Bangladesh J. Bot. **47**(4): 893-901, 2018 (December)

CONSTITUENTS OF ESSENTIAL OILS FROM LEAVES AND SEEDS OF *LAURUS NOBILIS* L.: A CHEMOTAXONOMIC APPROACH

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Keywords: Laurus nobilis, Essential oil, Eucalyptol, α-terpinyl acetate

Abstract

In the present study, chemical compositions of essential oils from seeds and leaves of laurel (*Laurus nobilis* L.) were evaluated using GC-GC/MS system. Sixty nine different compounds were identified constituting 86.7% of the total oil from the seed, while 76 compounds were determined, constituting 95.8% of the total oil extracted from the leaves. The major compounds of essential oil from laurel seeds included eucalyptol (17.2%), α-terpinyl acetate (9.0%), caryophyllene oxide (6.1%), spathulenol (5.0%) and methyl eugenol (4.2%), constituting 41.5% of the total oil. However, eucalyptol (18.0%), α-terpinyl acetate (13.1%), sabinene (7.8%), α-pinene (4.5%), 2 (4-methoxyphenyl)-N,N,2-trimethyl-1-pyrroline (4.4%) were identified as the major compounds in the oil from laurel leaves, constituting 47.8% of the total oil. Eucalyptol and α-terpinyl acetate, belonging to monoterpenoids, were determined in the highest concentrations within both oils. However, the other principle compounds differ between the two volatile oils.

Introduction

Laurus nobilis L. is the member of the family Lauraceae which comprises 32 genera and about 2,000 - 2,500 species (Chahal *et al.* 2017, Garg *et al.* 1992). The leaves of *L. nobilis* are short, thick and its fresh leaves are in red tinged yellow color which turns into light green with light green veins. They also have little aromatic odor. The fresh shoots are green, while the following is red, black and hairless. Their maximum length is known as 2 cm (Yılmaz and Deniz 2017, Yazıcı 2002). *L. nobilis*, commonly known as bay, sweet bay and laurel, is an evergreen tree native to the south part of Europe and the Mediterranean region specially in Turkey, Greece, Spain, Italy and France. Turkey is one of the main producers and suppliers of bay leaves (Demir *et al*. 2004).

The essential oil (EO) and extracts from medicinal and aromatics plans may contain a wide variety of free radical scavenging molecules, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids, and some other endogenous metabolites, which are rich in antioxidant activity (Pirbalouti *et al.* 2013).

L. nobilis is a plant of industrial importance and used in foods, drugs, and cosmetics. The dried leaves and essential oils are used extensively in the food industry for seasoning of meat products, soups and fishes. Its antimicrobial and insecticidal activities are among the factors for which bay is used in the food industry as a food preservative. The fruits contain both fixed and volatile oils, which are mainly used in soap making (Bozan and Karakaplan 2007). Traditionally it is used against rheumatism and dermatitis (Kilic *et al.* 2004), gastrointestinal problems, such as epigastric bloating, impaired digestion, eructation, and flatulence. The aqueous extract is used in Turkish folk medicine as an anti-hemorrhoidal, anti-rheumatic and diuretic, as an antidote in snake bites and for the treatment of stomachche (Gulcin 2006, Baytop 1984, Aqili 1992). Recently it is used in treating diabetes and preventing migraine (Duke 1997, Patrakar *et al.* 2012). In addition, recent studies of laurel seeds and leaves extracts have been carried out for antioxidant, antiepileptic

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(Simic *et al.* 2003), gastro protective (Afifi *et al.*1997), antibacterial, antifungal (Erturk 2006), analgesic and anti-inflammatory activities (Sayyah *et al.* 2003, Isbilir *et al.* 2008). Since Turkey is one of the main producers and suppliers of the plant, it is important to provide the good quality of laurel products, in which essential oils constitute a big portion. Thus the aim of the present study was to carry out the chemical composition of essential oils from leaves and seeds of *Laurus nobilis*.

Materials and Methods

Laurus nobilis specimens were collected from natural habitats in Black Sea region, Sinop, Turkey. The leaves and seeds were dried in the shade at room temperature. The specimens were studied at Sinop University, Scientific and Technological Research Application and Research Center, Sinop and Firat University, Plant Products and Biotechnology Research Laboratory (BUBAL), Elazig, Turkey.

Air-dried aerial parts of the plant materials (100 g) were subjected to hydro distillation using a Clevenger-type apparatus for 3 hrs to extract the essential oil.

The essential oil was analyzed using HP 6890 GC equipped with a FID detector and an HP-5 MS column (30 m × 0.25 mm i.d., film thickness 0.25 μm) and the capillary column were used. The column and analysis conditions were the same as in GC-MS. The percentage composition of the essential oils was computed from GC–FID peak areas without correction factors.

The oil samples were analyzed by GC-MS, using a Hewlett Packard system. HP-Agilent 5973N GC-MS system with 6890 GC is in Plant Products and Biotechnology Res. Lab. (BUBAL) at Fırat University. HP-5 MS column (30 m × 0.25 mmi. d., film thickness 0.25 μm) was used with helium as the carrier gas. Injector temperature was 250^0C, split flow was 1 ml/min. The GC oven temperature was kept at 70^0C for 2 min and programmed to 150^0C at a rate of 10^0C/min and then kept constant at 150^0C for 15 min to 240^0C at a rate of 5^0C/min. Alkanes were used as reference points in the calculation of relative retention indices (RRI). MS were taken at 70 EV and at a mass range of 35 - 425. Component identification was carried out using spectrometries electronic libraries (WILEY, NIST). The identified constituents of the essential oils are listed in Table 1. The chromatograms were obtained and are shown in Figs 1 and 2.

Results and Discussion

For the purpose of studying the chemical composition of the essential oils (EOs) of different parts of *Laurus nobilis* were collected from Sinop, Turkey. EOs of dried leaves and seeds of *L. nobilis* were analyzed by GC and GC-MS in terms of their chemical composition. The results of the analysis of EOs of *L. nobilis* leaves are presented in Table 1 and in Fig. 1. A total of 107 compounds were identified, in which 38 of them were in common. In the essential oil of laurel leaves, 76 compounds were identified, representing 95.8% of the total oil (Fig. 1). While eucalyptol (1,8-cineole) was at the highest percentage (18.0), α-terpinyl acetate (13.1%), sabinene (7.8%), α-pinene (4.5%), 2 (4-methoxyphenyl)-N, N, 2-trimethyl-1-pyrroline (4.4%) were also detected as the major compounds in the oil profile (Table 1). In the case of *L. nobilis* EO from the seeds, 69 compounds were identified, representing 86.7% of the total oil (Fig. 2). Eucalyptol was detected to be present at the highest percentage (17.2) in the laurel seed EO. The presence of α-terpinyl acetate (9.0%), caryophyllene oxide (6.1%), spathulenol (5.0%) and methyl eugenol (4.2%) was also important for the oil profile (Table 1).

While the main components of EO of the leaves of laurel consist of monoterpenes, the main components of the seed EO are mono-(1,8-cineole, α-terpinyl acetate) and sesquiterpenes (sabinene, α-pinene). 2-(4-methoxyphenyl) -N, N, 2-trimethyl-1-pyrroline was the major compound in the EO of leaves, but it was not detected in the EO of the seeds. Comparison of the compositions between two oils showed that the amounts of sabinene, linalool and eugenol were higher in the EO of leaves than those of the seeds. In addition, the seed oil contained caryophyllene oxide and spathulenol with higher concentration than oils of leaves.

There are a many studies on chemical compositions of the EO obtained from the leaves of *L. nobilis* from different locations of the world, but studies on seed EOs are scarce (Bouzouita *et al.* 2001, Verdian-rizi 2009, Yalçın *et al.* 2007, Conforti *et al.* 2006, Kovacevic *et al.* 2007, Ramosa *et al.* 2012, Sellami *et al.* 2011, Caputo *et al.* 2017, Pinheiro *et al.* 2017, Yılmaz and Deniz 2017, Peixoto *et al.* 2017). The present results are more or less similar to the results previously reported.

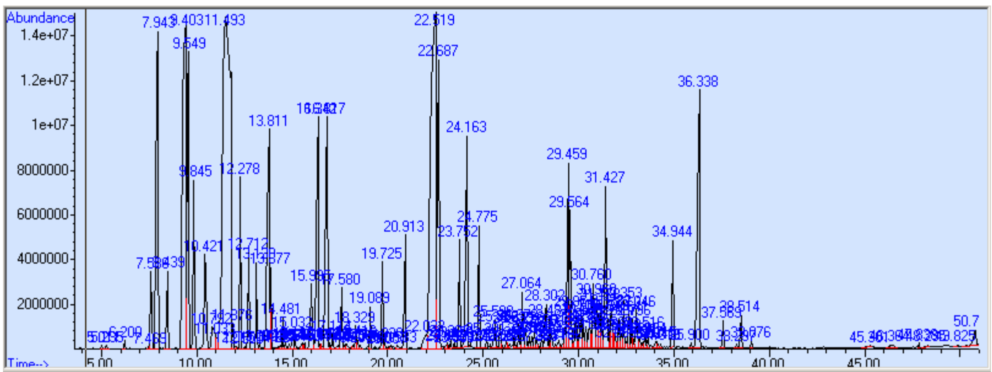

Fig. 1. Gas chromatographic-flame ionization detector (GC-FID) profile of the essential oil of leaves of *Laurus nobilis.*

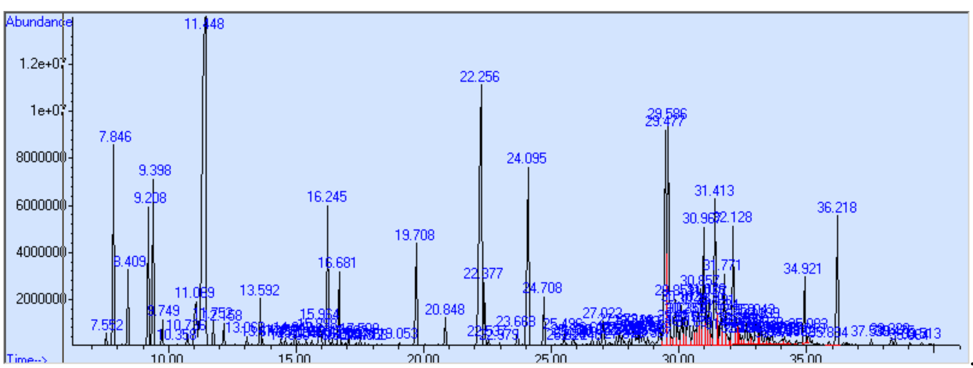

Fig. 2. Gas chromatographic-flame ionization detector (GC-FID) profile of the essential oil of seeds of *Laurus nobilis.*

In different studies, the essential oil constituents of *L. nobilis* leaves from Turkey, namely eucalyptol (59.94, 44.72, 35.63%), α-terpinyl acetate (16.33, 12.95, 20.26%), sabinene (8.70, 12.82, 6.10%), α-pinene (2, 61, 3.44, 8.02%) were reported to be the major compounds, respectively (Sangun *et al.* 2007, Ozcan *et al.* 2010, Yılmaz and Deniz 2017). In this study, *L. nobilis* leave and seed EOs have similar chemical compositions with much lower concentration of the first major compound, eucalyptol (18.10-17.2 %), followed by low or high concentrations of the other constituents (Table 1).

Table 1. Chemical composition of the leaves and seeds of *Laurus nobilis*.

No.	Compounds	RI	Leaves (%)	Seeds (%)
1	2-hexenal	964	0,1	-
2	Isopropyl-2-methyl butyrate	985	0,1	-
3	Tricyclene	1013	0,1	-
4	α-thujene	1016	0,8	0,2
5	α-pinene	1022	4,5	3,3
6	Camphene	1034	0,7	1,1
7	Sabinene	1052	7,8	2,2
8	β-pinene	1059	3,4	2,7
9	β-myrcene	1066	1,6	0,4
10	α-phellandrene	1077	1,5	0,1
11	α-terpinene	1085	0,6	0,3
12	*o*-cymene	1092	0,1	-
13	*m*-cymene	1093	-	1,5
14	Eucalyptol (1,8-cineol)	1102	18	17,2
15	β-ocimene	1110	0,1	0,3
16	γ-terpinene	1117	1,1	0,3
17	*trans*-sabinene-hydrate	1129	0,7	-
18	α-terpinolene	1138	0,5	0,1
19	2-nonanone	1141	0,1	-
20	Linalool	1148	4,2	0,7
21	6-methyl- 3,5 heptadien-2-one	1150	-	0,1
22	1-terpineol	1154	0,4	-
23	*p*-mentha-trans-2,8 dien-diol	1165	0,1	-
24	*p*-menth-2-en-1-ol	1166	-	0,1
25	4-acetyl-1-methyl cyclohexene	1170	-	0,1
26	*trans*-pinocarveol	1178	-	0,2
27	Pinocarvone	1193	-	0,1
28	δ-terpineol	1199	-	0,3
29	Borneol	1200	-	0,4
30	4-terpineol	1209	2,9	2,7
31	α-terpineol	1216	3,1	1,4
32	1-cyclohexylpropadiene	1222	-	0,1
33	*cis*-piperitol	1225	0,1	0,1
34	(+) *trans*-carveol	1231	0,1	0,1
35	Nerol	1234	-	0,1
36	*trans*-geraniol	1236	0,5	-
37	Isocarveol	1239	-	0,1
38	Homoveratrole	1242	-	0,1
39	Carvol	1250	0,1	-
40	Linalyl acetate	1252	0,2	-

(Contd.)

41	Geraniol	1254	0,1	-
42	Piperitone	1258	0,1	-
43	Bornyl acetate	1283	0,7	2,1
44	2-undecanone	1289	0,1	-
45	Perilla alcohol	1300	0,1	-
46	α-terpinyl acetate	1338	13,1	9
47	Eugenol	1341	4,2	1,1
48	Neryl acetate	1345	-	0,1
49	Ylangene	1354	-	0,1
50	Geranyl acetate	1361	0,1	-
51	β-bourbonene	1367	0,1	-
52	β-elemene	1371	0,9	0,3
53	Methyl eugenol	1380	2,6	4,2
54	α-gurjunene	1384	0,1	-
55	β-caryophyllene	1394	0,9	0,9
56	α-guaiene	1403	0,1	-
57	Epizonaren	1407	0,1	-
58	*trans*-cinnamyl acetate	1410	-	0,2
59	*trans*-isoeugenol	1412	0,3	-
60	Epizonarene	1413	-	0,1
61	α-humulene	1418	0,2	0,1
62	10s,11s-himachala-3(12),4-diene	1419	-	0,1
63	Aromadendrene	1420	0,1	-
64	α-selinene	1429	0,1	-
65	(+) epibicyclosesquiphellandrene	1434	-	0,2
66	Germacrene-D	1435	0,2	-
67	β-selinene	1440	0,2	0,2
68	Methyl isoeugenol	1443	-	0,6
69	Bicyclogermacrene	1444	0,6	-
70	Eremophila-1(10),11-diene	1452	0,1	-
71	α-amorphene	1455	0,1	0,2
72	δ-cadinene	1458	0,2	0,3
73	L-calamenene	1460	-	0,2
74	*cis*-αbisabolene	1471	0,6	-
75	α-copaen-11-ol	1473	-	0,3
76	β-asarone	1475	0,2	0,3
77	Hedycaryol	1477	-	0,2
78	Elemol	1478	0,2	-
79	Corodane	1486	-	0,2
80	β-humulene	1491	0,1	-
81	Spathulenol	1497	1,8	5
82	Caryophyllene oxide	1500	1,4	6,1
83	Ledol	1505	0,7	1,8

(Contd.)

84	4-Bromo-1-naphthylamine	1517	0,3	-
85	γ-selinene	1521	0,3	0,4
86	Epiglobulol	1523	-	0,4
87	Alloaromadendrene	1525	1,1	0,9
88	Aromadendrene	1528	-	1,2
89	Caryophylladienol I	1530	0,5	2,1
90	*trans*-isoelemicin	1532	-	1,1
91	Thujopsene	1534	-	0,8
92	Valencene-1	1535	0,6	-
93	t-muurolol	1540	1,9	3,7
94	γ-gurjunene	1545	0,3	-
95	Aromadendrene epoxide	1547	0,5	-
96	2-hexyl-1-decen-3-yne	1558	-	0,5
97	1-amino-4-bromonaphtalene	1576	0,6	0,8
98	β-oplopenone	1592	-	0,1
99	(+)-β-costol	1597	-	0,2
100	(+)-eremophilene	1603	-	0,1
101	β-panasinsene	1604	0,1	-
102	3-ethyl-6(methoxycarbonyl)2-naphthol	1617	0,9	3,9
103	2-(4-methoxyphenyl)-N,N,2-trimethyl-1-pyrroline	1648	4,4	-
104	*n*-hexadecanoic acid	1691	0,1	0,2
105	Heptadecane	1901	0,1	-
106	17-pentatriacantone	1911	0,1	-
107	Cyclotetracosane	1944	0,1	-
	Total		95,8	86,7

RI: Retention indices.

On the other hand, isoeugenol (53.5, 57.0%) and linalool (42.61%) were the main compounds of EOs of *L. nobilis* leaves from Brazil and India. Furthermore, 1,8-cineole and α-pinene have been described as minor compounds in *L. nobilis* essential oils, which is in contrast with the results of the present study (Peixoto *et al.* 2017, Pinheiro *et al.* 2017, Choudhary *et al.* 2013, Chahal *et al.* 2017). In this study, the lowest concentration of 1,8-cineole was reported from Turkey (Karık *et al.* 2015). Although the major compounds in EO scan vary, several studies have shown that the major compounds of *L. nobilis* essential oil are mono- and sesquiterpene hydrocarbons (Basak and Candan 2013, Cherrat *et al.* 2014, Goudjil *et al.* 2015, Peixoto *et al.* 2017), which are in agreement with the present results. Saab *et al.* (2012) reported that, β-ocimene, 1,8-cineolẹ,α-pinene and β-pinene were the main compounds of seed essential oils of *L. nobilis* from Italy, in which 27 compounds were identified in the essential oil of the seeds (Saab *et al.* 2012). The present samples constituted 1,8-cineole and α-pinene as the major compounds, while β-ocimene and β-pinene were determined as minor compounds.

The yield and composition of essential oil varies with genetic and environmental factors, as well as developmental stage and extraction methods like steam distillation, hydro distillation and soxhlet extraction (Woolf 1999). Karık *et al.* (2015) reported 1,8-cineole as the first major compound of the EO of *L. nobilis* from five different locations of Sinop province. However, in this report, sabinene, α-pinene and α-terpinyl acetate were not determined in some of the samples of

L. nobilis leaves. Therefore, it may be suggested that seasonal variations affect the chemical profile of the EO.

The results obtained from this study represent that the EOs obtained in different phenological stages displays similar compositions. The main compounds of *L. nobilis* EO were determined as 1,8-cineole, trans-sabinene hydrate, α-terpinyl acetate, methyl eugenol, sabinene, eugenol, α-pinene and α-terpineol (Verdian-rizi 2009). A number of studies on the chemical composition of the EO obtained from different parts of *L. nobilis* harvested from various regions of Turkey were published. However, this study is the first one presenting the chemical composition of the EO of *L. nobilis* seeds from Black Sea region of Turkey.

In conclusion, it may be mentioned that EO composition of the members of *Laurus* displays similarity. The essential oil compositions of different parts of *Laurus* samples have also shown that they can be used as raw material for medicinal and pharmaceutical purposes and natural products.

Acknowledgments

This work was supported by Sinop University Scientific Research Coordination Unit. Project Number: 1901. 14-05, 2015.

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(Manuscript received on 5 may, 2018; revised on 20 May, 2018)