Comparison of *Eucalyptus camaldulensis* Dehn. Oils from Mozambique As Obtained by Hydrodistillation and Supercritical Carbon Dioxide Extraction

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Leaf oils of *E. camaldulensis* Dehn. from Mozambique obtained by hydrodistillation and by supercritical carbon dioxide extraction under different conditions were compared with regard to their major components. The oil obtained by hydrodistillation showed high concentrations of 1,8-cineole (43%), α-pinene (5.5%), *β*-pinene (3.4%), *p*-cymene (5.2%), terpinen-4-ol (3.1%), and globulol (4.1%). The extracts obtained by supercritical carbon dioxide extraction have lower amounts of 1,8-cineole, α-pinene, *β*-pinene, and terpinen-4-ol, but have higher amounts of *allo*-aromadendrene and globulol. At the same time, distilled oil of *E. radiata* was extracted from an inert matrix (Celite) and the extract showed a higher content of 1,8-cineole at 80 bar and 60 °C than the feed, suggesting that a multiple-stage supercritical carbon dioxide extraction of the *E. camaldulensis* studied can produce an oil close to the market requirements set by the European pharmacopoeias.

Keywords: *Eucalyptus; Camaldulensis; Radiata; Supercritical Carbon Dioxide; Extraction; Mozambique*

INTRODUCTION

The genus *Eucalyptus* includes more than 700 species and belongs to the family of Mitraceae. Originally from Australia, it has spread worldwide, particularly in Africa, because of its easy adaptability and fast growth (*1*). *E. camaldulensis* is grown in Mozambique and exploited especially for wood purposes, building material, and soil fixation (*2*).

Many works have been already published (*2*−*12*) about the composition of this species. Among the different *E. camaldulensis* studied, three chemotypes can be distinguished: one rich in 1,8-cineole (28−84%); one rich in *p*-cymene (20−30%), and one rich in spathulenol (18%)(*8*). The cultivar grown in Mozambique belongs to the 1,8-cineole rich chemotype (40%), as its composition fits into this group (*2*).

However, the European pharmacopoeias require eucalyptus oils with at least 70% of 1,8-cineole for market purposes (*13*). Thus, common distilled oils from eucalyptus leaves must fill this requirement in order to compete with other suppliers. Many species, including some subspecies of *E. camaldulensis* like the one grown in Mozambique, do not yield such purity of the oil, so further work on purification or enrichment must be carried out after distillation. Traditionally, subjecting the crude oil to further re-distillations under different conditions carries out this task (*14*).

Although distillation is a very convenient method for extracting essential oils from plant materials, it also has disadvantages because of artifacts produced during the process, specially when thermolabile components are involved (*15*).

In the present work we have studied the composition of *E. camaldulensis* oil obtained by both hydrodistillation and supercritical fluid extraction (SFE) in different combinations of temperature and pressure and observed the composition variations of the different oils obtained in regard to their major components. SFE has the advantage of utilizing relatively low temperatures, and so the oil recovered is more close to the natural essential oil and free of artifacts. Furthermore the easy possibility of manipulating the pressure and temperature makes it possible to extract oils of desired characteristics (*16*). Again, SFE acting as a solvent can extract components from the plant material which are not extractable by distillation, what may or may not be desirable depending on the intended use of the oil.

The aim of this study is to determine whether any of the supercritical conditions used can provide a greater amount of 1,8-cineole, providing a possible alternative method for obtaining oil which may fill the European pharmacopoeias' requirements for the market.

MATERIALS AND METHODS

Materials. *E. camaldulensis* Dehn. leaves were harvested from adult plants (12 years old) on April 1999 (cold and dry season) at INIA (Instituto Nacional de Investigação Agronómica) in Maputo, Mozambique. Carbon dioxide, 99.99% grade, was supplied by Alfa (Sweden), and Hexane (C_6H_{12}), 99% grade was supplied by Kebob AB (Sweden). G. R. Davis PTY Ltd., Australia, supplied the crude extract of E. radiata.

Sample Preparation. After harvesting, the eucalyptus leaves were allowed to dry indoors for 20 days at 20 °C and subsequently stored in a cold room at 4 °C. For both hydrodistillation and supercritical carbon dioxide extraction, the *Eucalyptus* leaves were milled in a KAMAS SKV200 hammer mill, manufactured in Malmö, Sweden.

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Table 1. Composition of Eucalyptus Leaf Oil as Obtained by Hydrodistillation (HD) and Supercritical CO_2 at Different Pressures at 40 °C

	Composition; (%) of peak area					
compound	HD	80 bar	100 bar	150 bar	200 bar	250 bar
α-pinene	5.5	2.6	1.5	1.7	1.8	2.1
β-pinene	3.4	1.8	1.1	1.2	1.2	1.4
limonene	2.5	2.5	2.4	2.5	1.8	2.9
1,8-cineole	43.4	39.1	34.0	32.2	32.5	33.4
γ-terpinene	3.9	2.5	1.3	1.7	1.1	1.9
p-cymene	5.2	2.1	1.7	1.5	1.3	1.5
C10H14	0.9	1.8	1.7	1.8	1.8	1.7
terpinen-4-ol	3.1	0.5	0.4	0.4	0.5	0.4
allo-aromadendrene	1.9	3.9	5.1	4.6	5.5	3.8
α-terpineol	2.6	0.4	0.5	0.5	0.5	0.5
globulol	4.1	5.1	4.5	4.8	4.8	5.0

Hydrodistillation. A 500-mL round-bottom glass flask was filled with 300 mL of distilled water and 50 g of milled eucalyptus leaves, coupled to a Karlsruher apparatus (*17*), and boiled at atmospheric pressure for about 4 h as recommended by the Dutch pharmacopoeia (*18*). The oil recovered was kept in a refrigerator for its further composition analysis by GC−MS.

Supercritical Fluid Extraction. *Extraction from E. camaldulensis Dehn. Leaves.* A supercritical extraction was performed in an apparatus as described by Dauksas (*19*). A 50-mL stainless steel extractor was loaded with 12 g of milled eucalyptus leaves. The system was operated at a constant temperature of 40 °C as an optimal operating temperature (*20*) for all the range of pressures used: 80, 100, 150, 200, and 250 bar. The flow rate was kept constant at 250 mL/min in all runs for 2 h, the total extraction time used. The collected samples were sealed and stored in a refrigerator for analysis by GC−MS.

Extraction from E. radiata Distilled Oil. For *E. radiata*, 1 g of oil was mixed in 3 g of Celite diatomaceous earth, filled into a 30-mL stainless steel extractor, and the extraction process was carried out at 250 mL/min carbon dioxide flow rate until 6 liters of CO_2 had passed throughout. The pressure was set to 80, 100, 150, and 250 bar, and the temperature was set at 40 °C and 60 °C for each pressure in each run. The collected samples were stored in a refrigerator for further GC−MS analysis

Gas Chromatography/Mass Spectrometry. CG analyses were carried out in a Shimadzu 14a GC filled with a DB-Wax column (30 m, 0.32 mm i.d., 0.15 μm film thickness). The oven temperature was programmed from 50 °C (5 min) and then rose at 4 °C/min up to 230 °C in a total time of 90 min. The injection was made in a split mode 1:50 at an on-column injector temperature of 250 °C.

Helium was used as the carrier gas at a pressure of 0.5 kg/cm^2. The mass spectrophotometer SX 102 was used as the detector, operating at 70 eV and a scan rate of 33 to 800 amu/s. The trials were performed in duplicate.

The compounds were identified by comparison of their retention times, mass spectra, and the known compounds previously published (*2, 6, 21*) and the amount taken as the percentage of TIC with the response factor estimated as 1 for all compounds.

Table 3. Extraction Yields of *E. Camaldulensis* Leaf Oils and *E. Radiata* Oil

P/T (bar/°C)	*E. radiata* (% w/w) re-extraction[a]	*E. camaldensis* (% w/w) extraction[b]
80/40	5.5	1.5
80/60	3.8	
100/40	4.7	1.4
100/60	4.6	
150/40	5.2	1.8
150/60	4.1	
250/40	4.9	2.0
250/60	4.6	

[a] mass of extract/mass of added oil. [b] mass of extract/mass of dry leaves.

RESULTS AND DISCUSSION

Hydrodistillation Results. *E. camaldulensis* Dehn. from Mozambique presents high amounts of 1,8-cineole (43%), α-pinene (6%), *β*-pinene (3%), *p*-cymene (5%), *γ*-terpinene (4%), and globulol (4%), among other compounds (Table 1). These results are in accordance with those published earlier by Pagula and co-workers (*2*) on the same species grown in the same place for the same components. As expected there are slight differences that can be attributed to the sample randomness and experimental conditions, as well as the sample harvesting time (*8, 9*).

Results of Supercritical Carbon Dioxide Extraction. Although in the CG−MS many compounds have been eluted, only those considered relevant for the present discussion have been reported in Table 1. For these compounds it is observed that, in general, supercritical carbon dioxide extraction has yielded lower amounts for the discrete compounds as compared with the yields from hydrodistillation (HD). This is excepted for limonene at 80 and 250 bar, and C10H14, *allo*-aromadendrene, and globulol for all the range of pressures considered. Additionally, it is observed that for the reported compounds, the amounts yielded are more closely comparable at 80 and 250 bar.

Yields. The yields on the distillation and SFE extraction processes of *E. camaldulensis* Dehn. leaf oils and those on the SFE treatment of the *E. radiata* distilled oils are displayed in Table 3.

DISCUSSION

The reduced percentage amounts of terpenes obtained in supercritical conditions in comparison to those from the distilled extracts are expected, because supercritical carbon dioxide, as a solvent, can extract other than volatile compounds. Similar results have been achieved earlier (*21*).

Alcohols such as terpinen-4-ol and α-terpineol (Table 1) are present in lower percentages in SFE extracts compared to hydrodistilled extract. This may be because

Table 2. Composition of *E. Radiata* Hydrodistilled Oil and Oil Treated at Different SFE Conditions

	P (bar)/T (°C)								
compound	HD	80/40	80/60	100/40	100/60	150/40	150/60	250/40	250/60
α-pinene	2.59	2.05	2.66	0.78	1.94	2.21	1.50	2.23	2.16
α-terpinene	1.39	1.29	1.70	1.08	1.30	1.30	1.32	1.37	1.32
limonene	5.59	1.38	1.70	1.08	1.30	1.30	1.32	1.37	1.32
1,8-cineole	76.36	80.91	86.51	74.24	80.86	81.23	80.74	80.84	80.54
terpinen-4-ol	1.30	1.46	0.88	2.26	1.56	1.38	1.54	1.41	1.47
δ-terpineol	1.31	1.65	0.60	2.80	1.58	1.61	1.80	1.52	1.72
α-terpineol	7.95	8.08	3.98	13.05	8.19	7 0.74	8.50	7.79	8.47

of the low polarity of CO_2. However, this observation is not applicable to globulol, for which the higher percentage occurred in supercritical carbon dioxide extracts. Regardless, in both cases there is an agreement with the literature available (*21*).

The sesquiterpene *allo*-aromadendrene, absent in distilled Egyptian *E. camaldulensis* (*21*) and less abundant in adult leaves of the Mozambican *E. camaldulensis* (*2*), was recovered in both cases in supercritical carbon dioxide extracts in our study. It was found in greater amounts in supercritical carbon dioxide extracts than in those obtained by hydrodistillation. Once again our results agree with those of Fadel and co-workers (*21*). In their study, *allo*-aromadendrene was completely undetected in distilled extracts, whereas it was detected in important amounts in supercritical carbon dioxide extracts. High-molecular-weight compounds including esters, fatty acids, and waxy-like compounds are more likely to be extracted by CO_2 rather than by hydrodistillation which is more likely to extract the volatile compounds (*20*, *22*−*24*).

At 80 bar, the concentrations of the compounds listed are the highest in the range of pressure variation. This is expected at the conditions set because all essential oils are freely soluble in compressed carbon dioxide, and their solubilities rise exponentially between 70 and 100 bar at 40 °C as a result of the rapid rise of density of the carbon dioxide (*25*). As the pressure increases, the density, which commands the solvating power, increases too, but slowly. This explains the fact that the compositions at higher pressures in the oil showed almost no change.

Apparently, the opposite effect is observed in Italian *E. globulus* (*26*). A higher cineole content was achieved in supercritical carbon dioxide extraction than was achieved in distillation. This is because, in their case, they performed two fractions during the extraction process, one of which was rich in terpene-like compounds and consequently had a higher cineole content. Otherwise, the processing of extracted oils yields richer contents in some terpenes. We have processed *E. radiata* distilled oil and the results showed an increase of cineole content in a reasonable percentage (Table 2). A remarkable difference in composition over the range of supercritical conditions used can be observed at 80 bar and 60 °C. The 1,8-cineole content (86.51%) of the oil has an increase of 13.4% compared to that of the distilled sample, whereas from 100 bar and 60 °C upward, there is a constant change of about 6% regarding this component as compared to the same base. The abrupt changes of the physical-chemistry characteristics of a compressed gas at the critical point occur in a restricted range of pressure, from the critical pressure (73 bar) to about 200 bar. A slow and steadily weakening increase of density is then observed at high pressures (*25*). This was also observed in our studies. The increase of pressure does not make great changes in the compositions of the obtained oils. This is valid for both the case of extraction of *E. camaldulensis* leaves as well as for the case of *E. radiata* distilled oil reprocessing. In the case of *E. radiata*, the temperature increase up to 60 °C favors the yields of 1,8-cineole, suggesting the existence of relative selectivity of CO_2 for the different compounds in this interval of study on the basis of their physical-chemistry characteristics. Somewhat in contradiction with what was observed with the eucalyptus extracts above-discussed, the terpene alcohol contents in *E. radiata* oils in supercritical carbon dioxide (Table 2) are higher than those in the original distilled oil. As mentioned earlier, high-MW compounds are more likely to be extracted by supercritical carbon dioxide than by distillation. Because the *E. radiata* oil had already been obtained by distillation, these alcohols are now components with high-MW in the mixture, so they are more likely to be extracted with the supercritical carbon dioxide than the present monoterpenes which are present in lower percentages.

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