VARIATION IN THE ESSENTIAL OIL COMPOSITION OF *LAURUS NOBILIS L.* OF DIFFERENT GROWTH STAGES CULTIVATED IN IRAN

Mohammadreza Verdian-rizi*
Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences,
Tehran, Iran

ABSTRACT

The chemical variations of the essential oil from the aerial parts of *Laurus nobilis* L.(Lauraceae) have been studied. Plant material has been harvested at each phenological status (vegetative, before anthesis, full flowering and seed-bearing). The oils were obtained by hydro distillation of the air-dried samples. Analysis by GC and GC/MS of the essential oils have allowed to identify 49 components. The main components were 1,8-cineole, *trans*-sabinene hydrate, α-terpinyl acetate, methyl eugenol, sabinene, eugenol and α-Pinene.

Keywords: *Laurus nobilis* L., Lauraceae, 1,8-Cineole**,** Essential oil.

INTRODUCTION

Laurus nobilis L. (Lauraceae) is an evergreen shrub indigenous to the south parts of Europe and Mediterranean area. This plant is cultivated in the north of Iran (Zargari, 1990). In Iranian folk medicine, the leaves of this plant have been used to treat epilepsy (Aqili khorasani, 1992; Zargari, 1990), neuralgia and parkinsonism (Aqili khorasani, 1992). The essential oil obtained from the leaves of this plant has been used for relieving hemorrhoid and rheumatic pains (Zargari, 1990). It also has diuretic (Aqili khorasani, 1992; Zargari, 1990), antifungal (Qamar and Chaudhary, 1991) and antibacterial (Seyed *et al*., 1991) activities.

There are many studies on chemical composition of the essential oil obtained from the leaves of Mediterranean and European *L. nobilis* (Riaz *et al*., 1989; Lin *et al*., 1990; Baghdadi *et al*., 1993; Putievsky *et al*., 1994; Fiorini *et al*., 1997). In the study of Riaz *et al*., the main components of the essential oil were cineol (44.12%), eugenol (15.16%), sabinene (6.20%), 4-terpineol (3.60%), a-pinene (2.74%), methyleugenol (2.48%), a-terpineol (2.19%) and b-pinene (2.05%) (Riaz *et al*., 1989). Pharmacological studies have demonstrated the anesthetic, hypothermic, muscle relaxant and anticonvulsant activity of eugenol and methyleugenol (Dallmeier and Carlini, 1981) and also anti-stress effect of eugenol (Sen *et al*., 1992). Furthermore, some analogs of a-pinene prevent the audiogenic seizures in susceptible rats (Consroe *et al*., 1981).

To the best of our knowledge, an investigation of the essential oil of *Laurus nobilis* L. from Iran has not been reported to date. As a part of our studies on the chemical composition of the essential oils and screening program for bioactive compounds from plants that grow in Iran, the present study describes the essential oil composition of aerial parts of *Laurus nobilis* L. in different growth stages. The plant was analyzed during three different stages of their development.

*****Corresponding author: e-mail: mverdian@razi.tums.ac.ir

MATERIALS AND METHODS

Plant materials

The aerial parts of *Laurus nobilis* L. were collected at four different stages of development respectively in May (vegetative plants) August (before anthesis), September (full flowering plants) and November (seed-bearing plants) of 2006 from Tabriz, northwest of Iran. A voucher specimen was deposited at the Herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences.

All of the solvents applied were of pro-analysis purity and were purchased from Fluka Chemical Co. (Buchs, Switzerland). Anhydrous sodium sulphate was obtained from Merck (Darmstadt, Germany).

Isolation of the essential oil

The aerial parts (100 g) were dried at 25°C in the shade and subjected to hydro distillation, using a Clevenger-type apparatus for 4 h. The oil was dried with anhydrous sodium sulphate, weighed and stored at 4–6°C in dark until use.

Gas chromatography/ mass spectrometry (GC-MS)

A Hewlett-Packard 6890 gas chromatography was used, with HP-5 capillary column (phenyl methyl siloxane of 25m length, 0.25 mm i.d., and 0.25 μm film thickness). Carrier gas was He; split ratio was 1:25, and the detector was flame ionization. Temperature program was 60°C (2 min) rising to 240°C at 4°C/min; injector temperature was 250°C, and detector temperature was 260°C. GC–MS was a Hewlett-Packard 6859 equipped with a quadruple detector, on a HP-5 column (see GC), operating at 70 eV ionization energy, using the same temperature program

Table 1. Chemical composition of *Laurus nobilis* L. essential oil.

Identifiedcompounds	RI	Phenological stage			
		Vegetative	Buds	Flowering	Seeding
α-Thujene	933	0.4	1.0	0.3	0.3
α-Pinene	941	3.2	2.6	2.6	2.7
Camphene	955	0.3	0.3	0.3	0.3
Sabinene	977	6.5	6.0	5.8	5.9
β-Pinene	981	2.9	2.5	2.4	2.5
Myrcene	992	1.1	0.9	0.8	1.0
3-Carene	1013	0.5	0.4	0.4	0.5
α-Terpinene	1020	0.2	0.2	0.2	0.2
p-Cymene	1028	0.2	0.2	0.2	0.2
Limonene	1032	1.3	1.4	1.3	1.3
1,8-Cineole	1035	35.7	34.9	31.4	35.7
γ-Terpinene	1064	0.4	0.4	0.3	0.4
cis-Sabinene hydrate	1070	0.6	0.6	0.6	0.6
Terpinolene	1089	0.2	0.2	0.2	0.2
trans-Sabinene hydrate	1100	9.7	11.9	9.8	11.4
δ-Terpineol	1166	–	0.4	0.6	0.4
Borneol	1167	–	0.2	–	0.2
4-Terpineol	1180	1.4	1.6	1.6	1.5
α-Terpineol	1190	2.8	3.2	3.3	3.0
Nerol	1227	0.2	0.3	0.3	0.2
Linalyl acetate	1258	0.3	0.4	0.4	0.4
Isobornyl acetate	1286	0.3	0.4	0.4	0.4
Terpinen-4-yl acetate	1291	0.1	0.2	0.2	0.2
α-Terpinyl acetate	1351	9.3	12.1	11.4	10.4
Eugenol	1356	4.8	3.8	5.5	4.3
β-Elemene	1390	0.1	–	–	–
Methyl eugenol	1404	6.8	8.1	9.4	7.9
β-Caryophyllene	1420	0.6	0.4	0.5	0.5
Valencene	1492	0.3	0.3	0.4	0.3
β-Bisabolene	1509	0.2	–	0.2	0.2
Elemicin	1554	0.6	0.5	0.7	0.5
Spathulenol	1577	0.6	–	–	–
Germacrene D	1574	1.2	1.2	1.6	1.2
Caryophyllene oxide	1581	0.2	0.7	0.8	0.7
Viridiflorol	1590	0.3	0.2	0.3	0.2
γ-Eudesmol	1630	0.2	0.1	0.2	0.2
Bisabolol	1637	0.2	0.2	0.2	0.2
β-Eudesmol	1647	0.5	0.5	0.5	0.5
α-Cadinol	1652	1.2	0.8	1.3	1.0
Total identified		95.8	98.8	95.5	97.3

and carrier gas as above. Retention indices were calculated by using retention times of C8-C22 that were injected after the oil at the same chromatographic conditions according to Van Den Dool method (Van Den Dool *et al.,* 1963).

Identification of components

The linear retention indices for all the compounds were determined by coinjection of the sample with a solution containing the homologous series of C8–C22 *n*-alkanes. The individual constituents were identified by their identical retention indices, referring to known compounds from the literature (Adams, 1995) and also by comparing their mass spectra with either the known compounds or with the Wiley mass spectral database.

RESULTS AND DISCUSSION

The essential oil content of aerial parts of *Laurus nobilis* L. obtained by hydro distillation, were 0.784%, 0.813%,

Table 2. Percentages of main chemical classes of volatiles.

Chemical classes	Phonological stage			
	Vegetative	Bud	Flowering	Seed-bearing
Oxygenated monoterpens	60.43	66.19	59.87	64.32
Monoterpene hydrocarbons	17.37	16.11	14.82	15.35
Phenylpropanoids	1.24	12.40	15.58	12.67
Sesquiterpene hydrocarbons	1.22	0.66	1.06	1.04
Oxygenated sesquiterpenes	4.76	3.60	4.38	3.94

1.132% and 0.654% in vegetative, bud, flowering and seed-bearing stages respectively, calculated on dry weight basis. The components of the essential oils are reported in Table 1. Thirty-seven components accounting for 95.8% of the total composition were identified in thevegetative stage. The major constituents of this oil were 1,8-cineole (35.7%), *trans*-sabinene hydrate (9.7%), α-terpinyl acetate (9.3%), methyl eugenol (6.8%), sabinene (6.5%) and eugenol (4.8%). In the volatile of bud stage, thirty-six compounds amounting 98.8% of total components were identified which included 1,8-cineole (34.9%), α-terpinyl acetate (12.1%), *trans*-sabinene hydrate (11.9%), methyl eugenol (8.1%), sabinene (6.0%) and eugenol (3.8%) as main components. In the oil obtained from flowering stage, thirty-six components were characterized, which represented about 95.5 % of the total composition. 1,8-Cineole (31.4%), α-terpinyl acetate (11.4%), *trans*-sabinene hydrate (9.8%), methyl eugenol (9.4%), sabinene (5.8%) and eugenol (5.5%) were the principal components of this oil. In the seed-bearing stage oil thirty-seven constituents accounting for 97.3% of the total oil were characterized that included 1,8-cineole (35.7%), *trans*-sabinene hydrate (11.4%), α-terpinyl acetate (10.4%), methyl eugenol (7.9%), sabinene (5.9%) and eugenol (4.3%) as main components. The majority of identified compounds belong to the monoterpene fraction (Table 2), with percentages ranging from 77.80% in the vegetative stage, to 72.30% before anthesis, 74.69% in the flowering stage and 79.67% in the seed-bearing stage. The oxygenated fraction is mainly composed of monoterpenes and bud stage oil has the highest percentage of oxygenated monoterpenes.

The results obtained from this study shows that the oils obtained from the different phenological stages were found to have similar compositions, the main compounds were 1,8-cineole, *trans*-sabinene hydrate, α-terpinyl acetate, methyl eugenol, sabinene, eugenol, α-Pinene and α-Terpineol. Thus the time of harvesting of this plant does not have a major effect on chemical composition of the essential oil but it effects on the essential oil content of the plant and the flowering stage is the best time for harvesting the plant and obtaining the essential oil because at this time the plant contains highest percent of the essential oil.

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