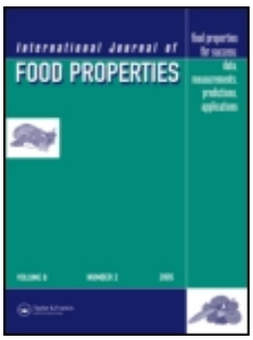

International Journal of Food Properties

ISSN: 1094-2912 (Print) 1532-2386 (Online) Journal homepage: https://www.tandfonline.com/loi/ljfp20

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To cite this article: Irene Peris & María Amparo Blázquez (2015) Comparative GC-MS Analysis of Bay Leaf (*Laurus nobilis* L.) Essential Oils in Commercial Samples, International Journal of Food Properties, 18:4, 757-762, DOI: 10.1080/10942912.2014.906451

To link to this article: https://doi.org/10.1080/10942912.2014.906451

International Journal of Food Properties, 18:757–762, 2015

ISSN: 1094-2912 print/1532-2386 online
DOI: 10.1080/10942912.2014.906451

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Comparative GC-MS Analysis of Bay Leaf (*Laurus nobilis* L.) Essential Oils in Commercial Samples

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Chemical composition of *Laurus nobilis* essential oils traded as spice and medicinal items was analyzed by gas chromatography-mass spectrometry. Sixty-four compounds accounting between 91–99% of the total oil was identified. Qualitative and quantitative differences were found among essential oils obtained from bay leaves used both for cooking and medicinal purposes. The oxygenated compounds were the principal fraction in all analyzed oils and consisted in oxygenated monoterpenes (73.13%), in medicinal essential oil and oxygenated monoterpenes (37.60 and 29.82%), oxygenated sesquiterpenes (15.98 and 22.99%), and phenylpropanoids (24.78 and 26.33%), respectively, in commercial food items. A high content of methyl eugenol (19 ± 4%; 21 ± 1%) and α-terpinyl acetate (18 ± 5%; 17 ± 7%) was found in commercial food items, whereas 1,8-cineole (51%) and α-terpinyl acetate (10%) were the main compounds in commercial pharmaceutical items.

Keywords: *Laurus nobilis* L, Essential oil, Bay leaf, 1,8-cineole, Lauraceae.

INTRODUCTION

Laurus nobilis L. (Lauraceae) is an evergreen tree, growing up to ten meters high widespread in the Mediterranean area, and widely cultivated in many countries with moderate and subtropical climate (Turkey, Algeria, Morocco, Portugal, Spain, Italy, France, or Mexico), mainly for the commercial value of its aromatic leaves.[1] It is well known as Bay, Bay Laurel, Sweet Bay, True Bay, Roman Laurel, Grecian Laurel, or Mediterranean Bay. The leaves around 5–10 cm long and 2–5 cm broad are leathery, elliptic-lanceolate and wavy at the margins. Dried bay leaves are mainly used as a spice, improve flavor for soups, meats, fish, vinegars, and beverages and has been an important part of the Mediterranean diet. Commercial essential oils generally obtained by hydrodistillation or steam distillation[2] are commonly employed by the pharmaceutical and food industries.

The bay leaf stimulates digestive functions, having also gastroprotective,[3] antidiarrheal,[4] antibacterial,[5] and antioxidant[6,7] properties. It also improves glucose and lipid profiles reduces serum glucose, total cholesterol, LDL cholesterol, and triglycerides profiles, and increases the HDL cholesterol levels of people suffering from type 2 diabetes.[8] On the other hand the high content of 1,8-cineol[9] or methyl eugenol[10] in bay leaf essential oil contributes to the antibacterial

Received 24 October 2013; accepted 17 March 2014
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activity against *Staphylococcus aureus, Staphylococcus intermedius, Klebsiella neumonia*,[11] as well as against *Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella typhimurium,* and *Staphylococcus aureus,* all responsible for foodborne diseases.[12] In addition it shows selective antiproliferative activity against cell line K562, the human chronic myelogenous leukaemia cells[13] without effect against cell line MCF7 the human breast adenocarcinoma cells.[14] As the pharmacological activity is correlated with the qualitative and quantitative composition of the essential oils, the aim of this study was to analyze the essential oils obtained by hydrodistillation from dried bay leaf traded for cooking and to compare them with the essential oil of *Laurus nobilis* L. traded for medicinal use.

MATERIALS AND METHODS

Plant Material

Two commercial dried bay leaf (6 samples of 100 g each) traded as spice were subjected to hydrodistillation for 3 h in a Clevenger-type apparatus. The essential oils were dried over anhydrous sodium sulphate and stored at 4°C until a gas chromatographic-mass spectrometry (GC-MS) analysis. A commercial sample of *Laurus nobilis* essential oil sold for medicinal use was also analyzed.

GC-MS Analysis

GC-MS analysis was carried out with a 5973N Agilent apparatus, equipped with a capillary column (95 dimethylpolysiloxane-5% diphenyl), Agilent HP-5MS UI (30 m long and 0.25 mm i.d. with 0.25 μm film thickness). The column temperature program was 60°C during 5 min, with 3°C/min increases to 180°C, then 20°C/min increases to 280°C, which was maintained for 10 min. The carrier gas was helium at a flow-rate of 1 mL/min. Split mode injection (ratio 1:30) was employed. Mass spectra were taken over the m/z 30–500 range with an ionizing voltage of 70 eV. Kovat's retention index was calculated using co-chromatographed standard hydrocarbons. The individual compounds were identified by MS and their identity was confirmed by comparison of their RIs, relative to C_8-C_{32} *n*-alkanes, and mass spectra with authentic samples or with data already available in the NIST 2005 mass spectral library and in the literature.[15]

RESULTS AND DISCUSSION

Hydrodistillation of six samples, coming from two commercial dried bay leaves type available for food use gave a yellowish essential oil (0.26 ± 0.16 and 0.23 ± 0.15, respectively), with a specific density lower than water and a strong odor. Generally, greater variability in yield was showed among samples of the same trademark than between the two different brands. This may probably be due to the different time of harvest required to provide the market with a continuous new herbs supply. Thus, 0.45% yield was obtained from samples with an expiration date of January 30, 2014, while more similar yields (0.20 and 0.15%) were found in samples with only one month of difference in the expiration dates (06/30/14 and 05/30/14, respectively). Sixty-four compounds accounting for 91.06–99.58% of the essential oils were identified by capillary GC-MS. Components are listed (Table 1) as homologous series of monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, and phenylpropanoids.

TABLE 1
Constituents of food supermarkets and pharmacy *Laurus nobilis* L. essential oil by GC-MS analysis

Compound	*RT*	*RI*	*Peak area (%) supermarket 1*	*Peak area (%) supermarket 2*	*Peak area (%) pharmacy EO*
Monoterpene hydrocarbons			7.12 ± 1.96	5.35 ± 1.57	20.34
α-Thujene	6.704	925	0.25 ± 0.04	0.19 ± 0.05	0.60
α-Pinene	6.967	933	2.07 ± 0.23	1.54 ± 0.51	6.81
Camphene	7.493	947	0.10 ± 0.01	0.07 ± 0.02	0.41
Sabinene	8.516	972	1.38 ± 0.91	0.65 ± 0.29	6.69
β-Pinene	8.627	975	1.13 ± 0.34	0.86 ± 0.26	2.36
Myrcene	9.236	988	0.22 ± 0.13	0.10 ± 0.04	0.58
α-Phellandrene	9.771	999	0.12 ± 0.04	0.07 ± 0.01	–
δ-3-Carene	10.024	1005	0.17 ± 0.04	0.20 ± 0.04	–
α-Terpinene	10.318	1013	0.34 ± 0.15	0.29 ± 0.07	1.52
p-Cymene	10.682	1022	0.07 ± 0.03	0.07 ± 0.01	–
Limonene	10.858	1026	0.41 ± 0.23	0.54 ± 0.18	–
cis-Ocimene	11.800	1048	0.03 ± 0.02	0.02 ± 0.01	0.30
γ-Terpinene	12.253	1058	0.59 ± 0.30	0.55 ± 0.13	0.78
Terpinolene	13.592	1084	0.23 ± 0.11	0.20 ± 0.06	0.27
Oxygenated monoterpenes			37.60 ± 19.12	29.82 ± 13, 17	73.13
1,8-dehydro-Cineole	9.192	987	–	0.06 ± 0.03	–
1,8-Cineole	11.113	1032	7.67 ± 7.93	3.52 ± 2.77	50.57
cis-Sabinene hydrate	13.148	1076	–	–	0.62
Linalool	14.472	1101	5.65 ± 5.46	3.53 ± 2.00	6.78
cis-p-Ment-2-en-1-ol	15.175	1118	0.05 ± 0.03	0.07 ± 0.01	0.05
trans-p-Ment-2-en-1-ol	15.997	1136	0.04 ± 0.03	0.04 ± 0.02	–
δ-Terpineol	17.324	1165	0.24 ± 0.15	0.22 ± 0.08	0.28
Terpinen-4-ol	17.864	1175	1.49 ± 1.09	1.52 ± 0.75	2.24
α-Terpineol	18.556	1189	2.34 ± 1.19	2.32 ± 0.87	1.42
Nerol	20.273	1227	0.04 ± 0.05	–	–
Linalyl acetate	21.484	1254	0.16 ± 0.14	0.08 ± 0.02	0.19
4-Thujanyl acetate	22.323	1272	–	0.05 ± 0.02	0.03
Bornyl acetate	22.753	1281	0.37 ± 0.33	0.14 ± 0.05	0.23
δ-Terpinyl acetate	24.199	1312	0.78 ± 0.33	0.73 ± 0.37	0.43
α-Terpinyl acetate	25.933	1353	18.32 ± 4.86	17.27 ± 6.79	10.25
Neryl acetate	26.331	1362	0.21 ± 0.09	0.11 ± 0.05	0.01
Geranyl acetate	27.142	1381	0.21 ± 0.06	0.15 ± 0.03	0.02
Sesquiterpene hydrocarbons			6.61 ± 1.95	6.39 ± 1.00	2.15
Isoledene	26.971	1377	–	–	0.04
α-Copaene	27.154	1381	–	–	0.02
β-Elemene	27.447	1387	0.31 ± 0.50	1.14 ± 0.17	0.40
β-Caryophyllene	28.577	1414	1.42 ± 0.28	1.77 ± 0.16	0.80
α-Guaiene	29.358	1433	0.30 ± 0.13	0.18 ± 0.07	0.07
6,9-Guaiadiene	29.547	1438	0.07 ± 0.02	0.06 ± 0.02	–
α-Humulene	29.942	1448	0.38 ± 0.11	0.35 ± 0.06	0.08
allo-Aromadendrene	30.607	1464	–	–	0.06
Germacrene D	31.068	1475	0.29 ± 0.09	0.24 ± 0.05	0.07
β-Selinene	31.272	1480	0.80 ± 0.43	–	0.10
α-Selinene	31.655	1488	–	–	0.06
Bicyclogermacrene	31.696	1490	1.06 ± 0.41	0.89 ± 0.16	0.14
α-Bulnesene	32.050	1498	0.56 ± 0.30	0.38 ± 0.10	0.03
γ-Cadinene	32.391	1507	0.33 ± 0.16	0.33 ± 0.11	0.08
δ-Cadinene	32.784	1517	1.08 ± 0.52	1.06 ± 0.29	0.19
trans-Cadina-1,4-diene	33.074	1535	–	–	0.01

(*continued*)

TABLE 1
(*Continued*)

Compound	*RT*	*RI*	*Peak area (%) supermarket 1*	*Peak area (%) supermarket 2*	*Peak area (%) pharmacy EO*
Oxygenated sesquiterpenes			15.98 ± 9.92	22.99 ± 7.86	0.29
Spathulenol	35.029	1575	3.18 ± 1.67	2.89 ± 0.19	0.11
Caryophyllene oxide	35.116	1577	3.17 ± 1.93	4.04 ± 0.73	0.16
Viridiflorol	35.451	1586	–	1.71 ± 0.67	0.02
Ledol	35.860	1596	0.86 ± 0.56	0.87 ± 0.29	–
β-Eudesmol	37.493	1641	3.73 ± 2.28	3.53 ± 1.22	–
α-Eudesmol	37.725	1647	1.19 ± 1.11	2.66 ± 1.07	–
α-Cadinol	37.854	1651	3.85 ± 2.93	7.29 ± 3.91	–
Phenylpropanoids			24.78 ± 5.76	26.33 ± 1.14	3.64
Methyl Chavicol	18.813	1194	0.07 ± 0.06	0.07 ± 0.01	0.09
Eugenol	26.140	1358	2.53 ± 0.84	2.30 ± 0.05	0.64
Hydrocynnamyl acetate	26.543	1367	0.08 ± 0.03	0.06 ± 0.03	–
Methyl Eugenol	28.386	1409	18.78 ± 4.18	21.35 ± 1.08	2.74
trans-Cynnamyl acetate	29.682	1441	0.59 ± 0.05	0.15 ± 0.07	0.05
trans-Isoeugenol	29.854	1446	0.18 ± 0.07	–	–
Ethyl-*trans*-Cinnamate	30.467	1461	0.10 ± 0.06	0.06 ± 0.01	0.01
trans-Methylisoeugenol	31.846	1493	0.80 ± 0.32	0.89 ± 0.16	0.05
Elemicin	34.261	1556	1.65 ± 0.72	1.45 ± 0.26	0.06
Others			0.18 ± 0.04	0.17 ± 0.05	0.05
2-Undecanone	23.186	1289	0.18 ± 0.04	0.17 ± 0.05	0.05
Total			92.28 ± 4.16	91.06 ± 4.26	99.58

Compounds listed in order of elution in the HP-5MS UI column; RI: retention index relative to C_8-C_{32} n-alkanes on the HP-5MS UI column.

Oxygenated monoterpenes represented quantitatively the main fraction in all analyzed essential oils, but the qualitative and quantitative differences found among the homologous series can establish two different groups. The first group includes essential oils obtained from commercial dried bay leaves, used as a flavor in Mediterranean diets, with oxygenated monoterpenes (37.60 ± 19.12 and 29.82 ± 13.17%), followed by phenylpropanoid (24.78 ± 5.76 and 26.33 ± 1.14%), and oxygenated sesquiterpenes (15.98 ± 9.92 and 22.99 ± 7.86%) as the main fractions. The second group corresponding to *Laurus nobilis* L. essential oil, sold for medicinal use, was rich in monoterpene compounds, both oxygenated monoterpene (73.13%) and monoterpene hydrocarbons (20.34%) and with low percentage in phenylpropanoid (3.64%) and oxygenated sesquiterpenes (0.29%). Although oxygenated monoterpenes are the principal fraction some differences in the amounts of the major compounds in the two established groups were observed as well. α-Terpinyl acetate (18.32 ± 4.86 and 17.27 ± 6.79%), 1,8-cineole (7.67 ± 7.93 and 3.52 ± 2.77%), and linalool (5.65 ± 5.46 and 3.53 ± 2.00%, respectively) were the main compounds in the essential oils from samples available for food use . However, in the commercial *Laurus nobilis* essential oil for medicinal use 1,8-cineole is by far the main compound with 50.57%, followed by α-terpinyl acetate (10.25%), and linalool (6.78%). Qualitative and quantitative differences were found in the other fractions, ledol (0.86 ± 0.56 and 0.87 ± 0.29%), β-eudesmol (3.73 ± 2.28 and 3.53 ± 1.22%), α-eudesmol (1.19 ± 1.11 and 2.66 ± 1.07%), and α-cadinol (3.85 ± 2.93 and 7.29 ± 3.91%, respectively) present in the essential oils from two commercial spices, were not found in essential oil for medicinal use. It is also interesting to note the quantitative differences found between the two established groups in the aromatic fraction of phenypropanoids (24.78 ± 5.76 and 26.33 ± 1.14% vs. 3.64%). Even though the main compounds are the same, in the essential oils from bay leaves of the first group, methyl eugenol reaches 18.78 ± 4.18 and 21.35 ± 1.08% and eugenol reaches 2.53 ± 0.84 and 2.30

± 0.05%, while in the second one, these compounds only represent the 2.74 and 0.64%, respectively . Such differences could also be responsible for modifications not only in the organoleptic characteristics but as well as in the pharmacological activity of *Laurus nobilis* essential oil. In this sense methyl eugenol has been reported as endowed with antioxidant, antimicrobial, anesthetic, and muscle relaxant effects,[10,16] whereas cineol, the main compound (50.57%) in the essential oil sold for medicinal use, possesses antibacterial, anti-inflammatory, and antinociceptive effects.[9,17]

CONCLUSION

The chemical composition of *Laurus nobilis* essential oils traded as spice and medicinal items has been analyzed. The presence of large amount of the biologically active compounds methyl eugenol for food use and 1,8-cineole for medicinal use can contribute to their pharmacological properties, muscle relaxant effect, and anti-inflammatory activity, respectively.

ACKNOWLEDGMENTS

The authors would like to thank the Central Service for Experimental Research of the University of Valencia (SCSIE) for providing the gas chromatography-mass spectrometry equipment.

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