointed out altitude seems to be another important that environmental factor influencing the essential oil content and chemical composition (Ben Jemâa et al. 2012). For example, 1.8 Cineol is increased from 58.660% (Kesab), to 62.059% (Slinfah), to 73.702% (Mont Nabi Yunis), this mean that the percentage of 1.8 Cineol is increased as increased the height of the area, where the samples gatherd (also P-Cymene,  $\gamma$ terpinene,  $\beta$ -Caryophllen and others).

But other volatile compounds such as Sabinen is decreased from 8.897% (Kesab), to 7.089% (Slinfah) to 3.560% (Mount Nabi Yunis), this result has the meaning that the percentage of Sabinen is decreased as increased the height of the area (also  $\alpha$ -humulene and Limonen, Myrcene and others.). These results ensure the direct effect of the heights on some essential oil components. It can be related to humidity in winter and sunlight in summer. This study leads to the result that the sample of *Laurus* nobilis L. which is grow in the mountains (Mount Nabi Yunis) showed equilibrium in its essential oil, more than the samples of other regions.

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