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Research Paper

Laurel Extracts Obtained by Steam Distillation, Supercritical Fluid and Solvent Extraction

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ABSTRACT

The chemical composition of laurel leaves and fruits essential oils (content of 0.917 and 0.747%, respectively) and laurel leaves extracts obtained at different pressures/temperatures by supercritical fluid extraction (SFE) were studied by GC-MS. The predominant compound in the essential oils and in CO_2 extracts obtained at pressures/temperatures of 100 bar/40°C and 250 bar/40°C was 1,8-cineole, but at 100 bar/60°C, α–terpineol acetate was dominant. The extraction yield of SFE increases from 0.68 to 2.54% by increasing the density of CO_2 (from 0.29 to 0.88 g/mL).

Keywords: Laurel; Extraction; SFE; Pressure and temperature; GC-MS

INTRODUCTION

Laurel is a small perennial tree native to Asia Minor and the Balkans. A bay leaf (*Laurus nobilis*) belongs to the family *Lauraceae*, and is one of the most widely used culinary spices in a any countries. Traditionally has been used as herbal medicine to treat rheumatism, earaches, indigestion, sprains, and to promote perspiration. Also, can be used in treating diabetes and preventing migraine. Bay leaf essential oil is one of main products from bay trees that are used in food, spice, flavoring and cosmetic industries (Sari, et al., 2006; Fang, et al., 2005).

The essential oil from the leaves (0.8 to 3%) contains mostly 1,8-cineol (up to 50%), also eugenol, acetyl and methyl eugenol, α- and β-pinene, phellandrene, linalool, geraniol and terpineol. The dried laurel fruits contain 0.6 to 10% of essential oil. The aroma of this essential oil is mostly due to terpenes (cineol, terpineol, α- and β-pinene, citral), but also cinnamic acid and its methyl ester. The potential role of laurel essential oil as an antimicrobial agent was investigated, too (Atanda, et al., 2007; Ozcan and Erkmen, 2001; Smith-Palmer, et al., 2001).

Components of laurel essential oil responsible for anticonvulsant activity are methyl eugenol, egenol and pinene. At the other hand, cineol, eugenol and methyl eugenol produced sedation and motor impairment (Sayyah, et al., 2002). Analgesic and

anti-inflammatory effects of laurel leaves essential oil are known, too (Sayyah, et al., 2003).

Methanolic extracts of *L. nobilis*, contains polar compounds (such are phenols, flavones and flavonols), and shows antioxidative activity (Simic, et al., 2003; Skerget, et al., 2005; Demo, et al., 1998). Same activity, as well as antimicrobial activity, was a topic of investigation of laurel extracts obtained by supercritical fluid extraction (SFE), using ethanol as modifier in extraction process (Santoyo, et al., 2006).

The classical procedures for active substances separation from the plant material by steam distillation and extraction with organic solvents have serious drawbacks. The distillation procedure allows only the separation of volatile compounds (essential oils), which, to a greater or lesser extent, are transformed under the influence of the elevated temperature. On the other hand, extraction with organic solvents can hardly render an extract free of traces of the organic solvent, which are undesirable for either organoleptic and/or health reasons. Besides, organic solvents are insufficiently selective, so that, in addition to the active substances, they also dissolve some concomitant compounds. Moreover, the absence of light and air during extraction reduce the risk of degradation reactions. For these reasons supercritical fluid extraction (SFE) with supercritical carbon dioxide (CO_2) has recently gained in importance as an alternative to the classical procedure. Extraction procedures involving supercritical CO_2 belong to "clean technologies", with no secondary products polluting the environment. CO_2 is most widely used in SFE because it is simple to use, inexpensive, non-flammable, nontoxic, chemically stable, shows great affinity to volatile (lipophilic) compounds, and can be easily and completely removed from any extract. By changing pressure and/or temperature above the critical point of CO_2 (T_c=31.3°C; p_c=72.8 bar; d_c=0.467 g mL^{-1}), a pronounced change in the density and dielectric constant, i.e. solvent power of supercritical CO_2, can be achieved (Zekovic, et al., 2000).

In present study, the chemical composition of laurel (*Laurus nobilis*) essential oil (EO) and the laurel extracts obtained at different pressures and temperatures, i.e. solvent densities, by supercritical fluid extraction (SFE) using CO_2 were studied. The chemical composition of laurel EO and CO_2 extracts were also compared.

MATERIALS AND METHODS

Plant material: The samples of laurel leaves and fruits were collected near Kaštelir (Croatia) in year 2007. Voucher specimens (*Laurus nobilis* L. 1753 No 2-1850, Croatia, Kaštelir, 19.09.2007. det.: Goran Anačkov were confirmed and deposited at the Herbarium of the Department of Biology and Ecology (BUNS Herbarium), Faculty of Natural Sciences, University of Novi Sad. Samples were air-dried, milled and mean particle size were determined by sieve set (Erweka, Germany).

***Chemicals*:** The commercial carbon dioxide (Tehno-gas, Novi Sad, Serbia) as the extracting agent were used. All other chemicals were analytical reagent grade.

Determination of essential oil content: The content of essential oil was determined by officinal procedure (Pharmacopoeia Jugoslavica, 1984).

***Extraction with methylene chloride*:** Laurel sample (20.0 g) was extracted by methylene chloride (200 mL) using Soxhlet apparatus. After 15 exchanges of the extract, the solvent was evaporated under vacuum and the obtained extract was dried under vacuum (50°C, 24 hours).

***Extraction with supercritical carbon dioxide*:** The supercritical fluid extraction (SFE) with carbon dioxide (CO_2) was carried out using a laboratory-scale high pressure extraction plant - HPEP (NOVA-Swiss, Effretikon, Switzerland), described previously (Pekić, et al., 1995). The main parts and characteristics (the specification of

manufacturer) of the plant are: the diaphragm-type compressor (up to 1000 bar), extractor with the internal volume of 200 mL (p_{max}=700 bar), separator with the internal volume 200 mL (p_{max}=250 bar), maximum CO_2 flow rate of about 5.7 kg/h. The laurel sample mass in the extractor: 70.0 g; pressure: 100 or 250 bar; temperature: 40 or 60°C; CO_2 flow rate: 1.629 dm^3/min; extraction time: 3 hours. Separator conditions were: pressure 15 bar and temperature 25°C.

TLC: Glass plates (20x20 cm) were coated in our laboratory using Silica gel G (Merck, Germany), thickness 0.25 mm. Investigated laurel extracts were dissolved in toluene (ratio 1 : 20) and different volume of samples (20 or 40 μL) were spotted on the plate as a start point or line. The mobile phase was toluene: ethylacetate (93:7; V/V). The development was at room temperature (approximately 20°C) in a glass chamber. Detection was done by spraying the plate using 1% vanillin solution (1 g of vanillin was dissolved in 99 g mixture of 95% ethanol and cc. sulfuric acid, ratio 9:1; w/w). After spraying, the plate was heated at 110°C for 5-10 min.

***GC-MS*:** The GC instrument was an Agilent 7890A with MSD model Agilent 5975C (Santa Clara, Calif, USA). A column HP-5 MS (30.0 m x 0.25 mm; film thickness 0.25 μm) was used. The helium pressure was 100 bar. The injector temperature was 250°C (split-ratio 20:1); the detector was set at 300°C, temperature program was set initially at 60°C and was increased linearly at 3°C per minute to 300°C. Total analysis time was 80 min. The injected volume of sample solution in methylene chloride (1 mg/mL) was 0.2 μL. The detector was set to 35-550 D. The compounds were identified using the databases Adams and NIST/EPA/NIH version 2.0d.

RESULTS AND DISCUSSION

The contents of essential oil (EO) determined by official procedure (Pharmacopoeia Jugoslavica, 1984) were 0.917% (V/w) for laurel leaves and 0.747% for laurel fruits (about 18% less than in leaves).

The total extract yields obtained by methylene chloride (Soxhlet extraction) of laurel leaves and fruits were 7.9% and 24.1% (w/w), respectively. Yield of fruits extract is about 3 times higher than that of leaves.

The dominant laurel compounds (terpineol, linalool, 1, 8-cineole, terpineol acetate, α– and β-pinene, limonene) were detected by TLC in all obtained samples of laurel (Table-1).

Table-1: hR_f value of laurel compounds.

Compound	Color	hR_f value
Terpineol	Violet-blue	22.8
Linalool	Blue, green-blue	34.5
1,8-Cineole	Blue-violet	47.6
Terpineol acetate	Dark blue	70.2
α– and β-Pinene, Limonene	Rose, violet	96.9

Because of higher essential oil content, laurel leaves were extracted by SFE using CO_2. For process the relatively low temperatures of 40 and 60°C (avoid thermal

decomposition) were chosen. The highest extraction yield (2.54%) was obtained at pressure of 250 bar and temperature of 40°C, i.e. solvent density of 0.88 g/mL. By decreasing solvent density, i.e. solubility power, to 0.63 g/mL (100 bar, 40°C) and to 0.29 g/mL (100 bar, 60°C), extraction yield decreases to 1.37% and 0.68% (less than determined essential oil content of 0.917%), respectively (Table-2). The highest extraction yield obtained by SFE of 2.54% is about 3 times lower than that obtained by Soxhlet extraction, i.e. by non-selective solvent as methylene chloride.

After 3 hours of each SFE, plant material after extraction was steam distillated (Pharmacopoeia Jugoslavica, 1984) in the aim to investigate a quantification of extraction process. In all cases, some essential oil remained in laurel leaves, less after extraction at 100 bar and 40°C (0.125%), and much more after 100 bar and 60°C (0.225%) and after extraction at 250 bar and 40°C (0.300%).

After GC-MS analysis of investigated essential oils and CO_2 extracts, the results of qualitative and quantitative composition were obtained (Table-2).

The predominant compounds of laurel leaves and fruits EOs were, in the first place, 1,8-cineole (38.15 and 32.30%, respectively) and α–terpineol acetate (16.06 and 11.41%, respectively). Methyleugenol was detected in leaves EO in high content (12.54%), as well as β–elemene (9.11%) in laurel fruits EO. Compounds detected only in EO of laurel leaves were: sylvestrene, eugenol and elemicin. At the other hand, borneol, Z-β- and E-β-ocimene were components detected only in laurel fruits EO.

CO_2 extract of laurel leaves, obtained at 100 bar and 40°C, in comparation to EO obtained by steam distillation, contains the same dominant compound, 1,8-cineole, but with twice lower content (19.14%), as well as α–terpineol acetate and methyleugenol in some higher content (17.60 and 15.57%, respectively). The composition of remained EO after SFE-CO_2 (yield of 0.125%) was different in comparation to laurel leaves EO obtained by steam distillation: much higher contents of methyleugenol (24.75%) and α–terpineol acetate (19.58%), and some lower of 1, 8-cineole (31.18%).

By increasing the temperature in SFE process from 40 to 60°C (i.e. by decreasing the solvent density from 0.63 to 0.29 g/mL), the extraction yield was a lower (0.68%), and obtained extract, compared to extract obtained at 40°C, has a following characteristics: lower contents of 1, 8-cineole (15.54%) and methyleugenol (15.38%), but higher (24.74%) of the predominant compound in this extract, α–terpineol acetate.

By increasing the pressure from 100 to 250 bar at constant temperature of 40°C (i.e. by increasing the solvent density from 0.63 to 0.88 g/mL), the highest extraction yield was obtained (2.54%). The content of 1, 8-cineole (18.96%) was a similar to extract obtained at 100 bar (19.14%). In the same way, contents of α–terpineol acetate (14.12%) and methyleugenol (12.70%) were lower than that in extract obtained at 100 bar (17.60 and 15.57%, respectively). Because of used extraction conditions, i.e. higher solubility power, phytol, pentacosane and nonacosane were extracted and detected only in this extract.

CONCLUSION

The essential oils of laurel leaves and fruits, beside different content, have a different qualitative and quantitative composition, but with a same predominant compound (1, 8-cineole). By supercritical fluid extraction (SFE) using carbon dioxide (CO_2) with different densities, i.e. pressure and temperature combination, extraction yield increases by increasing the solvent density, i.e. by increasing solubility power. The dominant compound of laurel leaves CO_2-extract obtained at lowest investigated

density of 0.29 g/mL is α–terpineol acetate. The extracts obtained by higher CO_2 densities have a same dominant compound as laurel leaves essential oil isolated by steam distillation, 1,8-cineole.

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Table-2: Results of laurel investigation.

Plant part		Leaves	Fruits	Leaves	Leaves	Leaves	Leaves
Particle mean diameter (mm)		0.3005	0.3556	0.3005			
Procedure		D*	D	SFE** (100, 40)	Remained after SFE	SFE (100, 60)	SFE (250, 40)
Yield of EO or extract (%)		0.917	0.747	1.37	0.125	0.68	2.54
t_R (min)	Compound	Compound content (%)					
5.622	α–Thujene	0.19	0.10	-	0.07	-	-
5.816	α–Pinene	2.34	1.96	0.12	0.78	t	0.45
6.228	Camphene	0.19	0.83	-	0.06	-	-
6.910	Sabinene	5.75	2.80	1.00	2.83	0.55	1.56
7.022	β–Pinene	2.23	1.60	0.31	1.17	0.16	0.55
7.398	β–Myrcene	0.54	0.14	t	0.43	-	-
8.545	o-Cymene	0.34	0.27	0.10	0.38	0.15	t
8.698	Limonene	0.62	1.04	0.49	1.19	0.40	t
8.792	Sylvestrene	1.65	-	t	t	t	0.55
8.792	1,8-Cineole (Eucalyptol)	38.15	32.30	19.14	31.18	15.54	18.96
8.992	Z-β-Ocimene	-	0.45	-	-	-	-
9.375	E-β-Ocimene	-	0.67	-	-	-	-
11.375	Linalool	2.78	2.29	2.29	3.44	3.40	1.81
14.139	Borneol	-	1.64	-	-	-	-
14.186	δ-Terpineol	0.46	0.33	0.50	0.36	0.68	0.41
14.645	4-Terpineol	1.67	1.29	1.35	1.91	1.76	1.02
14.216	α-Terpineol	3.19	3.78	3.89	2.73	4.28	3.10
19.468	Bornyl acetate	0.47	2.91	0.37	0.71	0.60	0.27
22.292	α–Terpineol acetate	16.06	11.41	17.60	19.58	24.74	14.12
22.615	Eugenol	2.08	-	5.15	1.07	5.00	5.01
24.168	β–Elemene	0.46	9.51	1.13	0.54	1.37	1.11
24.715	Methyleugenol	12.54	2.48	15.57	24.75	15.38	12.70
25.350	E-Caryophyllene	1.25	0.89	3.75	1.62	4.63	2.83
26.797	α-Humulene	0.37	0.47	0.30	0.17	0.44	0.27
27.985	Germacrene D	0.76	0.72	0.90	0.33	1.09	0.56
28.650	Bicyclogermacrene	0.43	1.01	0.85	0.41	0.87	0.29
29.773	δ-Cadinene	0.38	4.27	0.32	0.39	0.76	0.26
31.126	Elemicin	0.36	-	0.70	0.24	0.75	0.61
31.967	Sphatulenol	1.77	2.14	1.66	2.68	1.26	1.71
50.995	Phytol	-	-	-	-	-	1.17
62.265	Pentacosane	-	-	-	-	-	1.13
72.312	Nonacosane	-	--	-	-	-	0.84
TOTAL		**94.90**	**87.30**	**77.49**	**99.02**	**83.81**	**71.29**

- * Steam distillation; ** Supercritical Fluid Extraction; t – trace
- All results are mean value of three analyses.

CO_2 densities:

Pressure (bar)	Temperature (°C)	Density (g/mL)
100	40	0.63
100	60	0.29
250	40	0.88