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Comparison between Supercritical CO_2 Extraction and Hydrodistillation for Two Species of Eucalyptus: Yield, Chemical Composition, and Antioxidant Activity

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Abstract: In this work, 2 Eucalyptus species extracts (*Eucalyptus cinerea* and *Eucalyptus camaldulensis)* were prepared by hydrodistillation (HD) and supercritical carbon dioxide extraction (SCE) techniques. The best yields of *E. cinerea* and *E. camaldulensis* (27.5 and 8.8 g/kg, respectively) were obtained using SCE at 90 bar, 40 °C compared to HD (23 and 6.2 g/kg, respectively). Extracts were quantified by gas chromatography-flame ionization detection and identified by gas chromatography-mass spectrometry. 1,8-cineole and p-menth-1-en-8-ol were the major compounds of *E. cinerea* essential oil obtained by HD (64.89% and 8.15%, respectively) or by SCE (16.1% and 31.87%, respectively). Whereas, in case of *E. camaldulensis*, 1,8-cineole (45.71%) and p-cymene (17.14%) were the major compounds obtained by HD, and 8,14-cedranoxide (43.79%) and elemol (6.3%) by SCE. Their antioxidant activity was assessed using 2 methods: 2,2-azino-di-3-ethylbenzothialozine-sulphonic acid radical cation ($ABTS^{\bullet+}$) and 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$). In the SCE extracts from both *E. cinerea* and *E. camaldulensis*, a promising radical scavenging activity was observed with $ABTS^{\bullet+}$, (65 and 128 mg/L, respectively). The total phenolics composition of the extracts was measured and the range was 2 to 60 mg of gallic acid equivalent/g dry plant material. The SCE method was superior to HD, regarding shorter extraction times (30 min for SCE compared with 4 h for HD), a low environmental impact, allows production of nondegraded compounds and being part of green chemistry.

Keywords: antioxidant activity, essential oil, Eucalyptus, GC-MS, hydrodistillation, supercritical carbon dioxide extraction

Practical Application: The natural products are important in different applications. Especially, the natural antioxidants provide an encouragement for the industry of natural substances, especially food, cosmetic, and pharmaceutical sectors. So, the use of supercritical carbon dioxide extraction (SCE) in industry applications can provide several benefits to final products. SCE presented important advantages compared to hydrodistillation: extraction is faster, improvement of the yield, a low environmental impact, producing of not degraded compounds, being part of green chemistry, and in the optimum process for obtaining extracts with high antioxidant quality.

Introduction

Plants remain an inexhaustible source of biologically active substances, among which are the aromatic and medicinal plants that possess a privileged status (Ujváry 2000). However, the quality and activity of extract is greatly dependent upon the process. Hydrodistillation (HD) and solvent extraction have been the major processes for a long time. During last decades, the use of supercritical fluids for extraction of plant volatile fraction has been increasingly proposed (Pourmortazavi and Hajimirsadeghi 2007; Xu and others 2011). This is due to the advantages of the supercritical carbon dioxide extraction (SCE) technique: rapid, selective, and convenient technique for extraction of natural compounds from aromatic and medicinal plants. Also, SCE extracts are considered as solvent free, which enhances their attractiveness for the consumer (Berka-Zougali and others 2012).

The Eucalyptus is a widely grown tree, well known for production of essential oil, with high biological activities used in various fields (Coppen 2002). Eucalyptus leaves provide extracts used as food additives and in cosmetic formulations (Gilles and others 2010). For extraction process, studies (for example, Ramezani and others 2002) focused on essential oil extracted by HD, and the study of Elaissi and others (2011) treated a large number of Eucalyptus species. However, only one study (Mann and others 2011) has investigated the extraction of Eucalyptus leaves by SCE and compounds were quantified by gas chromatography-flame ionization detection (GC-FID). In other studies (Fadel and others 1999; da Cruz and others 2001), compounds were quantified by

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gas chromatography-mass spectrometry (GC-MS) to compare HD and SCE.

Eucalyptus species are an important part in the forest area of Tunisia where the most abundant species are *Eucalyptus camaldulensis* and *Eucalyptus cineria*. The study of the production of volatile oils extracted from leaves of these 2 species of Eucalyptus has been found interesting because of the economic, food and medicinal interests of these species (Babu and Singh 2009), and the availability of the plant material.

The objective of this work is the comparison of essential oil extracted by SCE and HD for the 2 species *E. cinerea* and *E. camaldelensus*. This comparison is based on the level of the yield, the volatile chemical composition, phenolics, and antioxidant activity.

Material and Methods

Plant material

Leaves of *E. camaldulensus* and *E. cineria* were gathered on October 2010 from the region of Korbos (Tunisia) and air-dried until constant weight and protected against direct sunlight (shade-dried). Specimens were identified in the Regional Station of the Natl. Inst. of Research in Farming genius, Waters and Forests. The voucher specimens were deposited at the Dept. of Biology. After grinding, the average particle size of the 2 species was 0.8 mm. The moisture of plant material (air-dried) was quantified by Karl Fischer method. The content was 10% w/w.

Chemicals

All chemicals purchased from Sigma-Aldrich-Fluka (Saint-Quentin, France) were an analytical reagent grade, gallic acid (≥99%), ascorbic acid (≥99%), 1,1-diphenyl-2-picrylhydrazyl free radical (99%), Folin–Ciocalteu reagent 2 N, potassium persulfate (≥99.0%), 2,2-azinobis-3-ethylbenzothiazoline-6-sulphonate (≥99%). Commercial carbon dioxide (purity 99.99%) was purchased from Air liquid (Toulouse, France) for SCE extraction.

Extraction methods

Traditional techniques for extraction: Clevenger-type apparatus (HD). After grinding, the *E. camaldulensis* or *E. cinerea* leaves (100 g of dried material) were submitted to HD with 1 L of water for 3 h, using a Clevenger-type apparatus (European Pharmacopoeia procedure, 1983). The volatile distillate was collected and dried by anhydrous sodium sulfate and stocked at 4 °C prior to analysis. The optimization of the operating conditions of HD was carried out for several parameters: extraction time, flow of condensation (mL/s) which is directly related to heating power, factor of division of the vegetable material, particle size of the vegetal material after grinding (Herzi and others 2012).

Supercritical carbon dioxide extraction (SCE). An extraction pilot SFE200 from SEPAREX was used. It is composed of 200 mL stainless steel extractor, which was loaded with 50 g of dried Eucalyptus leaves. The system was operated at a constant temperature of 40 °C and pressure of 90 bar. During analyses, the temperature and pressure oscillations were ± 2 units (°C and bar). Pure CO$_2$ was passed into the cell during 30 min with the flow rate kept constant at 20 g/min in all runs. We have chosen the same conditions optimized in our previous study about SCE of volatile fraction extraction of *Tetraclinis articulata* (Herzi and others 2012). Each experiment was triplicate using new Eucalyptus raw material.

The recovery of extract was made through 3 cyclonic separators placed in series on decreasing pressures. The 1st and the 2nd separator were set at 50 bar and the 3rd at 2 bar. The small quantities of the extracts obtained were recovered by washing the pilot with ethanol. The extracts were concentrated with a rotary evaporator under reduced pressure at 35 °C.

GC and GC-MS. Quantitative analysis was carried by GC-FID (Varian Star 3400 Cx chromatograph, Les Ulis, France) fitted with a fused silica capillary DB-5MS column (5% phenyl-methylpolysyloxane, 30 m × 0.25 mm, film thickness 0.25 μm). The column temperature was started from 60 °C to 260 °C with a gradient rise of 5 °C/min followed by 15 min isotherm at 260 °C, a 2nd gradient step was applied up to 340 °C at 40 °C/min. Total analysis time was 57 min. Essential oil was dissolved in petroleum ether, 1 μL was injected in the split mode ratio of 1:10. Helium (purity 99.999%) was used as carrier gas at 1 mL/min.

Qualitative analysis of the essential oil was carried out by GC-MS. The same chromatographic conditions for GC-FID were used. The injector was operated at 200 °C. The mass spectrometer (Varian Saturn GC/MS/MS 4D) was adjusted for an emission current of 10 μA and electron multiplier voltage between 1400 and 1500 V. The trap and the transfer line temperatures were set at 220 °C and 250 °C, respectively. Mass scanning range was from 40 to 650 amu.

The compounds were identified by (i) comparison of their retention indices (RI) relative to C5-C24 *n*-alkanes compared to those provided in the literature and (ii) by comparison of their mass spectra with those recorded in NIST 08 (National Inst. of Standards and Technology) or reported in literature or by co-injection of reference compounds. The samples were analyzed in duplicate.

The quantification of compounds was computed by the normalization method from the GC peak areas, assuming identical mass response factor for all compounds. The results were calculated as mean values of 3 injections from essential oil, without using correction factors.

Determination of total phenolics content. Total phenolics content of extracts was quantified by the Folin and Ciocalteu (1927) method. Each extract (0.1 mL) was diluted and mixed with Folin–Ciocalteu reagent (0.5 mL at 0.2 N). After 5 min at room temperature, sodium carbonate solution (0.4 mL at 75 g/L) was added. After 1 h of incubation, the absorbances were measured at 765 nm against water blank using a spectrophotometer (Helios, Unicam, Cambridge, U.K.). Gallic acid (0 to 200 mg/L) used as standard calibration curve. The total phenolics content in extracts was expressed as mg of gallic acid equivalent (GAE)/g of dry mass.

Antioxidant activity. *DPPH$^{\bullet}$ free radical scavenging activity.* 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH$^{\cdot}$) was used to quantify antioxidant activity by Blois (1958) method with some modifications. Extracts (1.5 mL) were mixed with a 0.2 mM methanolic DPPH$^{\cdot}$ solution (1.5 mL). The absorbance, $A_{(\text{sample})}$, was recorded at 520 nm after an incubation period of 30 min at 25 °C. The blank, ($A_{(\text{blank})}$), was carried out by applying the same procedure to a solution without extract. The antioxidant activity of each solution was calculated as percentage inhibition according to the following equation: $\%\ \text{inhibition} = 100 \times [(A_{(\text{blank})} - A_{(\text{sample})})/A_{(\text{blank})}]$

The concentration of the test material required to cause 50% decrease in initial DPPH$^{\cdot}$ concentration, is named IC$_{50}$. Ascorbic acid was used as a standard.

ABTS$^{\bullet+}$ radical scavenging. The antioxidant capacity of extracts for the ABTS$^{\bullet+}$ (2,2-azinobis-3-ethylbenzo-thiazoline-6-sulfonate) was determined as described by Re and others (1999). ABTS$^{\bullet+}$ was generated by mixing a solution of ABTS (7 mM) at

Table 1–Extraction yield for *E. cinerea* and *E. camaldulensis*.

	E. cinerea		*E. camaldulensis*	
	HD	**SCE**	**HD**	**SCE**
Extraction time (min)	180	30	180	30
Temperature (°C)	100	40	100	40
Yield (g extract/kg dry plant material)	23 ± 0.16^b	27.5 ± 0.24^a	6.2 ± 0.1^d	8.8 ± 0.21^c
Color	Yellow	Dark yellow	Yellow	Dark yellow

Each experiment was triplicate using new *Eucalyptus* raw material. Values within lines with different superscripts (a to d) were significantly different.

pH 7.4 (5 mM NaH_2PO_4, 5 mM Na_2HPO_4 and 154 mM NaCl) with of potassium persulfate (2.5 mM) followed by storage in the dark at room temperature for 16 h. The mixture was diluted with water to give an absorbance of 0.70 ± 0.02 units at 734 nm using spectrophotometer. For each extract, methanol solution (100 μL) was added to $ABTS^{\bullet+}$ solution (900 μL), and the absorbance was measured after 6 min. The free radical scavenging capacity, IC_{50}, was determined using the same equation used previously for the $DPPH^{\cdot}$ method. Ascorbic acid was used as a reference.

Results and Discussion

Extraction process

The global yield and the color parameter of each extract from the leaves of *E. cinerea* and *E. camaldulensis* obtained by supercritical fluid extraction (SFE) and HD are given in Table 1. The color intensity of the extracts from both species was different, the HD extracts were yellow, and the extracts by SCE exhibited a dark yellow color that can be attributed to the differences in chemical composition. The global yield of essential oil extracted from *E. cinerea* and *E. camaldulensis* leaves obtained by HD were 23 and 6.2 g/kg, respectively. These results are in accordance with literature data on *E. cinerea* leaves essential oil yield obtained by HD (Mann and others 2011) which is about 3% (30 g/kg) as well as for *E. camaldulensis* leaves essential oil yield (Farah and others 2002) which is about 8 g/kg.

The global yields of volatile fraction from each species obtained using SFE extraction (40 °C and 90 bar) were 27.5 and 8.8 g/kg for *E. cinerea* and *E. camaldulensis*, respectively. For both plants, higher yield was obtained using SCE during 30 min compared to HD during 180 min. This is in accordance with other publications in terms of capacity of SCE extraction. Fadel and others (1999) have obtained 11 g/kg for *E. camaldulensis* yield but the experimental conditions were 50 °C, 200 bar during 120 min. In the work of da Cruz and others (2001), a high yield of 20 g/kg (250 bar, 40 °C) was obtained during 120 min.

In this study, the SCE extraction for the 2 species, *E. cinerea* and *E. camaldulensis* improves the extraction yield by about 20% and 40%, respectively. This is probably due to the properties of SCE technique including the low temperature and rapidity (40 °C, 30 min), which minimize the exposition to high temperature and consequently the thermal degradation as encountered with HD (around 100 °C, 240 min) (Yamini and others 2002; Mirzaei and others 2007).

Chemical composition

According to GC-MS and GC-FID analyses, the chemical composition of extracts obtained by SCE and HD was different qualitatively and quantitatively (Table 2). *E. cinerea* (HD, SCE) and *E. camaldulensis* (HD, SCE) extracts revealed 37 (94.22%), 39 (96.54%), 43 (97.17%), and 41 compounds (100%), respectively.

The major compounds of *E. cinerea* HD essential oil were 1,8-cineole (64.89%), p-menth-1-en-8-ol (8.15%), terpinen-4-ol (4.30%), and alpha-pinene (4.07%), then the major compounds of *E. cinerea* SCE extract were p-menth-1-en-8-ol (31.87%) 1,8-cineole (16.1%), 8,14- cedranoxide (5.68%), terpinene-4-ol (5.34%), and caryophyllene alcohol (5.39%).

In the case of HD, the major compounds of *E. camaldulensis* were 1,8-cineole (45.71%), p-cymene (17.14%), alpha-pinene (7.88%), and limonene (5.06%), whereas in the case of SCE the major compounds of *E. camaldulensis* were 8,14-cedranoxide (43.79%), elemol (6.3%), and myrtanal (4.38%).

For a more detailed comparison, 1,8-cineole was the main component of the essential oil for the 2 species for HD extracts (64.89% of *E. cinerea* and 45.71% of *E. camaldulensis*). Alpha-pinene and limonene were present at high concentrations in both samples, but p-menth-1-en-8-ol and terpinene-4-ol were present at high concentrations only in *E. cinerea* essential oil, and p-cymene and myrtanal in *E. camaldulensis*. Conversely, in the SCE extracts, 1,8-cineole (16.1%) was detected at high concentration but it is not the major compound.

In literature, only one study for Eucalyptus has compared the chemical composition of compounds obtained by SCE and HD, quantified by GC-FID and identified by GC-MS (Mann and others 2011). By HD, the extract consisted of 1,8-cineole (85.1%) as the main component, in the presence of alpha-terpineol (7.2%) and of limonene (4.4%), whereas SCE extracts consisted of a relatively lower quantity of 1,8-cineole (70.4%) and a relatively higher quantity of alpha-terpineol (8.6%) in the presence of globulol (3.1%). We noticed a great difference in the chemical composition and quantity of our extracts compared to those of Mann and others (2011). For example, this difference was in major compounds such as 1,8-cineole (16.1% with SCE in our study and 70.4% in study of Mann and others 2011) and the p-menth-1-en-8-ol was identified in our study (31.87% in SCE extract) but it is not detected in study of Mann and others (2011).

In other works (Fadel and others 1999; da Cruz and others 2001), *E. camaldulensis* compounds were quantified by GC-MS, thus we cannot compare them with our samples quantified by GC-FID.

Results showed an important difference in monoterpene and sesquiterpenes (hydrocarbons and oxygenated) identified in each extract. Indeed, the sesquiterpenes were much better extracted by SCE than HD, 33.31% compared to 3.46% in *E. cinerea* and 77.36% compared to 7.74% in *E. Camaldulensis*. The opposite was noted for the monoterpenes: 90% in HD compared to 58.47% in SCE for *E. cinerea* and 88.72% in HD compared to 14.8% in SCE for *E. camaldulensis*. Monoterpene hydrocarbons were 1% for all SCE extracts.

In the literature, in the most cases, the comparison between HD and SCE extraction showed a difference of yields but a few variations in chemical composition for the same major compounds in the extract. We report here for the 1st time an enormous variation for the major compounds.

SCE extracts from the 2 species, especially for *E. Camaldulensis,* were characterized by higher contents of heavier molecular weight compounds (retention time in the range 30 to 40 min) than in the case of the HD essential oils. In parallel, lighter components, for example, alpha-thujene, alpha-pinene, camphene, limonene, and gamma-terpinene, present in the HD essential oils, were weakly detected in the SCE extracts. This does not mean that such light

Table 2–Chemical composition, percentage and amounts, of *E. cinerea* and *E. camaldulensis* essential oils obtained by HD and SCE.

		E. cineria-HD		*E. cineria*-SCE		*E. camaldulensis*-HD		*E. camaldulensis*-SCE	
RI	**Compounds**	**Area**	**mg/kg**	**Area**	**mg/kg**	**Area**	**mg/kg**	**Area**	**mg/kg**
928	Alpha-thujene					0.17 ± 0.00^{a}	11		
937	Alpha-pinene	4.07 ± 0.18^{b}	936			7.88 ± 0.34^{a}	489		
952	Camphene	0.15 ± 0.00^{a}	35			0.12 ± 0.00^{a}	7		
962	1-ethyl-4-methylbenzene			0.35 ± 0.01^{a}	95				
982	Beta-pinene					0.11 ± 0.00^{a}	7		
997	1,2,3-trimethylbenzene			0.43 ± 0.02^{a}	119			0.16 ± 0.00^{b}	14
1007	Alpha-phellandrene	0.17 ± 0.00^{a}	39			0.21 ± 0.00^{a}	13		
1018	Alpha-terpinene					0.13 ± 0.00^{a}	8		
1025	p-cymene	1.52 ± 0.06^{b}	350	0.37 ± 0.01^{d}	102	17.14 ± 0.69^{a}	1063	0.90 ± 0.03^{c}	79
1029	Limonene	3.05 ± 0.09^{b}	702			5.06 ± 0.21^{a}	314		
1033	1,8-cineole	64.89 ± 3.06^{a}	14925	16.10 ± 0.65^{c}	4428	45.71 ± 2.11^{b}	2834	1.74 ± 0.06^{d}	154
1055	Gamma-terpinene	0.08 ± 0.00^{b}	18			0.27 ± 0.01^{a}	17		
1070	artificial ketone	0.08 ± 0.00^{a}	18						
1084	Alpha-terpinolene	0.17 ± 0.00^{b}	39			0.25 ± 0.01^{a}	16		
1092	Linalool	0.17 ± 0.00^{a}	39			0.19 ± 0.00^{a}	12	0.13 ± 0.00^{b}	11
1109	Fenchol	0.20 ± 0.00^{b}	46	0.26 ± 0.01^{a}	72	0.21 ± 0.00^{b}	13		
1114	1-S-beta-fenchol					0.27 ± 0.01^{a}	17	0.29 ± 0.01^{a}	26
1132	Alpha-campholenal	0.51 ± 0.01^{b}	117	0.53 ± 0.02^{b}	144	1.19 ± 0.05^{a}	74	0.33 ± 0.01^{c}	29
1140	Veratrole					0.21 ± 0.00^{a}	13		
1154	2-methyl-1-phenylpropan-2-ol	0.04 ± 0.00^{b}	10			0.33 ± 0.01^{a}	20		
1156	Isoborneol	0.64 ± 0.02^{b}	147	0.94 ± 0.03^{a}	258	0.34 ± 0.01^{c}	21		
1166	lavandulol	0.45 ± 0.01^{d}	104	0.59 ± 0.02^{c}	162	1.46 ± 0.07^{a}	91	0.88 ± 0.02^{c}	78
1172	Ethyl benzoate	0.04 ± 0.00^{c}	8	0.58 ± 0.01^{a}	159	0.16 ± 0.00^{b}	10		
1174	Myrtanal	0.38 ± 0.01^{c}	87			3.36 ± 0.15^{b}	209	4.38 ± 0.17^{a}	385
1177	Terpinen-4-ol	4.30 ± 0.18^{b}	989	5.34 ± 0.21^{a}	1467	0.56 ± 0.02^{c}	34		
1192	Z-piperitol					0.07 ± 0.00^{b}	4	0.20 ± 0.00^{a}	17
1202	E-dihydrocarvone	0.15 ± 0.00^{b}	35	0.22 ± 0.01^{a}	60	0.12 ± 0.00^{b}	7		
1210	E-carveol	0.41 ± 0.01^{b}	94	0.74 ± 0.03^{a}	204	0.27 ± 0.01^{d}	17	0.33 ± 0.01^{c}	29
1213	Verbenone	0.28 ± 0.01^{a}	65	0.26 ± 0.01^{a}	72	0.20 ± 0.01^{b}	13		
1222	Cuminaldehyde					1.69 ± 0.05^{a}	105	1.31 ± 0.06^{b}	115
1226	Eucarvone	0.06 ± 0.00^{a}	14						
1235	Neral					0.19 ± 0.00^{b}	12	0.36 ± 0.01^{a}	32
1254	Carvenone					0.45 ± 0.01^{a}	28		
1266	p-tert-butylphenol					0.40 ± 0.01^{b}	25	1.40 ± 0.05^{a}	124
1274	Perilla aldehyde	0.30 ± 0.01^{d}	69	0.63 ± 0.02^{b}	174	0.54 ± 0.01^{c}	33	0.98 ± 0.03^{a}	86
1281	Methyl nerolate							0.56 ± 0.01^{a}	49
1289	Undecan-2-one			0.16 ± 0.00^{b}	43			2.91 ± 0.08^{a}	256
1291	1-tridecene			0.39 ± 0.01^{a}	107				
1292	Thymol	0.20 ± 0.00^{a}	46						
1318	p-menth-1-en-8-ol	8.15 ± 0.37^{b}	1875	31.87 ± 1.07^{a}	8764	0.17 ± 0.00^{c}	11		
1330	p-mentha-1,4-dien-7-ol							0.91 ± 0.03^{a}	80
1344	2,6-dimethoxyphenol							0.56 ± 0.01^{a}	49
1356	Citronellyl acetate			0.23 ± 0.01^{b}	64			0.59 ± 0.01^{a}	52
1366	3-phenylpropyl acetate	0.28 ± 0.01^{a}	64						
1374	Longicyclene			0.33 ± 0.01^{a}	91				
1377	E-4,5-epoxy-E-2-decenal							0.62 ± 0.03^{a}	55
1383	Beta-bourbonene	0.11 ± 0.00^{b}	25	2.42 ± 0.09^{a}	666	0.09 ± 0.00^{b}	6		
1401	Longifolene	0.07 ± 0.00^{d}	16	1.76 ± 0.05^{b}	485	0.54 ± 0.01^{c}	33	2.52 ± 0.08^{a}	221
1413	Isocaryophyllene			0.34 ± 0.01^{a}	93				
1421	Beta-gurjunene			0.96 ± 0.03^{b}	264	0.27 ± 0.01^{c}	17	3.30 ± 0.15^{a}	290
1453	Alpha-caryophyllene					0.19 ± 0.00^{b}	12	0.75 ± 0.02^{a}	66
1456	Beta-acoradiene	0.19 ± 0.00^{c}	44	5.03 ± 0.24^{a}	1383			1.14 ± 0.04^{b}	101
1458	E-2-dodecenal			0.43 ± 0.01^{a}	119				
1477	Gamma-muurolene			0.56 ± 0.02^{a}	155				
1483	(E,E,Z)-2,4,7-decatrienal			1.15 ± 0.04^{a}	317				
1492	Anisic acid			1.67 ± 0.06^{a}	460			0.33 ± 0.01^{a}	29
1517	Cubebol					0.35 ± 0.01^{a}	22		
1520	Lilial							1.31 ± 0.05^{a}	115
1528	1,4-cadinadiene	0.16 ± 0.00^{b}	37	1.28 ± 0.04^{a}	352	0.17 ± 0.00^{b}	11		
1538	8,14-cedranoxide	0.72 ± 0.03^{d}	165	5.68 ± 0.21^{b}	1563	2.54 ± 0.11^{c}	158	43.79 ± 2.07^{a}	3853
1545	Elemol	0.62 ± 0.01^{d}	143	4.15 ± 0.19^{b}	1142	2.01 ± 0.08^{c}	125	6.30 ± 0.27^{a}	554
1555	Caryophyllene alcohol	0.74 ± 0.02^{b}	170	5.39 ± 0.25^{a}	1482	0.85 ± 0.03^{b}	53		
1564	E-nerolidol	0.23 ± 0.00^{b}	53	0.82 ± 0.03^{a}	226				
1573	Spathulenol							1.80 ± 0.07^{a}	158

Table 2–Continued.

RI	Compounds	*E. cineria*-HD Area	*E. cineria*-HD mg/kg	*E. cineria*-SCE Area	*E. cineria*-SCE mg/kg	*E. camaldulensis*-HD Area	*E. camaldulensis*-HD mg/kg	*E. camaldulensis*-SCE Area	*E. camaldulensis*-SCE mg/kg
1587	Viridiflorol	0.25 ± 0.01^{c}	58	1.68 ± 0.07^{a}	462	0.33 ± 0.01^{b}	20		
1603	Cedrenol	0.16 ± 0.00^{c}	37	0.61 ± 0.02^{b}	167			1.01 ± 0.05^{a}	89
1616	E-isolongifolanone							0.33 ± 0.01^{a}	29
1632	Hinesol	0.22 ± 0.00^{d}	51	1.15 ± 0.04^{b}	316	0.40 ± 0.01^{c}	25	1.80 ± 0.07^{a}	158
1660	14-hydroxy-9-epi-E-caryophyllene			0.30 ± 0.01^{b}	82			1.63 ± 0.07^{a}	144
1714	cis-beta-santalol			0.36 ± 0.01^{b}	100			3.05 ± 0.14^{a}	268
1717	Z,Z-farnesol							2.21 ± 0.10^{a}	194
1724	Alpha-cyperone							2.09 ± 0.08^{a}	184
1728	E-gamma-curcumen-12-ol							3.30 ± 0.15^{a}	290
1754	E,Z-farnesol			0.47 ± 0.01^{b}	130			1.93 ± 0.06^{a}	170
1766	Dehydrovomifoliol							0.75 ± 0.02^{a}	66
1774	Guaiazulene							0.78 ± 0.02^{a}	69
1800	5,13-dimethylheptadecane							0.36 ± 0.01^{a}	32
	Monoterpene hydrocarbons	9	2119	1	221	31	1943	1	94
	Monoterpene oxygenated	81.21	18679	57.47	15804	57.72	3579	13.80	1214
	Sesquiterpenes hydrocarbons	0.53	122	12.69	3490	1.61	100	7.71	678
	Sesquiterpenes oxygenated	2.94	676	20.62	5669	6.13	380	69.65	6129
	Phenolics	0	0	0	0	0	0	0.78	69
	Others	0.32	73	4.96	1364	0.37	23	6.99	615
	Total	94.22	21669	96.54	26548	97.17	6025	100	8800

Values within rows with uncommon superscripts (a to d) were significantly different ($P < 0.05$). Uncommon superscripts (a to d) posted for the percentages columns are valid for the columns of masses (mg/kg dry matter).

Table 3–Amount of the total phenolics (mg GAE/g dry mass) and antioxidant activity of the *E. cinerea* and *E. camaldulensis* extracts.

	E. cinerea HD	*E. cinerea* SCE	*E. camaldulensis* HD	*E. camaldulensis* SCE
Total phenolics (mg GAE/g dry plant material)	43.85 ± 1.03^{b}	3.9 ± 0.07^{c}	60.19 ± 1.33^{a}	2.16 ± 0.03^{d}
IC_{50} DPPH$^{\bullet}$ (mg/L)	1519 ± 52^{a}	468 ± 21^{d}	1146 ± 45^{b}	606 ± 30^{c}
IC_{50} ABTS$^{\bullet+}$ (mg/L)	399 ± 15^{a}	65 ± 3^{d}	183 ± 3^{b}	128 ± 4^{c}

Values within rows with uncommon superscripts (a to d) were significantly different ($P < 0.05$).

compounds are not present in SCE extracts, but the presence of other compounds, especially heavy, will make these compounds in trace quantity. Indeed, it would be surprising that SCE does not extract such kind of lighter compounds, which are known for being soluble in CO_2 in these conditions.

Determination of total phenolic content

The amounts of the total phenolics in leaves extracts were determined and the results were presented as GAEs (mg/g dry mass) in Table 3. The quantity of total phenolics has varied in the different extracts. By HD, essential oils from the 2 species contained a higher amount of total phenolics than SCE extracts (43.85 compared to 3.9 mg GAE/g extract from *E. cinerea*, respectively; 60.19 compared to 2.16 mg GAE/g extract from *E. camaldulensis*). This may be due to the low polarity of CO_2, which hinders extraction of heavy polar compounds. In addition, these compounds are not detected by GC-MS, which indicates that they are not volatile. Most studies upon HD and SCE extracts, which use GC-MS analyses to identify the volatile compounds, were not considered for analysis of phenolics. To our knowledge, no study quoted the quantification of phenolics in the extracts obtained by HD or SCE starting from these 2 species of Eucalyptus. We note the low total phenolics from SCE extracts compared to HD extracts. Similarly, Santos and others (2012) obtained 10.92 mg GAE/g extract in *E. globulus*.

Antioxidant activity

The DPPH$^{\cdot}$ and ABTS$^{\bullet+}$ assays are used to evaluate the antioxidant activity of plant extracts. The antioxidant activity by DPPH and ABTS assays of *E. camaldulensis* and *E. cinerea* essential oils has not been reported in the literature. The results of antioxidant activity of the 2 species of Eucalyptus obtained by HD and SCE are presented in Table 3. A high antioxidant activity was obtained with ABTS$^{\bullet+}$ assay in the SCE extracts from both *E. cinerea* (65 mg/L) and *E. camaldulensis (*128 mg/L) but all extracts of the 2 species exhibited very low DPPH$^{\cdot}$ radical-scavenging activity (468 to 1519 mg/L).

Poor antioxidant activities were obtained from other species of Eucalyptus, with DPPH assay. *E. gunnii* showed an IC_{50} of 272.93 mg/mL (Guimarães and others 2010), *E. grandis* had an IC_{50} of 13.63 mg/mL (Sewanu, 2012) and *E. citriodora* showed an IC_{50} of 425.4 mg/L (Singh and others 2012). Similarly, with ABTS assay, Sewanu (2012) obtained a little antioxidant activity ($IC_{50} = 97$ mg/mL) for *E. grandis*.

The antioxidant activity of SFE extracts is more important than that of the HD extracts. These results are in accordance with literature where the SCE extract from *E. camaldulensis* (Fadel and others 1999) and *E. globulus* (Santos and others 2012) were more active than HD essential oil.

Antioxidant activity is influenced by the phenolic composition of the extract (Ahmadi 2010). Although the SCE extracts contained lower amount of total phenolics than HD extracts, non-phenolics can take part to this activity. From all results obtained in this study it is clear that the chemical composition and antioxidant properties of the volatile oil from 2 Eucalyptus species are influenced by the methods of extraction. The SCE method is preferred to HD because SCE extracts give more important antioxidant activity. This particular result, gives an interest of the use of the SCE to concentrate the high antioxidant extracts.

In order to find new antioxidant extracts to use in food industry, the principal aim of this study was the application of SCE technology for extraction of natural antioxidants from medicinal and aromatic plants. We have achieved our objective with success for 2 Eucalyptus species (not previously studied) and we have determined the benefits of this technology and its efficiency to extract antioxidants.

Conclusion

The influence of the extraction process, HD or SCE, was studied for 2 species of Eucalyptus. SCE compared to HD presented some advantages: (i) faster extraction, (ii) improvement of the yield, and (iii) preparation of extracts with a powerful antioxidant activity.

Moreover, most of the disadvantages of conventional extraction methods of essential oil can be avoided by using SCE. This process offers an additional advantage over conventional methods because it is more efficient and cleaner. The antioxidant compounds are easily extractable by SFE rather than by HD. The natural products are important in different applications. The natural antioxidants are especially important for food industry, cosmetic, and pharmaceutical sectors. The use of SCE in industrial applications can provide several benefits to final products.

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