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ESSENTIAL OIL COMPOSITION OF *LAURUS NOBILIS* L. SAMPLES FROM DIFFERENT POPULATIONS IN ALBANIA BASED ON GC-FID ANALYSIS

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Abstract: In this study chromatographic data of *Laurus nobilis* L. essential oil from different areas of Albania were presented. *Laurus nobilis* is an aromatic evergreen tree, part of the flowering plant family Lauraceae. It is native to the Mediterranean region and can be found almost in all areas of Albania. It has been used in culinary and traditional medicine since ancient times. Leaves of *Laurus nobilis* were sampled in June, 2018 in six different areas of Albania (Tirana, Durres, Lezhe, Lushnje, Elbasan and Pogradec). The air dried leaf samples were subjected to European Pharmacopoeia apparatus (Clevenger type) for 4 hours to obtain *Laurus nobilis* essential oil. The leaves contained 1.21 - 1.44% essential oil. The chemical composition of the essential oils was analyzed using GC/FID technique. *Laurus nobilis* essential oil samples were injected manually in a Varian 450 GC. VF-1ms capillary column (30 m x 0.33 mm x 0.25 um) were used for separation of terpene compounds. Oxygenated monoterpenes were in higher percentage for all studied samples. 1,8-Cineole (Eucalyptol) was the main compound followed by Linalool, Limonene, Linalyl acetate, alfa- and beta-Pinene. Their profile was similar for all studied areas despite the differences in geographic and atmospheric conditions. Profile and percentage of terpenes in *Laurus nobilis* L. samples from Albania was similar with other reported studies from Balkan and Mediterrean area.

INTRODUCTION

Laurus nobilis is an aromatic evergreen tree or a large shrub with green, smooth and hairless leaves, in the Lauraceae flowering plant family. It sometimes reaches height of 7-18 meters. *Laurus nobilis* is dioecious (unisexual), with male and female flowers on separate plants. Each flower is pale yellow-green, about 1

cm in diameter, and they are borne in pairs beside a leaf. The leaves are glabrous, 6-12 cm long and 2-4 cm wide, with an entire (toothed) margin. The fruit is a small, shiny black berry-like drupe of about 1 cm long that contains one seed. It is native to the Mediterranean region and is used as a bay leaf for seasoning in cooking. It was found almost in all Albania areas (Asllani 2004; Kathe *et al* 2003). *Laurus nobilis* was known in ancient times. It figures prominently in classical Greek-Roman culture. It was used as symbol of peace and victory and to make wreaths for emperors, generals and poets. The plant is the source of several popular herbs and one spice used in a wide variety of recipes, especially among Mediterranean cuisines. Whole bay leaves have a long shelf life of about one year under normal temperature and humidity.

The health benefits of *Laurus nobilis* essential oil can be attributed to its properties as an antiseptic, antibiotic, anti-neuralgic, anti-spasmodic, analgesic, astringent, cholagogic, insecticide, sedative, stomachic and a tonic substance (Bakkali *et al* 2008; Bozin *et al* 2006). 1,8-Cineole (Eucalyptol) was reported as the main compound followed by Linalool, Limonene, Linalyl acetate, alfa- and beta-Pinene (Caputto *et al* 2017; Marzouki *et al* 2009; Sangun *et al* 2007). Oxygenated monoterpenes are thought to play an important role in health benefits of *Laurus nobilis* (Dadalioglu *et al* 2004).

MATERIALS AND METHODS

Sampling of *Laurus nobilis* samples

Leaves of *Laurus nobilis* were sampled in June, 2018 in six different areas of Albania. Twenty-seven different samples were taken from population of Albania: Tirana (6 samples), Durres (4 samples), Lezhe (3 samples), Lushnje (5 samples), Elbasan (5 samples) and Pogradec (4 samples) respectively. Laurus nobilis leaves were air dried in shadow to preserve their morphological characteristics.

Isolation of *Laurus nobilis* essential oil

Plant material (50 g of ground dry leaves) was subjected to hydrodistillation for 4 h, using a modified Clevenger-type apparatus to produce essential oil, recommended by Pharmacopoeia. 150 ml of water and 2 ml Toluene was added to the balloon for isolation of oregano essential oils. The oil was dried by anhydrous sodium sulphate (Na_2SO_4) and kept sealed in dark glass vial at +8^{o}C until use. Diluted essential oil in toluene was used for GC-FID analyses.

Chromatography

Gas chromatographic analyses of Laurus nobilis essential oil were realized with a Varian 450 GC instrument equipped with a flame ionization detector and PTV injector. The temperature of PTV injector was 280^{o}C. One µl of *Laurus nobilis* essential oil diluted in Toluene was injected manually in splitless mode.

A temperature for FID was held at 280°C. Nitrogen was used as carrier (1 ml/min) and make-up gas (25 ml/min). Hydrogen and air were flame detector gases with 30 ml/min and 300 ml/min, respectively. VF-1ms capillary column (30m x 0.33mm x 0.25µm) was used to separate compounds of *Laurus nobilis* essential oil. The oven temperature was programmed as follows: 40°C (held for 2 minutes) to 150°C (with 4°C/min), after that to 280°C with 10°C/min and held for 2 minutes. The identification of the compounds was based on comparison of their Kovats indices (KI), their retention times (RT) and literature (Adams, 2001; David *et al.,* 2010, Caputo *et al* 2017; Marzouki *et al* 2009; Konig *et al* 1999). Mixture of n-alkanes (C6-C20) was used for determination of retention indices.

RESULTS AND DISCUSSION

Essential oil of 27 different *Laurus nobilis* samples from six different areas of Albania were analyzed using GC/FID technique. Average of result for the same areas was presented in this study. The data present main compounds that were identified for all *Laurus nobilis* samples. Data show percentage for the total of peaks except the peak of Toluene (solvent used for extraction/dilution of essential oil). The peaks lower than 0.1% was not present in this study. Table 1 shows average percentage for the main compounds in analyzed *Laurus nobilis* essential oil samples from Tirana, Durresi, Lezha, Lushnja, Elbasani and Pogradeci areas (June 2018). Chromatograms of *Laurus nobilis* essential oil were noted to have 50-60 compounds. This study includes 29 main compounds that were from 92.6% (Lushnja) to 99.2% (Tirana) for all analyzed samples. The most abundant compounds were: Eucaliptol (41.8 – 48.2%) > Linalool (8.8 – 11.9%) > Sabinene (8.9 – 11.7%) > Terpinyl acetate (8.4 – 10.8%) > alfa-Pinene (4.8 – 6.0%), etc. Other compounds were account for 0.5 to 3%. Oxygenated monoterpenes (Eucaliptol, Linallol and Terpinyl acetate) were found in high percentage for all analyzed samples of *Laurus nobilis.* Distribution of main components for all samples of Albania population were similar. Monoterpenes were the largest group ranging from 91.1% to 98.8%. Sesquiterpene (beta-Cariophyllene, delta-Cadinene and Cariophyllene oxide) were found from 1.1% to 1.5%. The largest monoterpene group was oxygenated monoterpenes (Ecualiptol, Linalool, Borneol, Terpilen-4-ol, Alpha-Terpineol, Bornyl Acetate) that were found from 68.0% to 74.4%. Aromatic monoterpenes (para-Cymene, Thymol and Carvacrol) were found from 3.1 to 3.6%. Monocyclic monoterpenes (alpha-Terpinene, Limonene, gamma-Terpinene) were the second group with range between 3.5-8.3%. Bicyclic monoterpenes (Phellandrene, alpha -Pinene, Camphene and beta-Pinene) was in the range 1.7 – 2.5%. The alicyclic monoterpenes (Myrcene and Ocimene) were from 0.9 to 1.2%. Differences in chemical composition of *Laurus nobilis* samples from different areas of Albania are related with these factors: geographical area, height from sea level, average temperatures, humidity, geological composition

of the soil, the maturity time, collection time, drying process, etc. Profile and levels of *Laurus nobilis* samples from Albania were s other reported studies from Balkans and Mediterranean area (Daferera *et al,* 2000; Dadalioglu 2004 Caputo e*t al* 2017; Marzouki *et al* 2009; Sangun et al 2007) where oxygenated monoterpene were reported to be in higher percentage.

Table 1. GC/FID data on *Laurus nobilis* samples from different areas of Albania, 2018

Compounds/ station	RT	Tirana	Durres	Lezhe	Lushnje	Elbasan	Pogradec
Tricyclene	3, 42	0.12 ± 0.03	0.04 ± 0.01	0.09 ± 0.01	0.08 ± 0.02	0.21 ± 0.04	0.11 ± 0.03
α-Thujene	3, 74	0.42 ± 0.04	0.56 ± 0.06	0.34 ± 0.03	0.87 ± 0.11	0.28 ± 0.05	0.19 ± 0.02
α-Pinene	3, 85	**5.21 ± 1.39**	**5.63 ± 1.08**	**4.98 ± 0.93**	**6.01 ± 2.01**	**5.22 ± 0.93**	**4.75 ± 1.84**
Camphene	3, 91	0.41 ± 0.05	0.65 ± 0.08	0.44 ± 0.06	0.29 ± 0.04	0.51 ± 0.06	0.74 ± 0.09
Sabinene	4, 15	**10.44 ± 3.82**	**9.56 ± 2.71**	**11.65 ± 3.29**	**9.42 ± 2.29**	**8.93 ± 2.57**	**11.59 ± 3.28**
β-Pinene	4, 30	0.12 ± 0.02	0.16 ± 0.03	0.28 ± 0.03	0.31 ± 0.06	0.17 ± 0.03	0.29 ± 0.04
β-Myrcene	4, 42	0.90 ± 0.11	1.12 ± 0.43	0.75 ± 0.09	0.93 ± 0.11	0.67 ± 0.09	0.71 ± 0.08
α-Phellandrene	5, 72	0.45 ± 0.06	0.51 ± 0.06	0.63 ± 0.08	0.27± 0.04	0.48 ± 0.05	0.61 ± 0.07
δ-3-Carene	6, 2	0.33 ± 0.04	0.28 ± 0.04	0.43 ± 0.07	0.18 ± 0.03	0.27 ± 0.04	0.18 ± 0.03
α-Terpinene	7, 19	0.52 ± 0.08	0.62± 0.09	0.69 ± 0.11	0.38 ± 0.04	0.57± 0.06	0.44 ± 0.05
p-Cymene	7, 73	0.72± 0.07	0.69 ± 0.07	0.51 ± 0.06	0.73 ± 0.08	0.82± 0.15	0.55 ± 0.06
Eucalyptol	8, 68	**48.21 ± 7.62**	**45.62 ± 6.39**	**43.65 ± 9.12**	**41.86 ± 7.06**	**45.38 ± 5.39**	**47.84 ± 6.43**
cis-Ocimene	9, 14	0.31 ± 0.04	0.12 ± 0.04	0.18 ± 0.03	0.41 ± 0.05	0.22 ± 0.04	0.51 ± 0.06
γ-Terpinene	10, 31	1.12 ± 0.09	0.95 ± 0.17	1.42 ± 0.32	0.83 ± 0.09	0.78 ± 0.09	1.52± 0.28
Terpinolene	10, 63	0.35± 0.05	0.30 ± 0.04	0.28± 0.06	0.22 ± 0.04	0.36 ± 0.05	0.13 ± 0.04
trans-Sabinenehydrate	10, 82	0.09± 0.01	0.15 ± 0.03	0.21± 0.05	0.06 ± 0.01	0.20 ± 0.04	0.11 ± 0.03
Linalool	12, 56	**10.33 ± 2.51**	**9.43 ± 3.04**	**8.75 ± 3.13**	**11.93 ± 4.27**	**10.18 ± 3.75**	**10.52 ± 2.55**
Borneol	14.33	0.15 ± 0.03	0.21 ± 0.04	0.18 ± 0.04	0.11 ± 0.02	0.27 ± 0.04	0.19 ± 0.04
δ-Terpineol	14.69	0.22 ± 0.04	0.31 ± 0.08	0.16 ± 0.03	0.47 ± 0.05	0.33 ± 0.06	0.18 ± 0.03
Terpinene-4-ol	16.32	2.61 ± 0.79	3.01 ± 0.83	2.77 ± 0.87	2.03 ± 0.32	1.83 ± 0.21	1.79 ± 0.66
α-Terpineol	17.37	1.91 ± 0.81	2.33 ± 0.97	1.53 ± 0.62	1.74 ± 0.15	2.04 ± 0.56	1.52 ± 0.73
Linalool acetate	19.37	0.11 ± 0.02	0.27 ± 0.04	0.09 ± 0.01	0.12 ± 0.03	0.08 ± 0.02	0.09 ± 0.02
Bornyl acetate	21.28	0.43 ± 0.05	0.37 ± 0.07	0.51 ± 0.06	0.82 ± 0.27	0.47 ± 0.09	0.29 ± 0.05
δ-Terpinyl acetate	24.23	0.53± 0.06	0.18 ± 0.05	0.26 ± 0.04	0.29 ± 0.05	0.38 ± 0.06	0.27 ± 0.06
α-Terpinyl acetate	24.88	**9.85 ± 2.61**	**8.43 ± 2.77**	**10.83 ± 3.19**	**8.63 ± 2.93**	**10.38 ± 3.23**	**9.53 ± 4.32**
Eugenol	25.49	0.42 ± 0.08	0.39 ± 0.04	0.63 ± 0.09	0.51 ± 0.06	0.38 ± 0.05	0.47 ± 0.06
Methyl eugenol	26.22	2.51 ± 0.91	2.65 ± 0.72	1.92 ± 0.86	1.83± 0.32	2.18 ± 0.78	1.79± 0.28
β-Caryophyllene	28.43	0.81 ± 0.07	1.03 ± 0.15	0.82 ± 0.09	0.76 ± 0.11	1.17 ± 0.43	0.93 ± 0.06
δ-Cadinene	29.93	0.20 ± 0.03	0.18 ± 0.03	0.31 ± 0.04	0.24 ± 0.04	0.19 ± 0.01	0.11± 0.03
Carryophyllene oxide	31, 12	0.12 ± 0.04	0.03 ± 0.01	0.09 ± 0.01	0.29 ± 0.05	0.17 ± 0.02	0.07 ± 0.02
Total		**99.92**	**95.78**	**95.38**	**92.62**	**95.12**	**98.02**
Total monoterpene		98.79	94.54	94.16	91.33	93.59	96.91
Monocyclic monoterpene		50.65	48.00	46.67	43.56	47.57	50.54
Bicyclic monoterpene		17, 02	16.99	18.33	17.14	15.58	17.85
Aliphatic monoterpene		1, 21	1, 24	0.93	1, 34	0.89	1, 22
Oxygenated monoterpene		26.14	24.54	25, 08	26.14	25.96	24.38
Aromatic monoterpene		3, 65	3, 73	3, 06	3, 07	3, 38	2, 81
Sesquiterpene		1, 13	1, 24	1, 22	1, 29	1, 53	1, 11

CONCLUSIONS

Laurus nobilis samples were analyzed, from different areas of Albania, using GC/FID technique. The data present average of 29 main compounds that were identified for all analyzed samples. Chromatograms of *Laurus nobilis* essential oil were noted to have 40-50 compounds. Monoterpenes were the largest group in essential oil of *Laurus nobilis.* The most abundant compounds for all analyzed samples were: Eucaliptol > Linalool > Sabinene > Terpinyl acetate > alfa-Pinene, etc. Other compounds were from 0.5 to 3%. Oxygenated monoterpenes, Eucaliptol, Linallol and Terpinyl acetate, were found in higher percentage for all *Laurus nobilis* samples. Distribution of analyzed components was similar for all samples from Albania population. Some differences were noted between different areas of Albania related with geographical position, height from sea level, average temperatures, humidity, geological composition of the soil, the maturity time, collection time, etc. Profile and levels of main constituents of *Laurus nobilis* samples from Albania were the same with recent studies from Balkan and Mediterranean areas.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this article. It was written after the proposal of the first author (J.S.) and all the other authors (A.N., A.D. and Dh.P.) contributed equally to the discussions of the text.

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