Chemical Composition of the Supercritical CO_2 Extract and Essential Oil of Bay (*Laurus nobilis* L.)

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In the present study supercritical carbon dioxide (SC-CO_2) extraction and hydrodistillation (HD) of dried leaves of bay (*Laurus nobilis* L.) were compared with respect to efficiency and selectivity. The yield of SC-CO_2 extract isolated at pressure of 10 MPa and at temperature of 40 °C was 1.37 mass% after 1.4 hours of extraction while yield of the essential oil (EO) was 1.43 %wt after 4 hours. Comparative analysis of chemical composition of SC-CO_2 extract and HD revealed significant difference. The most abounded components in the essential oil were monoterpenes and their oxygenated derivates (98.4 %wt), principally 1,8-cineole (33.4 %), linalool (16.0 %), α-terpinyl acetate (13.8%), sabinene (6.91 %), methyl eugenol (5.32 %), α-pinene (4.39 %) i β-pinene (3.52 %). The SC-CO_2 extract comprised twice less monoterpenes and their oxygenated derivates (43.89 %) besides sesquiterpenes (12.43 %), diterpenes (1.33 %) and esters (31.13 %). The most abounded components of the SC-CO_2 extract were methyl linoleate (16.18 %), α-terpinyl acetate (12.88 %), linalool (9.00 %), methyl eugenol (8.67 %), methyl arachidonate (6.28 %) and eugenol (6.14 %).

INTRODUCTION

Laurus nobilis L. (bay, bay laurel, laurel, sweet bay), a member of family Lauraceae which comprises 32 genera and about 2,000-2,500 species, is 15-20 m high evergreen shrub native to the southern Mediterranean region [1,2]. Dried leaves of bay and the essential oil are used extensively in the food industry for seasoning of meat products, soups, and fishes [3]. Several studies have evaluated the potential role of bay essential oil as an antimicrobial and antifungal agent [4-7], as well as the antioxidant properties of some leaves extracts [8-11]. Recently bay extracts have been studied for its cytotoxic activity [1,12,13]. Because of their high fatty acid content berries are generally utilized for the production of perfumed soaps (for acne and dandruff treatment) and candle manufacture [14].

The essential oil is generally obtained by hydrodistillation and solvent extraction although they suffer from certain disadvantages. Hydrodistillation, induces extensive phenomena of hydrolysis and thermal degradation, giving in any case a product with a characteristic off-odour. Solvent extraction can give oil, but due to a high content of waxes and/or other high molecular mass compounds, often gives rise to a concentrate with a scent very similar to the material from which it was derived. A further drawback of this technique is that small amounts of organic solvents can pollute the extraction product. Supercritical fluid extraction (SFE) can be used for the production of flavours and fragrances from natural materials and can constitute a valid alternative to both of the above-mentioned processes [15]. Tuning of the process parameters (p, T) enables tuning of the selectivity of supercritical carbon dioxide (SC-CO_2) towards desirable fractions as well as completely separation of the phases so that solvent-free extract can be obtained. Several research groups investigated SC-

CO_2 extraction in order to isolate biologically active compounds from *Laurus nobilis* leaves [7,11,15], berries [16] and seeds [17]. The chemical composition of the bay oil obtained by different methods has been studied by different researchers [7,15,18-24].

This paper was aimed to compare SC-CO_2 extraction and hydrodistillation of dried bay leaves with respect to ther efficiency and selectivity. Therefore, yield and chemical composition of SC-CO_2 extract and essential oil (EO) obtained by hydrodistillation (HD) of the bay leaves were invenstigated and discussed.

MATERIALS AND METHODS

Plant material. Dried leaves of bay (*Laurus nobilis* L.) originated from Montenegro were used for SC-CO_2 extraction and HD. The plant material was milled and sieved to the fraction with average particle diameter of 0.8-0.9 mm.

Hydro distillation. Plant material (24 g) and water (500 mL) were placed in a Clevenger-type apparatus. The essential oil was isolated by hydrodistillation for 4 hours. The obtained essential oil (EO) was in a sealed vial at 4°C until required.

Supercritical carbon dioxide extraction. Extraction with SC-CO_2 was preformed in a pilot-plant-scale supercritical fluid extractor (Autoclave Engineers SCE Screening System) with a 150 ml extraction cell previously described [25]. Commercial carbon dioxide (99% purity, Messer Tehnogas, Belgrade, Serbia) was used for the extraction. The SC-CO_2 extraction was carried out under the pressure of 10 MPa and at the temperature of 40°C (density of SC-CO_2 630 kg/m^3). The initially used mass of the plant material was 24 g and the solvent rate was 0.3 kg/h.

GC/FID/MSD. Qualitative and quantitative analyses of the EO and SC-CO_2 extract of bay was carried out using a Hewlett-Packard GC-FID and GC-MS analytical methods. In the first instance, model HP-5890 Series II, equipped with a split-splitless injector, HP-5 capillary column (25m 0.32mm, film thickness 0.52 μm) and a flame ionization detector (FID), was employed. Hydrogen was used as the carrier gas (1ml/min). The injector was heated at 250°C, the detector at 300°C, while the column temperature was linearly programmed from 40°C to 260°C (4°C/min). GC-MS analyse was carried out under the same analytical conditions, using model HP G 1800C Series II GCD analytical system equipped with HP-5MS column (30m×0.25mm×0.25μm). Helium was used as the carrier gas. The transfer line (MSD) was heated at 260°C. The EI mass spectra (70 eV) were acquired in the scan mode in the m/z range 40–400. In each case, sample solution in hexane (1μl) was injected in split mode (1:30). The identification of constituents was performed by matching their mass spectra and Kovats indices (I_K) with those obtained from authentic samples and/or NIST/Wiley spectra libraries, different types of search (PBM/NIST/AMDIS) and available literature data (Adams). Area percents, obtained by the integration of corresponding chromatograms (FID), were used for quantification of individual components.

RESULTS

The yield of the essential oil (EO) obtained by HD was 1.43 mass% after 4 h, while yield of the SC-CO_2 extract was 1.37 mass% after 1.4 hours of extraction (m_{CO2}/m_{solid}=16.67). Yield of bay EO in this study was higher than those previously reported in the literature. Carreda *et al*. [15] isolated 0.90 % of the EO from bay leaves (southern Sardinia, Italy) after 4 h. Recently, a novel microwave method has been applied to the hydrothermal extraction of essential oil from bay leaves [20]. The mentioned study [20] revealed that the yield of EO obtained by HD in a Clevenger-type apparatus equipped with an electric mantle heater after 1 h (traditional method) was 0.784 % while yields of EO obtained by hydrodistillation with a

200 W and 300 W microwave system after 1 h were 0.813 % and 1.132 %, respectively. Verdian-rizi *et al.* [21] obtained 0.654-1.132 % of essential oil by HD of aerial parts of bay in different vegetative stages after 4 h. in their study the highest yield was observed for flowering stage. Carreda *et al.* [15] reported yield of essential oil fraction of 0.82 % after 4 h (m_{CO2}/m_{solid}=21.44) obtained by SC-CO_2 extraction at 9 MPa and 50°C (waxes were entrapped in the first separator set at 9 MPa and -10 °C; the oil was recovered in the second separator at 1.5 MPa and 10°C.).

The results of chemical analyses from obtained SC-CO_2 extract and essential oil (EO) accomplished by GC-FID and GC-MSD are presented in Table 1. Thirty four components were detected and identified in the EO of bay obtained by HD. The EO comprised mostly oxygenated monoterpenes (78.77 %) and hydrocarbon monoterpenes (19.68 %). Sesquiterpenes (1.06 %) and their oxygenated (0.53 %) were also found in EO of bay. The main components in EO were 1,8-cineole (33.4 %), linalool (16.0 %), *α*-terpinyl acetate (13.8%), sabinene (6.91 %), methyl eugenol (5.32 %), *α*-pinene (4.39 %) and *β*-pinene (3.52 %). In the earlier papers [15,19-23], 1,8-cineole is reported to be the main component in the bay EO isolated by HD whereby its content was in the range of 23.51-60.72 %.

Table 1: Chemical composition (mass%) of the SC-CO_2 extract and essential oil (EO)

				SC-CO_2 extract		**EO**	
Components	**$I_{K,E}$**	**$I_{K,L}$**	**RT/MS**	**RT/FID**	**mass%**	**RT/FID**	**mass%**
p-xylene	871.6	866	5.40	10.358	0.44		
α-thujene	919.2	924	6.93			12.835	0.55
***α*-pinene**	924.8	932	7.10			13.156	**4.39**
camphene	938.9	946	7.54			13.794	0.30
sabinene	965.0	969	8.36			14.758	**6.91**
***β*-pinene**	967.2	974	8.42			14.944	**3.52**
dehydro-1,8-cineole	984.4	988	8.94			15.353	0.21
β-myrcene	985.1	988	8.97			15.450	0.14
α-phellandrene	997.1	1002	9.34			16.011	0.17
δ^3-carene	1002.7	1008	9.54			16.268	0.24
α-terpinene	1009.3	1014	9.77			16.513	0.42
p-cymene	1017.7	1020	10.06			16.839	0.41
limonene/*β*-phellandrene	1020.9	1024	10.17			17.029	1.59
1,8-cineole	1025.0	1026	10.31	16.889	2.53	17.169	**33.4**
γ-terpinene	1051.3	1054	11.22			18.232	0.74
cis-sabinene hydrate	1061.5	1065	11.57	18.334	0.25	18.604	0.30
terpinolene	1080.7	1086	12.24			19.467	0.33
linalool	1096.3	1095	12.77	19.530	**9.00**	19.796	**16.0**
δ-terpineol	1161.0	1162	15.03	22.401	0.49	22.674	0.57
terpinen-4-ol	1170.3	1174	15.36	22.840	0.90	23.107	2.38
p-cymen-8-ol	1175.5	1179	15.70	23.068	0.23		
α-terpineol	1184.5	1186	15.85	23.326	2.54	23.594	2.83
nerol	1227.0	1226	17.30	24.608	0.44	24.914	0.19
linalyl acetate	1250.4	1254	18.10	25.579	0.58	25.836	0.34
4-thujen-2a-yl acetate	1296.1	n/a	18.73	26.441	0.20	26.703	0.28
bornyl acetate	1278.7	1287	19.05	26.971	0.27	27.235	0.47
δ-terpinyl acetate	1310.1	1316	20.10	28.055	0.55	28.311	0.68

exo-2-hydroxycineole ac.	1335.8	n/a	20.94	28.918	0.31	29.177	0.20
α-terpynil acetate	1343.8	1346	21.19	29.212	**12.88**	29.464	**13.8**
eugenol	1352.8	1356	21.45	29.509	**6.14**	29.773	1.77
β-elemene	1383.8	1389	22.48	30.870	0.69		
methyl eugenol	1400.4	1403	22.99	31.006	**8.67**	31.252	**5.32**
n.i.				31.641	0.18		
β-caryophyllene	1409.8	1417	23.32	32.029	0.87	32.284	0.43
n.i.	1427.3		23.85	32.509	0.29		
α-guaiene	1429.7	1437	23.93	32.678	0.18		
α-humulene	1444.1	1452	24.37	33.271	0.71		
allo-aromadendrene	1451.2	1458	24.58	33.451	0.16		
germacrene D	1472.0	1484	25.22	34.070	0.55		
β-selinene	1476.8	1489	25.37	34.300	0.33		
bicyclogermacrene	1487.3	1500	25.69	34.589	0.72	34.833	0.36
germacrene A	1493.0	1508	25.87	34.803	0.39		
γ-cadinene	1504.7	1513	26.22	35.119	0.29		
δ-cadinene	1514.4	1522	26.51	35.317	0.32	35.564	0.27
trans-cadina-1,4-diene	1522.5	1533	26.75	35.746	0.41		
α-cadinene	1534.0	1537	27.09	36.017	0.79		
dauca-5,8-diene	1565.9	1573	28.04	37.152	0.56		
spathulenol	1567.9	1577	28.09	37.262	0.79	37.521	0.27
caryophyllene oxide	1572.7	1582	28.24	37.509	0.46	37.765	0.26
viridiflorol	1581.4	1592	28.50	37.776	0.49		
ledol	1592.3	1602	28.82	38.142	0.21		
dihydro-*cis*-α-copaene-8-ol	1608.7	n/a	29.33	38.889	0.20		
eremoligenol	1619.5	1629	29.58	39.107	0.37		
β-eudesmol	1640.0	1649	30.15	39.561	1.45		
n.i.	1644.7		30.29	39.866	0.17		
shyobunol	1680.3	1688	31.26	40.591	0.25		
n.i.	1653.2		31.50	40.809	0.20		
sedanenolide	1712.4	1719	32.13	41.568	1.21		
neocnidilide (sedanolide)	1717.7	1722	32.26	41.752	0.36		
oplopanone	1729.1	1739	32.56	42.100	0.17		
n.i.	1799.8		34.40	43.711	0.16		
neophytadiene isomer I	1806.8	1807	34.58	43.954	0.26		
dehydrosaussurea lactone	1823.8	n/a	35.01	44.307	0.35		
hexahydrofarnesyl acetone	1835.0	1845	35.30	44.507	0.40		
methyl palmitate[1]	1915.4	1921	37.32	46.600	**1.49**		
n.i.	1972.9		38.70	49.320	0.30		
eremanthin (vanillosimin)	1981.0	n/a	38.89	49.489	0.20		
methyl linoleate	2087.2	2095	41.32	51.005	**16.18**		
methyl petroselinate[2]	2092.2	n/a	41.44	51.112	5.95		

phytol	2102.4	2114	41.67	51.440	1.33		
methyl stearate[3]	2117.5	2124	42.00	51.666	1.23		
methyl arachidonate	2215.1	2217	44.14	53.609	**6.28**		
n.i.	2272.0		45.35	55.476	4.73		
n.i.	2280.4		45.50	56.107	0.29		
n.i.	2316.3		46.27	56.441	0.20		
n.i.	2331.6		46.57	56.717	0.25		
n.i.	2372.0		47.40	57.608	0.23		

1-methyl hexadecanoate; 2-methyl cis-6-octadecenoate; 3-methyl octadecenoate; IK,E, IK,L-Kovatsindices (experimental and literature values); RT/MS, RT/FID- retention times; mass%- mass percent of components.

Sixty three components were detected and fifty two components were identified (93.47 %) in the SC-CO_2 extract of bay. Supercritical extract comprised mostly oxygenated monoterpenes (43.2 %) and fatty acid esters (31.13 %), followed by sesquiterpene hydrocarbons (7.26 %) and their oxygenated derivates (5.17 %), hydrocarbons (2,60 %), phtalides (1.57 %), diterpenes (1.33 %) and monoterpene hydrocarbons (0.69 %). The most abounded components in the SC-CO_2 extract were methyl linoleate (16.18 %), α-terpinyl acetate (12.88 %), linalool (9.00 %), methyl eugenol (8.67 %), methyl arachidonate (6.28 %) and eugenol (6.14 %). Comparative study of the SC-CO_2 extract and EO chemical composition revealed significant differences.The SC-CO_2 extract comprised more than two times less monoterpene hydrocarbons and oxygenated monoterpenes (43.89 %) in comparison to EO (98.4 %). Caredda *et al*. [15] reported that the lighter compounds (hydrocarbon monoterpenes) are extracted almost completely during the first extraction hour, content of oxygenated monoterpenes decreases to a minor extent during time, content of hydrocarbon sesquiterpenes increases significally during time, while oxygenated sesquiterpenes content doesn't change much after 3rd hour. Same authors also reported the most remarkable differences of contents of 1,8-cineole and methyl eugenol during extraction after the first and fourth hour (30.98 versus 2.05% for 1,8-cineole and 6.85 versus 16.42% for methyleugenol) [15]. It was reported that 1,8-cineole is the major aroma component of bay oil, followed by linalool, substances present in lower concentration such as eugenol and (E)-isoeugenol, as well as the non-identified compounds at trace level with a pepper-like odor [26]. In this study content of eugenol and methyl eugenol was two times higher then in EO. The significant difference of 1,8-cineole content in the EO (33.4 %) and in the SC-CO_2 extract (2.53 %) was observed as well. The SC-CO_2 extract in this study comprised much lower content of 1,8-cineole but higher content of eugenol and methyl eugenol than previously reported for SC-CO_2 extract of the bay [15].

CONCLUSION

In this study similar yields of EO and SC-CO_2 extract were obtained from dried leaves of bay, although supercritical extraction was less time-consuming process. According to chemical analysis content of lighter compounds, monoterpenes and their oxygenated derivates in EO (98.4 %wt), was two times higher in comparison to SC-CO_2 extract (43.89 %). The main components in EO were 1,8-cineole, linalool, α-terpinyl acetate, sabinene, methyl eugenol, α-pinene and β-pinene. The most abounded components of the SC-CO_2 extract were methyl linoleate, α-terpinyl acetate, linalool, methyl eugenol, methyl arachidonate and eugenol.

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