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Full Length Research Paper

Comparative survey on the essential oil composition from the leaves and flowers of *Laurus nobilis* L. from Kerman province

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The chemical composition of the essential oils of the leaves and the flowers of *Laurus nobilis* L. (Lauraceae) from Kerman province, Iran were obtained by hydrodistillation method and analyzed by gas chromatography and gas chromatography mass spectrometry. Thirty-three compounds, accounting for 95.75% of the total oil with 1.8% oil yield were identified in the essential oil of the leaves. The major components were 1,8-cineole (25.7%), Sabinene (8.7%) and α-pinene (5.25%). Twenty-seven compounds, accounting for 87.36% of the total oil with 1.2% oil yield were identified in the essential oil of the flowers. The major components were 1,8-cineole (18.69%), β-elemene (8.87%), Sabinene (7.93%), α-pinene (7.38%) and β-Caryophyllene (5.35%).

Key words: *Laurus nobilis* L., essential oil, medicinal properties, analytical chemistry.

INTRODUCTION

The sweet bay or bay laurel is (*Laurus nobilis* L.) an evergreen shrub. This ornamental tree is indigenous to the Mediterranean area and southeast part of Europe and cultivated in the north of Iran. In Iranian folk medicine, the essential oil obtained from *L. nobilis* L. has been used for rheumatic pains, treatment of epilepsy, neuralgia, Parkinsonism and muscular convulsion. Laurel is usually considered as the natural source of this compound, used in the flavor, cosmetic and fragrance industries. The essential oil obtained from the leaves of this plant has been used for relieving haemorrhoid and rheumatic pains. It also has diuretic, antifungal and antibacterial activities (Zargari, 1995). The plant is the source of several popular spices used in a wide variety of recipes, particularly among Mediterranean cuisines. Most commonly, the aromatic leaves are used, fresh or dried. For cooking purposes, whole bay leaves have a long shelf life of about one year, under normal temperature and humidity. Bay leaves are used almost exclusively as flavor agents during the food preparation stage; even when cooked, whole bay leaves can be sharp and abrasive enough to damage internal organs, so they are typically removed from dishes before serving, unless used as a simple garnish. Bay laurel is a pyramid-shaped tree or large shrub with aromatic, evergreen leaves and shiny gray bark. It can reach 60 ft (18.3 m) in height in its native range, but generally is much smaller, 3-10 ft (0.9-3.1 m) tall. In culture Bay laurel sometimes produces suckers from the base. The leaves are elliptic, 3-4 in (7.6-10.2 cm) long, rather thick and leathery, and shiny dark green. Clusters of small yellow flowers are produced in spring, followed, on the female plants, by shiny black or purple berries about 0.5 in (1.3 cm) long. The laurel is dioecious (unisexual), with male and female flowers on separate plants (Green, 2006). The essential oil of *L. nobilis* L. plant has been widely studied in Iran and other countries but the chemical composition of the essential oil of *Laurus nobilis* grown in Kerman province is yet to be determined. In the present work we have analyzed the chemical composition of the leaves and the flowers of *L.*

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nobilis L. that grow in Kerman province in Iran and then the results were compared with various origins in other countries.

MATERIALS AND METHODS

Plant material collection and isolation of their essential oil

The leaves and flowers of *L. nobilis* L. plant were obtained from plants grown in a village in Kerman province, Iran at full flowering stage in April 2011. The samples were cleaned in shade condition to prevent volatility of the plant material constituents and to keep the natural color of the sample fixed. Then they were air- dried and were powdered using a milling machine and kept in a cool dry place until ready for extraction of the essential oil. Afterwards, essential oil was taken from 150 g of the powdered sample in hydrodistillation method with the help of Clevenger set for three hours. Following the sample oils were dried with anhydrous sodium sulfate and kept in sterile sample tubes in refrigerator. The oil yields from leaves were calculated.

Analysis of essential oil

Gas chromatography

GC analysis was performed using a model HP-439 gas chromatograph equipped with column CP Sil. 5CB in 25 m length, internal diameter of 0.25 mm and film thickness o.39 µm. Oven temperatures was from 60 to 220°C at a rate of 7°C slope per minute. Injector temperature was 280°C and detector (FID) temperature was 270°C and carrier gas was helium.

Gas chromatography/mass mass spectrometry

In order to analyze and identify the combinations forming the essential oil, the chromatograph gas set attached to a mass spectrometry, Model Hewlett Packard-5973 was used. The conditions of analysis and specifications of the GC/MC set were as follows: Capillary column HP 5MS in 60 m length, internal diameter of 0.25 mm and layer thickness of 0.25 µm, thermal program of oven (3 min) in 60°C, then 60 to 220°C with a 6°C slope per minute, then 3 min in 220°C, the temperature of place of injection 280°C, gas conveying helium, the speed of gas move 1.0 milliliter per minute, the ratio of fission 1 to 43, the rate of injection 0.1 µl, temperature of the reservoir of ionization 230°C, ionization mode EI, Ionization energy 70eV. The series of normal Alkans C8-C17 were also injected to the set under the same condition with that of essential oil injection to calculate restrictive index (RI) of components of essential oil. The Restrictive Index of components of the sample was calculated by using a computerized program. Finally, the components of essential oil was identified by comparing the mass spectrums obtained with the existing standard mass spectrums at electronic library of Wiley 2000 existing in Absolution software of GC/Ms set and calculation of standard restrictive index in accordance with C_8-C_{17} Alkans and comparing them with the existing standard figurers in references (Adams, 2001).

RESULTS AND DISCUSSION

The identified combinations in essential oil, RI, and quantitative percentage of the compounds from leaves and flowers are listed in Table 1. A total of 33 compounds were identified in the essential oil from the leaves of the plant with a total of 95.75%. The combinations consist of 1,8-cineole (25.7%), Sabinene (8.7%) and α-pinene (5.25%) with 39.65% constituting the highest percentage of essential oil. Also from twenty-seven compounds have been identified in the essential oil from flowers of this plant with 87.36%, the combinations of 1,8-cineole (18.69%), β-elemene (8.87%), Sabinene (7.93%), α-pinene (7.38%) and β-Caryophyllene (5.35%) with 48.22% constitute the highest percentage of essential oil. The quality and quantity of the materials forming *L. nobilis* L. essential oil had some differences and similarities with the cases reported in other regions. The studies of the ingredients of the essential oil of botanical populations with ecological and genetic differences can be of great importance in identifying the variety of essential oil inside the population of specie. It seems that the geographical origin of *L. nobilis* L. greatly influences the oil quality. The essential oil of *L. nobilis* L. plant has been widely studied in Iran and other countries but the chemical composition of the essential oil of *L. nobilis* grown in Kerman province is yet to be determined. In present study, results showed the major oil constituents of the leaves and the flowers of *L. nobilis* L. from Kerman province, Iran were 1,8-cineole and Sabinene. It has been reported as the main constituents in many other countries (Ozcan and Chalchat, 2005; Derwich et al., 2009) but in this study, in addition to α-pinene and β-elemene, we also identified β-caryophyllene and β-pinene as the major compound. The results of this study can be interested for further phytochemical and biological investigation of *L. nobilis* L. taking into account that 1,8-cineole oil showed marked antimicrobial activity.

In comparison by other studies, it is reported that the yield of essential oil of *L. nobilis* L. studied in Iran, was 0.8 to 1.5 v/w% and the major compounds were; 1,8-cineol, α-terpinyl acetate and Sabinene (Amin et al., 2007). Previous research studies showed that the main components of aerial parts of *L. nobilis* L. by phenological variation were 1,8-cineole, *trans*-sabinene hydrate, α-terpinyl acetate, methyl eugenol, sabinene, eugenol and α-pinene (Verdian-rizi, 2008). Verdian-rizi (2009) reported that the chemical composition of the essential oil in different growth stages cultivated obtained from the aerial parts of *L. nobilis* L. 1,8-cineole was the major component in the oil together with β-terpinyl acetate, terpinene-4-ol, α-pinene, β-pinene, *P*-cymene, linalool and terpinene-4-ylacetate (Verdian-rizi, 2009). It has been reported the oil yields from leaves of *L. nobilis* were collected from Botanical Garden of Noshahr and National Botanical Garden of Iran in Tehran Iran for stem, leaf and fruit were 0.22, 0.43 and 1.35% respectively (for Noshahr samples) and 3.86% for the leaves of Tehran sample. Twenty -four, 27, 37 and 20 components were identified in the essential oils of stem, leaf, fruit (for Noshahr) and leaf (for Tehran) of *L. nobilis* (representing 95, 98.5, 95.6 and 98.4% of the oils), respectively. Also it reported that

Table 1. Combinations identified in the essential oil of *L. nobilis* L. from Kerman.

Compound name	(RI)	Leaf (%)	Flower (%)
(E)-2-hexenal	845	1.32	-
Tricyclene	925	2.27	0.15
α-thujene	928	0.38	1.97
α-pinene	937	5.25	7.38
Camphene	958	3.86	2.46
Sabinene	983	8.7	7.93
β-pinene	1005	3.99	3.8
Myrcene	1018	1.68	1.65
α-phellandrene	1022	0.37	0.53
α-terpinene	1025	2.12	-
P-cymene	1029	0.31	0.31
Limonene	1032	3.47	2.47
1,8-cineole	1037	25.7	18.69
phenylacetaldehde	1043	-	1.79
γ-terpinene	1068	3.48	-
Terpinolene	1092	0.22	0.22
Linalool	1123	1.56	2.9
Sabinol	1138	2.45	-
Borneol	1154	2.37	1.57
δ-terpineol	1175	0.19	2.52
Terpinene-4-ol	1192	1.21	-
α-terpineol	1201	3.79	1.79
Linalyl acetate	1256	-	1.26
Bornyl acetate	1257	1.79	1.98
γ-terpinyl acetate	1321	0.24	2.41
Eugenol	1368	1.69	2.33
α -terpinyl acetate	1386	-	1.97
β -elemene	1395	2.30	8.87
β-caryophyllene	1425	0.87	5.35
α-humulene	1476	2.19	1.43
Germacrene A	1498	1.53	-
γ-cadinene	1514	2.68	0.68
Germacrene D-4-ol	1549	1.59	2.95
Spathulenol	1573	3.38	-
Caryophyllene oxide	1589	0.58	-
Humulene epoxid-2	1597	2.22	-
Total		95.75	87.36

The indexes of restrictive have been calculated by injecting the mixture of normal hydrocarbons (C_8-C_{17}) to HP-5MS column,

the main components in the stem oil were 1,8-cineole (42.9%), α-terpinyl acetate (16.8%) and sabinene (4.7%) and main compounds in leaf oil were 1,8-cineole (58.2%), α-terpinyl acetate (10%) and sabinene (7.2%) and main compounds in leaf oil in Tehran sample were 1,8-cineole (47%), sabinene (13.9%) and a-terpinyl acetate (11.5%). The major components in fruit oil were E-β-ocimene (20.8%), 1,8-cineole (14.4%), α-terpinyl acetate (8.5%), germacrene B (7.8%), α-pinene (6.6%), germacrene D (6%), sabinene (5.4%) and β-pinene (5.1%) (Naderi-Hajibaghercandi et al., 2009). In other studies in the world reported that the oil yields of essential oil components of *L. nobilis* L. from Turkey were between 1.4 to 2.6%. in this research main component was 1,8-cineole (51.73 to 68.48%); and other predominant components were α-terpinyl acetate (4.04 to 9.87%), sabinene (4.44 to 7.75%), α-pinene (2.93 to 4.89%), β-pinene (2.58 to 3.91%), terpinene-4-ol (1.33 to 3.24%), and α-terpineol (0.95 to 3.05%) (Ozcan and Chalchat, 2005). In a research in Tunisia yields essential oil of stems, leaves, buds and flowers of *L. nobilis* L. were between 0.4 and 1.1%. The component identified were 1,8-cineole, α-

terpinyl acetate, methyl eugenol, eugenol and linalool (Marzouki et al., 2009). In a research, α-Pinene, β-Pinene, α-Phellandrene, 1,8-cineole and trans-β-osimen have been reported major components of fruits of *L. nobilis* L. harvested from Antakya, Yayladagi and Samandagi and trans-β-osimen was the major component of fruits essential oil of Samandagi (28.35%) (Sangun et al., 2006). The chemical composition of the essential oil from the leaves of the *L. nobilis* L. were monocyclic monoterpenes such as 1,8-cineole (58.59%), α-terpinyl acetate (8.82%), and terpinene-4-ol (4.25%) the main components (Yalcin et al., 2007). In a report chemical composition of *L. nobilis* L. by supercritical carbon dioxide extraction were 1,8-cineole (22.8%), linalool (12.5%), α-terpinyl acetate (11.4%), and methyleugenol (8.1%) (Caredda et al., 2002). In a study in France the composition of the essential oil of flowers *L. nolilis* L. were β-caryophyllene (10.0%), viridiflorene (12.2%), germacradienol (10.1%), β-elemene (9.7%) and (E)-ocimene (8.0%) (Fiorini et al., 1997). In a research in 2004 the main components of *L. nolilis* L. reported α-eudesmol, β-elemene, and β-caryophyllene in flowers, (*E*)-β-ocimene and biclyclogermacrene in fruits (*E*)-β-ocimene and germacrene D in buds (Kilic et al., 2004). In other study the major component of *L. nobilis* L. were 1,8-cineole (52.43%), α-terpinyl acetate (8.96%), sabinene (6.13%), Limonene (5.25%), α-pinene (3.72%), linalool (3.14%), terpinene-4-ol (2.56%), α-terpinene (2.12%), α-pinene (1.98%), α-terpineol (1.56%), bornyl acetate (1.89%), α-phellandrene (1.28%), myrcene (1.13%), camphene (1.05%), *P*-cymene (0.94%), α-terpinene (0.98%) and eugenol (0.56%) (Derwich et al., 2009).

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