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Influence of Phenological Stages and Method of Distillation on Iranian Cultivated Bay Leaves Volatile Oil

Gh. Amin, M.H. Salehi Sourmaghi, S. Jaafari, R. Hadjagaee and A. Yazdinezhad
Department of Pharmacognosy and Medicinal Plants Research Center,
Faculty of Pharmacy, Medical Sciences/University of Tehran, Iran

Abstract: Leaves of Bay (*Laurus nobilis* L.) were collected in different phonological stages and air-dried. Volatile oil of the leaves were obtained using hydro- and steam distillation and the chemical composition were analyzed by GC and GC/Mass and identified in comparison with authentic compounds. The yield of essential oil were 0.8 to 1.5 v/w% and the major compounds were; 1,8 Cineol, alpha-terpinyl acetate and Sabinene. Because of the interesting yield of the oil and presence of 1,8-Cineol as the major compounds, the bearing ripe fruit stage in the mid of September is the best time for harvesting the Bay leaves in Iran.

Key words: Bay, laurel, *Laurus nobilis*, phenology, essential oil

INTRODUCTION

Laurus nobilis L. (Lauraceae) is an evergreen shrub up to 2-15 m height and commonly named as Bay or Laurel. Leaves are about 8-14 cm long and 2.5-4.5 cm wide, leathery and dark green with wavy margin (Ghahreman, 1997). Bay is native to Mediterranean region especially Italy and Greece and North America (Simpson and Ogorzaly, 2001; Riaz *et al*., 1989) and has cultivated as a beautiful evergreen plant over two hundred years ago in the gardens of Tehran and in the north part of Iran. The Bay leaves that is called *Barg-e bu* in Persian, is a commonly used herb in Iranian folk medicine as a diaphoretic, analgesic for kidney's pain also as a flavoring agent and spice (Amin, 2005). The Bay leaf oil is used as a cure for rheumatic pain, dermatitis antimicrobial and food preservative (Riaz *et al*., 1989; Kilik *et al*., 2004). According to the literatures, the chemical composition of the essential oil of Bay leaves belonging to the different regions and different seasons has been studied (Hokwerda *et al*., 1982; Muller-Riebau *et al*., 1997; Fiorini *et al*., 1997; Akgul *et al*., 1989; Riaz *et al*., 1989; Caredda *et al*., 2002; Kilic *et al*., 2004) and the results showed the relationship between the chemical components and the seasonal effects. As the leaves of Laurel is used in Iranian folk medicine and also in food industries as a flavoring agent and there is no data about the best time for harvesting based on influence of phenological stage and the method of distillation with the essential oil in Iran, so in this research the volatiles of the cultivated Bay in Iran, was studied using two methods of hydro-and steam distillation during the deferent seasonal stages.

MATERIALS AND METHODS

Plant material and essential oil: Leaves of the selected specimen of *Laurus nobilis* L. (Code No. 83412) cultivated in the Herboratum (Educational and Research Medicinal Plants Garden) Faculty of Pharmacy, Medical Sciences/ University of Tehran, were picked in several phenological stages; mid of March (before blossoming), first of July (Full flowering) and the mid of September (bearing ripe fruit), during the years of 2004-2006. The air dried leaves (100 g) were isolated by hydro-and steam distillation for 3 h, using a Clevenger and steam- type apparatus in thrice for each sample. The essential oil were dried over anhydrous sodium sulfate and stored in tightly closed dark containers at 4°C until analyzing time.

GC analysis: GC analysis was performed by using a thermoquest gas chromatography Shimadzu 9A, with a flame Ionization Detector (FID) and carried out using fused silica capillary DB-5 column (60 m × 0.25 mm i.d., film thickness 0.25 μm). The operating conditions were as follows: Injector and detector temperatures were 250 and 300°C, respectively. Nitrogen was used as carrier gas at a flow rate of 1 mL min^{-1}; oven temperature programmed 60-250°C at the rate of 5°C min^{-1} and finally held isothermally for 10 min.

Corresponding Author: Gh. Amin, Department of Pharmacognosy and Medicinal Plants Research Center,
Faculty of Pharmacy, Medical Sciences/University of Tehran, Iran

GC-MS analysis: GC-MS analysis was performed by using a thermoquest-finigan gas chromatograph Varian 3400, equipped with above mentioned column and coupled to trace Mass quadrupled detector. Helium was used as carrier gas with ionization voltage of 70 ev. Ion source and interface temperatures were 200 and 250°C, respectively. Mass range was form m/z 43-456. Gas chromatographic conditions were as given for GC.

Identification of compound: The chemical compounds of essential oil were identified by calculation of their retention indices under temperature-programmed conditions for *n-alkanes* (C8-C24) and the oil on a DB-5 column under the same chromatographic conditions. Identification of individual compounds was made by comparison of their Mass spectra with those of the internal reference Mass spectra library or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literatures (Bagheri *et al*., 2007; Iranshahi *et al*., 2006; Salehi Sourmaghi *et al*., 2006; Bazargani *et al*., 2006; Sarkhail *et al*., 2006; Amin *et al*., 2005; Adams, 2004; Kilic *et al*., 2004; Riaz *et al*., 1989; Akgul *et al*., 1989). For qualification purpose, relative area percentages obtained by FID were used without the use of correction factors.

RESULTS

The average yield of essential oil obtained with two methods, hydro-and steam distillation is shown in Table 1 and the identified compounds of essential oil with two methods of distillation showed 90 well known components (Table 2) equal to 98% of the total oil. Significant difference between the percentage of the main compounds in two methods of distillation and the phonological stages were detected and shown in Table 3.

Table 1: Total essential oil percentage (V/W) of Bay leaves cultivated in Iran, in different seasonal time, obtained with two methods of distillation

Methods	Before flowering stage	Full flowering stage	Mature fruiting stage
Hydro-distillation	0.8	1	1.5
Steam distillation	0.8	1	1.5

Table 2: Chemical composition of the essential oil of Bay leaves cultivated in Iran with two methods of distillation

No.	RT	RRI	Compound	(GC/MS) (%)
1	5.41	803	n-Hexenal	tr
2	7.06	852	E-2-Hexenal	tr
3	7.15	855	Z-3-Hexenol	tr
4	7.46	865	E-2-Hexenol	tr
5	7.52	867	1-Hexanol	tr
6	9.86	927	Tricyclene	tr
7	9.97	931	α-Thujene	0.13
8	10.37	941	α -Pinene	2.73
9	11.01	959	Camphene	0.27
10	**12.10**	**981**	**Sabinene**	**5.44**
11	12.31	986	β-Pinene	2.44
12	12.62	994	Myrcene	0.19
13	12.75	997	dehydro-1,8-Cineole	tr
14	13.41	1012	α-Phellandren	tr
15	13.69	1018	δ-3-Carene	0.19
16	13.98	1024	α-Terpinene	0.23
17	14.41	1030	ρ-Cymene	tr
18	**15.00**	**1050**	**1,8-Cineole**	**27.58**
19	15.29	1052	trans-β-Ocimen	tr
20	15.94	1066	γ-Terpinene	0.70
21	16.36	1075	Z-Sabinene hydrate	0.36
22	17.33	1096	Terpinolene	0.24
23	17.87	1107	Linalool	4.00
24	17.95	1110	E-Sabinene hydrate	tr
25	18.98	1130	cis –ρ-Menth-2-en-ol	0.38
26	19.17	1134	α-Campholenal	tr
27	19.59	1143	ρ-Z -Mentha-2,8-dien-1-ol	tr
28	19.66	1145	Unknown	0.18
29	19.94	1150	trans-Pinocarveol	0.43
30	20.11	1154	trans-Verbenol	tr
31	20.38	1160	Neroloxide	tr

Table 2: Continued

No.	RT	RRI	Compound	(GC/MS)(%)
32	20.52	1163	Unknown	0.24
33	21.01	1173	Pinocarvone	0.16
34	21.24	1178	Borneol	0.63
35	21.84	1191	1,4-Terpineol	4.20
36	22.01	1194	Thuj-3-en-10-al	tr
37	22.17	1200	ρ-E-Mentha-1(7)8-dien-2-ol	0.23
38	22.40	1202	α-Tepineol	1.64
39	22.65	1208	Myrtenol	0.45
40	23.06	1217	trans-Piperitol	tr
41	23.58	1228	trans-Carveol	tr
42	23.87	1234	Nerol (Geraniol)	tr
43	24.02	1238	ρ-Z-Mentha-1(7)8-dien-2-ol	0.25
44	24.13	1239	cis-Carveol	tr
45	24.64	1251	Cumin aldehyde	tr
46	24.78	1254	Carvone	tr
47	25.01	1259	Linalyl acetate	0.43
48	25.80	1270	Geranial	tr
49	26.31	1285	Phellandral (ρ-Menth-1-en-7-al)	tr
50	26.69	1295	Bornyl acetate	0.72
51	27.00	1302	Cymen-7-ol	tr
52	27.63	1316	Carvacrol	tr
53	28.10	1327	δ-Terpinyl acetate	1.79
54	**29.85**	**1366**	**α-Terpinyl acetate**	**20.62**
55	30.13	1373	Eugenol	2.64
56	30.76	1387	Neryl acetate	tr
57	30.95	1391	α-Copaene	tr
58	31.42	1402	β-Borbonene	tr
59	31.56	1405	β-Elemene	0.24
60	**32.07**	**1417**	**Methyl eugenol**	**9.72**
61	33.03	1420	β-Caryophyllene	1.35
62	33.58	1453	α-Guaiene	tr
63	33.81	1458	Aromadendrene	tr
64	34.43	1473	α-Humulene	0.21
65	34.73	1480	allo-Aromadendrene	tr
66	35.56	1499	Germacrene-D	0.75
67	35.78	1504	trans- Methyl isoeugenol	0.26
68	36.02	1511	epi-Cubebol	tr
69	36.14	1514	Bicyclogermacrene	0.24
70	36.46	1522	δ-Amorphene	tr
71	36.82	1531	γ-Cadinene	tr
72	37.08	1538	δ-Cadinene	0.25
73	37.48	1548	10- epi-Cubebol	tr
74	37.69	1553	cis-α-Bisabolene	0.82
75	38.11	1562	Elemicine	0.40
76	39.42	1597	Germacrene-D-4-ol	0.41
77	39.54	1598	Spathulenol	tr
78	39.84	1607	Caryophyllene oxide	2.29
79	40.13	1615	Viridiflorol	0.23
80	40.71	1630	β-Oplopenone	tr
81	40.78	1632	Humulene oxide II	tr
82	41.70	1659	Unknown	0.52
83	41.85	1661	Caryophylla-4(14),8(15)dien-ol	1.16
84	42.41	1672	β-Eudesmol	0.70
85	42.56	1679	Unknown	0.31
86	42.76	1682	Selin-11-en-4-alpha-ol	tr
87	43.06	1692	Unknown	0.26
88	43.67	1708	Unknown	0.34
89	47.02	1797	α-Eudesmol acetate	tr
90	47.38	1802	Octadecane	tr
___	___	___	Total percentage of non-oxygenated monoterpens	12.56
___	___	___	Total percentage of oxygenated monoterpens	76.89
___	___	___	Total percentage of non-oxygenated sesquiterpens	3.86
___	___	___	Total percentage of oxygenated sesquiterpens	4.79
___	___	___	Total percentage of identified compounds	98.01

RT: Retention time, RRI: Relative Retention indices, tr: Trace, % (GC/MS) : Percentage according to GC/MS spectrum

Table 3: Percentage of major essential oil compounds of Iranian cultivated Bay leaves in different seasonal times and with two method of distillation, based on GC data.

No.	Compound	H1	S1	HF2	SF2	HD2	SD2	H3	S3
1	α-Pinene	4.67	5.63	3.99	4.04	5.10	5.33	2.35	4.42
2	Camphene	tr	tr	0.42	tr	0.53	tr	0.27	0.45
3	Sabinene β-Pinene	11.20	11.88	11.36	12.32	12.68	13.42	9.43	12.81
4	Myrcene	0.44	0.73	0.50	0.14	0.54	tr	0.43	0.68
5	**1,8-Cineole**	47.53	33.37	49.44	51.57	53.46	53.85	**58.12**	51.03
6	γ-Terpinene	1.20	0.28	0.73	0.64	0.88	0.94	0.32	0.68
7	Z-Sabinene hydrate	0.27	0.18	0.59	0.39	0.53	0.39	0.59	0.51
8	Linalool	3.10	3.96	2.79	2.57	2.49	2.26	5.84	3.66
9	cis –ρ-Menth-2-en-o	l0.39	0.12	0.39	0.28	0.30	0.27	0.28	0.23
10	trans-Pinocarveol	0.52	0.98	0.56	0.56	0.51	0.49	0.53	0.40
11	Pinocarvone	tr	tr	0.23	0.25	0.46	0.47	0.52	0.35
12	δ-Terpineol	0.34	tr	0.31	0.31	0.34	0.30	0.31	0.34
13	1,4-Terpineol	3.47	0.95	3.25	3.06	2.70	2.83	2.32	2.29
14	α-Tepineol	1.73	0.69	1.75	1.28	1.25	1.26	1.27	1.18
15	Linalyl acetate	0.25	0.34	0.37	0.25	tr	0.22	0.12	0.44
16	Bornyl acetate	0.42	1.15	0.36	0.26	0.79	0.25	0.20	0.26
17	δ-Terpinyl acetate	0.96	0.58	1.00	0.97	0.97	0.82	0.77	0.82
18	α-Terpinyl acetate	12.49	10.01	10.70	10.66	6.98	9.57	9.72	10.78
19	Eugenol	0.44	2.74	1.51	1.28	1.25	1.02	1.21	1.72
20	Methyl eugenol	4.69	6.51	4.40	4.48	3.60	3.08	4.08	4.32
21	Elemicine	0.54	tr	0.11	0.12	0.00	0.07	0.10	tr
22	Caryophyllene oxide	1.22	1.97	1.38	1.06	0.75	0.62	0.55	0.97
23	β-Eudesmol	0.93	1.82	0.79	0.49	0.32	0.33	0.28	0.52
___	Total percentage	96.80	83.89	96.93	96.98	96.40	98.09	99.61	98.87

H: Hydro-distillation, S: Steam distillation, D: Dried leaves, F: Fresh leaves, 1: Before blossoming, 2: Full flowering, 3: Bearing ripe fruit, tr: Trace

DISCUSSION

Present investigation showed that there is a interesting increasing of the yield of the oil (up to 1.5 equal) for Iranian cultivated Bay in comparison with the yield of Bay in other countries (Riaz *et al*., 1989; Kilic *et al*., 2004). The maximum rang of the oil was in full-flowering (1.5%) and will going decreased in fruiting time (1%) then goes to the minimum (0.8%) in before blossoming, but there was no significant difference between to method of distillation (Table 1). This investigation allowed the identification of 90 chemical components (Table 2) that are the same in the literature data (Kilic *et al*., 2004; Caredda *et al*., 2002; Muller-Riebau *et al*., 1997; Fiorini *et al*., 1997; Riaz *et al*., 1989; Hokwerda *et al*., 1982; Akgul *et al*., 1989). Comparison of the percentage of 28 major compounds of the different seasoning samples, on the basis of GC data (Table 3) showed that there is a significant difference between two methods of distillation especially for the major compounds, 1,8-Cineol (58.12% for hydro- and 53.85% for steam distillation). However these results were the same as before studies, but the maximum percent of 1,8-Cineol (42.24%) that is reported from Lahore (Riaz *et al*., 1989) was about 58.12% in our investigation with hydro-distillation method.

It is mentioned that the major compound, 1,8-Cineol, will increased during the vegetative stage and it will be in most percentage with the full flowering time (Kilic *et al*., 2004; Muller-Riebau *et al*., 1997; Riaz *et al*., 1989) and this subject was the same rolled for our study and was more over for the hydro-distillation.

Some researcher noted that the alpha terpinyl acetate will decrease and eugenol will increased during the leaves drying processing (Diaz-Maroto *et al*., 2002), it was the same for alpha terpinyl acetate but it was not significant for eugenol.

The increasing of mixed compounds, sabinene + beta pinene and also linalool and decreasing of the alpha pinene during the phenological stages, was interesting.

According to a reference data (Dictionary of Food, 2005) the Safrol (toxic compound of the oil) should be absence in the obtained essential oil of bay leaves and according to present study it is unidentified in the Bay leaves of Iran.

In advanced present study declare that because of the total percent of the oil and the presence of the chief compounds specially 1,8-Cineol, Sabinene+beta Pinene and Linalool in the matured fruiting time that is occur in the mid of September, it is the best time for harvesting the Bay leaves in Iran.

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