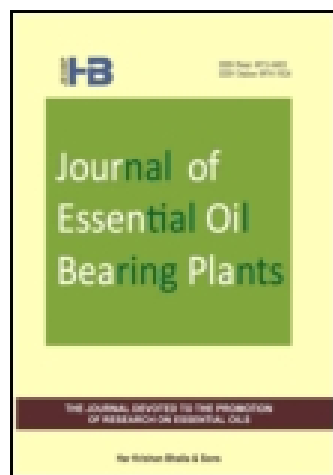


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## Chemical Constituents of Essential Oils from Three Eucalyptus Species Acclimatized in Ethiopia and Kenya

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**Abstract:** Essential oils obtained by steam distillation of three Eucalyptus species acclimatized in Ethiopia and Kenya were analyzed using GC and GC-MS. The species were *Eucalyptus citriodora*, *Eucalyptus globulus* and *Eucalyptus camaldulensis*. Results showed that the oils from *E. globulus* and *E. citriodora* from trees grown in the two countries were quite comparable. The major component of the essential oil of *E. globulus* was 1,8-cineole while that for *E. citriodora* was citronellal. The oils from *E. camaldulensis* grown in the two countries, however, were found to differ. While trees from Kenya afforded an oil rich in 1,8-cineole, those from Ethiopia yielded *p*-cymene and cryptone as major components. Oils from *E. camaldulensis* grown in two different locations in Ethiopia were also found to vary in their *p*-cymene content; one had 35 % while the other gave 21.2 %.

**Key words:** *Eucalyptus citriodora*; *E. globulus*; *E. camaldulensis*; Myrtaceae; Essential oils; Chemical composition.

**Introduction:** The genus *Eucalyptus* (Myrtaceae) is a well known source of eucalyptus oil which is widely used in pharmaceutical, confectionery and cosmetic industries <sup>1,2,3</sup>. 1,8-cineole, the most widely used component of the oil is an important ingredient in many cough and expectorant preparations <sup>4</sup>. Many species of the genus *Eucalyptus* have been introduced both in Ethiopia and Kenya; among them are *E. globulus*, *E. citriodora* and *E. camaldulensis*. The species *E. globulus* is known to contain 1,8-cineole while *E. citriodora* yields citronellal, both used in perfumery industry <sup>5,6,7,8,9,10</sup>.

The essential oil obtained from the leaf of *E. camaldulensis* vary in composition

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depending on the geographical location<sup>8,11</sup>. Species acclimatized in Burundi were reported to contain 1,8-cineole (43.0 %) as the major component along with limonene (24.0 %), *p*-cymene (13.0 %),  $\alpha$ -pinene (10.0 %) and  $\gamma$ -terpinene (5.0 %) <sup>12</sup>. The same species grown in Morocco were reported to contain essential oils of varying chemical compositions. In one report, 1,8-cineole was the major component with a concentration range of 50-70 %,  $\alpha$ -pinene (5-26 %),  $\alpha$ -terpineol (1-8 %) and trace amount of  $\gamma$ -terpinene <sup>5</sup>. In another report, though 1,8-cineole was the major component, its concentration was lower (45.0 %); other components reported in appreciable amounts were *p*-cymene (23.0 %),  $\alpha$ -pinene (10.0 %),  $\gamma$ -terpinene (8.0 %) and limonene (6.0 %) <sup>13</sup>. The leafy oil of *E. camaldulensis* grown in the Mediterranean region of Turkey contains 1,8-cineole (75.0 %), isovaleraldehyde, geraniol,  $\alpha$ -phellandrene, cuminal and phellandral <sup>14</sup>. Chemical analysis of oil from the same species endemic to South Africa afforded *p*-cymene (48.0 %) as the major component in addition to 1,8-cineole (7.0 %),  $\alpha$ -pinene (10.0 %),  $\beta$ -pinene (17.0 %), myrcene,  $\alpha$ -phellandrene and limonene <sup>4</sup>.

On the other hand, chemical composition of oils from Australian species afforded  $\alpha$ -pinene,  $\beta$ -pinene,  $\alpha$ -phellandrene,  $\beta$ -phellandrene, 1,8-cineole (18.0 %), *p*-cymene (17.0 %), myrcene and limonene (11.0 %) as the major components <sup>15</sup> while those from India have been reported to contain 1,8-cineole (28.0 %), *p*-cymene (25.0 %),  $\alpha$ -pinene (16.0 %),  $\beta$ -pinene (16.0%) and  $\alpha$ -terpinene (7.0 %) <sup>16</sup>.

In this study we report the variation in chemical composition of three eucalyptus species grown in the two neighboring countries, Ethiopia and Kenya.

### Experimental

**Plant materials from Ethiopia:** Fresh leaves of *E. globulus* (5 kg; voucher number, Abera A. S.-767) were collected from Arat Kilo Science Campus; *E. citriodora* (5 kg; Abera A. S.-698) was collected from Wondo Genet EORC farm site and *E. camaldulensis* (5 kg; voucher Abera A. S. - 808) was collected from Wondo Genet and also from Arat Kilo Science Campus (7 kg; voucher number, Pauline M. S.- 924). All voucher specimens were deposited at the National herbarium, Addis Ababa University.

**Plant materials from Kenya:** Fresh leaves of *E. globulus* (10 kg; voucher number, Manguro EG/4/2001); *E. citriodora* (10 kg; voucher specimen, Manguro ECI/4/2001) and *E. camaldulensis* (10 kg; voucher number, Manguro ECA/4/2001) were collected from the forest near Kenya Forestry Research Institute (KEFRI) and were confirmed with authentic specimens at the Kenya National Museum herbarium.

**Oil extraction:** Fresh leaf materials (approximately 10 kg) of each plant from Kenya were subjected to hydro-distillation using Cleveenger apparatus for 4 h. The volatile distillates were dehydrated ( $\text{Na}_2\text{SO}_4$ ) and kept in screw-capped vials in a deep freezer prior to analysis. *E. globulus* produced the highest quantity of oil (20 ml), followed by *E. citriodora* (15 ml) and *E. camadulencis* (13.5 ml). Ethiopian plants were similarly subjected to hydro-distillation for 4 h and produced oils in the following order: *E. globulus* (7.5 ml), *E. citriodora* (6.0 ml), *E. camadulensis* (from Hondo, 3.5 ml) and *E. camadulensis* (from Genet Arat Campus, 3.2 ml).

**Gas chromatography:** GC analyses were performed using Varian model 3700 GC (FID detector, 270°C) with fused silica HP-1 capillary column (30 x 0.25 mm, I.D) and N<sub>2</sub> (40 ml per minute) as carrier gas. Injection temperature 220°C, oven temperature was programmed from 50-210°C at a rate of 3°C per minute.

**Gas chromatography-Mass spectrometer:** GC-MS analyses were carried out on a fusion GC model 8000 series chromatograph coupled to MD 800 mass selective detector using DB-17 (30 x 0.25 mm) fused silica capillary column coated with medium polarity liquid phase. Helium was used as a carrier gas (5 psi). Injection temperature 220°C, oven temperature initially at 50°C rising to 210°C at 4°C per minute. The mass spectra were acquired at 70 eV ionization at a rate of 2°C per second.

The oil constituents were identified by matching their mass with those in NIST and Willy libraries, and further confirmed by peak enhancement and GC retention time, and also by comparison of their mass spectra with those of authentic reference compounds.

**Isolation of major components:** Isolation of major components of *E. globulus* and *E. citriodora* oils was achieved using column chromatography; eluent n-hexane to give 1,8-cineole (50 mg) and citronellal (25 mg), respectively. The structures of the isolates were determined using NMR and MS techniques as previously reported<sup>17</sup>.

**Results and discussion:** Examination of results (Table 1.) showed that *E. globulus* oils of Ethiopian and Kenyan origin had 1,8-cineole of 81.6 % and 79.6 % concentration, respectively. Although, the composition of the oils from trees grown in the two countries are quite comparable, the percentage of minor components such as  $\alpha$ -pinene, limonene,  $\alpha$ -terpineol and myrcene were found to be slightly higher in the Kenyan oil, while  $\alpha$ -terpinyl acetate and 1,8-cineole were higher in the Ethiopian one. Components such as  $\beta$ -pinene and  $\gamma$ -terpinene were not found in the oil from Ethiopian plant. Only two sesquiterpenes,  $\beta$ -eudesmol and alloaromadendrene, present in the oil from the Kenyan plant, could be identified clearly from their retention times.

The species *E. citriodora* had citronellal as the major component: 76.4 % (Ethiopia) and 84.9 % (Kenya). The oils were also found to be comparable in both cases, with citronellal and citronellyl acetate higher in the oil from Kenyan plant while citronellol and isopulegol were higher in the oil from Ethiopia species.  $\alpha$ -Pinene was only present in the oil from plant acclimatized to Ethiopia, while *trans*-ocimene, limonene and  $\gamma$ -terpinene were only found in the oil from Kenya.

It is interesting to note that the constitution of oils of *E. camaldulensis* from the two countries differ considerably. The oil from Kenya was rich in 1,8-cineole (> 92.0 %) while that from trees grown in Ethiopia had p-cymene (35.0 %) and cryptone (26.0 %) as the major components. It was also found that even the leaf oils from the two localities in Ethiopia yielded relatively different results. p-Cymene was absent in the Kenyan oil while 1,8-cineole was not found in oil from Ethiopian trees.

Terpinen-4-ol,  $\alpha$ -pinene and myrcene were found in the *E. camaldulensis* oils from the three localities but the percentage of terpinen-4-ol was higher in the oil from Ethiopian

trees than the Kenyan one. Limonene and  $\alpha$ -terpinyl acetate were absent in the oil from Wondo Genet, but present in the other two oils.  $\alpha$ -Terpinyl acetate was highest in the oil from Arat Kilo Campus.  $\alpha$ -Terpineol and  $\beta$ -pinene were absent in oil from Arat Kilo Campus but present in the other two oils.

Phellandral, p-cymen-7-ol, carvacrol, caryophellene oxide and spathulenol could be detected only in the oil from Wondo Genet while sabinene,  $\alpha$ -terpinene,  $\gamma$ -terpinene and  $\beta$ -phellandrene were found in the oil from Arat Kilo. p-Cymene,  $\alpha$ -thujene,  $\alpha$ -phellandrene and cuminaldehyde were common in the Ethiopian oils but absent in the Kenyan one. p-Cymene and  $\alpha$ -phellandrene were similarly in higher percentages in oils from Arat Kilo (35.0 % and 5.0 %) than in the oil from Wondo Genet (21.0 % and 0.2 %). The percentage of  $\alpha$ -thujene and cuminaldehyde were higher in the oil from Arat Kilo.

From the results, it can be seen that essential oils of *E. globulus* and *E. citriodora* from the two neighbouring countries were similar except for minor differences which might be due to other factors not considered here such as age of the trees. The leaf oil of *E. camaldulensis*, from the two countries and also from different localities in the same country was found to vary considerably.

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Table 1. Chemical composition (%) of Eucalyptus essential oils obtained from three species grown in Ethiopia and Kenya

RI	Component	Chemical composition %									Identification method
		<i>E. globulus</i>			<i>E. citriodora</i>			<i>E. camaldulensis</i>			
		Ethiop	Kenya	Ethiop	Kenya	W/Genet	Arat Kilo	Kenya			
921	<i>trans</i> -Ocimene	-	-	-	0.1	-	-	-	-	PE	
926	$\alpha$ -Thujene	-	-	-	0.3	0.5	0.9	-	-	PE	
930	$\alpha$ -Pinene	2.8	6.9	0.1	-	0.5	0.6	0.4	-	PE	
975	Sabinene	-	-	-	-	-	0.7	-	-	PE	
980	$\beta$ -Pinene	-	0.2	0.2	tr	1.4	-	tr	-	PE	
989	Myrcene	tr	0.2	tr	0.1	0.3	0.5	0.1	-	PE	
1004	$\alpha$ -Phellandrene	-	-	-	-	0.2	5.0	-	-	PE	
1018	$\alpha$ -Terpinene	-	-	-	-	-	0.7	-	-	PE	
1023	p-Cymene	-	-	-	-	21.2	35	-	-	PE,	
1032	$\beta$ -Phellandrene	-	-	-	-	-	11.3	-	-	PE	
1035	1,8-Cineole	81.6	79.6	tr	0.3	-	-	> 92	-	PE, <sup>1</sup> H& <sup>13</sup> C NMR	
1047	Limonene	1.1	2.7	-	0.1	-	1.1	2.2	-	PE	
1061	$\gamma$ -Terpinene	-	0.6	-	0.1	-	0.8	-	-	PE	
1084	Terpinolene	-	-	0.1	0.1	-	-	-	-	PE	
1100	Linalool	-	0.1	0.2	0.3	-	-	-	-	PE	
1130	Isopelugol	tr	-	4.7	3.7	-	-	-	-	PE	
1148	$\alpha$ -Terpineol	0.5	3.8	-	-	0.5	-	2.0	-	PE	
1157	Cryptone	-	-	-	-	-	26.3	-	-	PE	
1184	Terpinen-4-ol	-	0.5	-	-	6.8	4.1	0.6	-	PE	
1191	p-Cymen-7-ol	-	-	-	-	1.7	-	-	-	PE	
1201	Citronellol	-	-	7.8	-	-	-	-	-	PE	
1229	Phellandral	-	-	-	-	4.2	-	-	-	PE	

table 1. (continued).

RI	Component	Chemical composition %						Identification method	
		<i>E.globulus</i>		<i>E. citriodora</i>		<i>E. camaldulensis</i>			
		Ethiop	Kenya	Ethiop	Kenya	W/Genet	Arat Kilo	Kenya	
1241	Citronellal	-	-	76.4	84.9	-	-	-	PE, <sup>1</sup> H& <sup>13</sup> C NMR
1291	Carvacrol	-	-	-	4.5	0.7	3.2	-	PE
1328	Terpinyl acetate	1.6	0.4	-	0.1	-	5.4	0.4	PE
1353	Cuminaldehyde	2.8	-	-	4.2	3.2	7.0	-	PE
1411	<i>trans</i> -Caryophyllene	2.5	-	0.2	0.1	1.7	-	-	PE
1430	Citronellyl acetate	-	-	1.9	2.5	-	-	-	PE
1462	Alloaromadendrene	-	0.2	-	-	-	-	-	PE
1544	Geranyl acetate	-	-	tr	-	-	-	-	PE
1578	Spathulenol	-	-	-	-	2.3	-	-	PE
1610	Caryophyllene oxide	-	-	-	-	3.4	-	-	PE
1652	$\beta$ -Eudesmol	-	0.3	-	-	-	-	-	PE

Ethiop. = Ethiopia

W/Genet = Wondo Genet

PE = confirmed by Peak Enhancement (co-injected with authentic samples)

Tr = trace (&lt;0.1).