Iranian Journal of Pharmaceutical Research (2007), 6 (2): 135-140
Received: October 2005
Accepted: June 2006

Short Communication

Chemical Composition of the Essential Oils of Four Cultivated *Eucalyptus* Species in Iran as Medicinal Plants *(E. microtheca, E. spathulata, E. largiflorens* and *E. torquata)*

Fatemeh Sefidkon*, Mohammad Hassan Assareh, Zahra Abravesh and Mohammad Mehdi Barazandeh

Research Institute of Forests and Rangelands,Tehran, Iran.

Abstract

The leaves of four cultivated *Eucalyptus* species, *Eucalyptus microtheca* var. *Microtheca F. Muell*., *Eucalyptus spathulata*, *Eucalyptus largiflorens* and *Eucalyptus torquata* were collected in spring from Kashan and Isfahan provinces (central region of Iran). After drying the plant materials in shade, their essential oils were obtained by hydro-distillation. The oils were analyzed by capillary gas chromatography, using flame ionization and mass spectrometric detection.

Twenty-two components were identified in the oil of *Eucalyptus microtheca* with 1,8-cineole (34.0%), p-cymene (12.4%), α-pinene (10.7%) and β-pinene (10.5%) as main constituents. Twenty-one compounds were identified in the oil of *Eucalyptus spathulata* with 1,8-cineole (72.5%) and α-pinene (12.7%) as main components. Twenty-six compounds were characterized in the oil of *Eucalyptus largiflorens* with 1,8-cineole (37.5%), p-cymene (17.4%) and neo-isoverbenol (9.1%) as main components. Sixteen compounds were characterized in the oil of *Eucalyptus torquata* with 1,8-cineole (66.9%) α-pinene (13.9%) and trans-pinocarveol (6.3%) as main constituents. The results showed that although the 1,8-cineole was the main component of the essential oils of all *Eucalyptus* species, but its relative content was higher in the oil of *Eucalyptus spathulata* and *Eucalyptus torquata.*

Keywords: *Eucalyptus microtheca*; *Eucalyptus spathulata; Eucalyptus largiflorens, Eucalyptus torquata*.

Introduction

There are over 700 different species of *Eucalyptus* in the world, of which at least 500 produce a type of essential oil. The leaves and oils of many *Eucalyptus* species are especially used for respiratory aliments such as bronchitis and croup (1-4) and the dried leaves are smoked like tobacco for asthma in some countries. Some of the *Eucalyptus* species are also used for feverish conditions e.g.(malaria, typhoid, cholera) and skin problems like burns, ulcers and wounds (5). Aqueous extracts are used for aching joints, bacterial dysentery, ringworms, tuberculosis, etc. They are applied for similar reasons in both western and eastern medicine. The *Eucalyptus* oils and their main component (1,8-sineole) are largely used in the preparation of liniments, inhalants, cough syrups, ointments, toothpaste and also as pharmaceutical flavours in veterinary practice and dentistry. While being used as fragrance component in soaps, detergents and toiletries, they have little use as perfumes. The

* Corresponding author:
E-mail:frsef@rifr.ac.ir

oils of *Eucalyptus* species have also antioxidant properties (6) and anti-inflammatory effects (7-8) because of 1,8-cineole.

The European Pharmacopoeia monograph for *Eucalyptus* oil specifies a chromatographic profile: 1,8-cineole (=eucalyptol; not less than 70%), limonene (4- 12%), α-pinene (2-8%), α-phellandrene (less than 1.5%), β-pinene (less than 0.5%), camphor (less than 0.1%) (9).

To meet these requirements and to minimize less desirable substances such as aldehydes, the oil obtained from initial steam distillation is rectified by alkaline treatment and fractional distillation. The rectified oil contains 70-90% of 1,8-cineole (10-12). Sesquiterpenes such as globulol and aromadendrene, which are usually present in unrectified, steam-distilled oil (13), were not detected in the rectified oils.

In this study the essential oils of four cultivated and adapted *Eucalyptus* species in warm regions of Iran were investigated for their essential oil content and composition. We have also reported the oil content and composition of five other cultivated *Eucalyptus* species from these locations previously (14).

There are many references about the composition of other *Eucalyptus* species in the literature. For example, the essential oils obtained by steam distillation from the leaves of nine *Eucalyptus* species (*E. cinerea* F. Muell., *E. baueriana* F. Muell., *E. smithii* R. T. Baker, *E. bridgesiana* R. T. Baker, *E. microtheca* F. Muell., *E. foecunda* Schau., *E. pulverulenta* Sims, *E. propinqua* Deane and Maiden, *E. erythrocorys* F. Muell.) of Moroccan origin have been analyzed using GC and GC-MS. A total of 83 constituents were identified. All the species investigated were found to possess an oil rich in 1,8-cineole (>68%). In five species (*E. cinerea* F. Muell., *E. baueriana* F. Muell., *E. smithii* R. *T. Baker*, *E. bridgesiana* R. T. Baker and *E. microtheca* F. Muell.), the 1,8-cineole content exceeded 80% (15).

The volatile oils of leaves of *Eucalyptus nutans, E. platypus* Hook. var. *platypus, E. platypus* Hook. var. *heterophylla* Blakely, *E. spathulata* Hook. subsp. *spathulata, E. spathulata* Hook. subsp. *grandiflora* (Benth.) L.A.S. Johnson and D.F. Blaxell, *E. steedmanii* C.A. Gardner, *E. eremophila* (Diels) Maiden subsp. *eremophila, E. salubris* F. Muell. subsp. *salubris, E. ravida* L.A.S. Johnson and K.D. Hill, *E. campaspe* S. Moore, *E. diptera* C.R.P. Andrews, *E. terebra* L.A.S. Johnson and K.D. Hill, *E. doratoxylon* F. Muell., and *E. decurva* F. Muell, isolated by vacuum distillation, were analysed by GC and GC-MS. All species contained α-pinene (2.8-32.5%), 1,8-cineole (8.2-51.2%), *p*-cymene (0.3-3.3%), aromadendrene (2.3-19.0%) and bicyclogermacrene (0.3-28.6%) as principal leaf oil components (16).

During the period 1995-1997, the essential oils of leaves of 16 taxa of *Eucalyptus* had monitored to see if their oil compositions were essentially stable. These species were *Eucalyptus tumida* Brooker & Hopper; *Eucalyptus histophylla* Brooker & Hopper; *Eucalyptus flavida* Brooker & Hopper; *Eucalyptus clivicola* Brooker & Hopper; *Eucalyptus varia* Brooker & Hopper subsp. *varia*; *Eucalyptus varia* Brooker & Hopper subsp. *salsuginosa* Brooker & Hopper; *Eucalyptus angustissima* F. Muell. subsp. *angustissima*; *Eucalyptus balladoniensis* Brooker subsp. *balladoniensis; Eucalyptus cyclostoma* Brooker; *Eucalyptus aequioperta* Brooker & Hopper; *Eucalyptus* species aff. *pileata* (E. sp. U in Brooker & Kleinig); *Eucalyptus* species aff. dumosa; *Eucalyptus calcicola* Brooker; *Eucalyptus ligulata* Brooker; *Eucalyptus aquilina* Brooker and *Eucalyptus preissiana* Schauer subsp. *lobata* Brooker & Slee. The main components in the oils were torquatone, bicyclogermacrene and 1,8-cineole. The results indicate that during the period of observation, the compositions of all the essential oils were quite stable, except for two species which exhibited a seasonal variation. By March 1997, all taxa had generated buds and six had flowered (17).

Experimental

Materials and Methods

Plant Material

The seeds of some *Eucalyptus* species (Origin: Australia) were cultivated in the years 1993-1994 in Kashan in the central region of Iran. Some of these species have good adaptability with the climatic condition of Kashan (Hot and dry weather). The fresh leaves of four adapted

Table 1. Plant materials used for this study

Species	Collecting Date	Oil Yield (v/w)	Oil Color
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]

Eucalyptus species were collected in the middle of spring (2005), as mentioned in Table 1. The voucher specimens have been deposited in the national herbarium of Iran (TARI).

Isolation procedure

Air-dried leaves of the plants (50-70 g, three times) were subjected to hydro-distillation for 2.5h using a Clevenger-type apparatus. The oils separated from water and dried over anhydrous sodium sulfate and stored in sealed vials at low temperature before analysis.

GC and GC/MS analysis

The oils obtained from three distillation of each *Eucalyptus* species were mixed and then injected to GC and GC/MS. GC analyses were performed using a Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d., film thickness 0.25 μm). Oven temperature was was programmed to be held at 40°C for 5 minutes and then increased to 280 °C at a rate of 4 °C/min.. Injector and detector (FID) temperatures were 290 °C, and helium was used as carrier gas with a linear velocity of 32 cm/s, and split ratio 1/60.

GC-MS analyses were carried out on a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d.). Oven temperature was 40 °C increasing to 250 °C at a rate of 4 °C, transfer line temperature 260 °C. The carrier gas was helium with a linear velocity of 31.5 cm/s, split ratio 1/60, Ionization energy 70 eV, scan time1 s and mass range of 40-300 amu. The percentages of compounds were calculated by the area normalization method, without considering response factors. The components of the oils were identified by comparison of their mass spectra with those of a computer library or with authentic compounds, and confirmed by comparison of their retention indices either with those of authentic compounds or with data published in the literature (18- 19). The retention indices were calculated for all volatile constituents using a homologous series of n-alkanes.

Results and Discussion

The oils isolated by hydro-distillation from the leaves of *Eucalyptus microtheca* var. *microtheca* F. Muell., *Eucalyptus spathulata*, *Eucalyptus largiflorens* and *Eucalyptus torquata* were found to be colorless to pale yellow liquids. These oils were analyzed by capillary gas chromatography, using flame ionization and mass spectrometric detection. The mean oil yields of each species are shown in Table 1.

Twenty-two components were identified in the oil of *Eucalyptus microtheca.* The major components were 1,8-cineole (34.0%), p-cymene (12.4%), α-pinene (10.7%), β-pinene (10.5%) and virdiflorene (5.2%). Twenty-one compounds were identified in the oil of *Eucalyptus spathulata.* The main components of this oil were 1,8-cineole (72.5%), α-pinene (12.7%) and trans-pinocarveol (3.3%. Twenty-six compounds were characterized in the oil of *Eucalyptus largiflorens.* The main components of this oil were 1,8-cineole (37.5%), p-cymene (17.4%), neo-isoverbenol (9.1%), limonene (6.5%) and terpinen-4-ol (3.6%). Sixteen compounds were characterized in the oil of *Eucalyptus torquata.* The main components of this oil were 1,8-cineole (66.9%) α-pinene (13.9%), trans-pincarveol (6.3%) and p-cymene (4.2%).

The chemical composition of the oils can be seen in Table 2. The components are listed in the order of their elution on the DB-5 column.

The results showed that although 1, 8-cineole was the main component of the essential oils of all *Eucalyptus* species, but its relative amount in

Table 2. Percentage composition of the oils of *Eucalyptus* species

No	Compound	RI	*E. microtheca*	*E. spathulata*	*E. leptophleba*	*E. torquata*	Method of Identification
1	α-thujene	928	0.4	-	1.3	-	MS, RI
2	α-pinene	935	10.7	12.7	2.4	13.9	MS, RI, Cal
3	camphene	951	-	-	-	0.1	MS, RI
4	sabinene	973	-	-	1.3	-	MS, RI
5	β-pinene	976	10.5	0.4	1.2	0.7	MS, RI, Cal
6	myrcene	987	0.6	0.1	1.3	-	MS, RI, Cal
7	α-phellandrene	1002	7.3	0.1	1.5	0.2	MS, RI, Cal
8	α-terpinene	1015	0.4	-	1.2	-	MS, RI
9	p-cymene	1025	12.4	0.4	17.4	4.2	MS, RI, Cal
10	limonene	1027	4.2	1.1	6.5	-	MS, RI, Cal
11	1,8-cineole	1030	34.0	72.5	37.5	68.9	MS, RI, Cal
12	(Z)-β-ocimene	1037	1.1	-	–	-	MS, RI
13	γ-terpinene	1059	1.2	0.3	1.4	0.2	MS, RI, Cal
14	Cis-sabinene hydrate	1065	-	-	1.5	-	MS, RI
15	terpinolene	1085	0.7	0.3	1.3		MS, RI, Cal
16	isopentyl-isovalerate	1100	0.3	-	-	-	MS, RI
17	male-fenchol	1110	-	-	-	0.3	MS, RI
18	β-thujone	1111	-	-	1.2	-	MS, RI
19	neo-fenchol	1115	-	0.1	-	-	MS, RI
20	nonan-3-on-1-ol (cis-p)	1118	-	-	1.8	-	MS, RI
21	α-campholenal	1122	-	0.3	-	-	MS, RI
22	trans pinocarveol	1136	1.6	3.3	-	6.3	MS, RI, Cal
23	nonan-3-on-1-ol (trans-p)	1138	-	-	1.8	-	MS, RI
24	pinocarvone	1160	0.7	1.3	-	1.7	MS, RI, Cal
25	borneol	1161	-	0.3	-	0.7	MS, RI, Cal
26	terpinen-4-ol	1174	2.1	0.5	3.5	0.6	MS, RI, Cal
27	neo-isoverbanol	1184	-	-	9.1	-	MS, RI
28	α-terpineol	1186	1.5	0.8	1.5	1.0	MS, RI, Cal
29	cumin aldehyde	1236	-	-	3.6	-	MS, RI
30	piperitone	1250	-	-	1.3	0.4	MS, RI
31	geraniol	1252	-	0.3	-	-	MS, RI
32	thymol	1287	-	-	1.9		MS, RI
33	α-gurjunene	1435	1.5	0.4	-	-	MS, RI
34	bicyclogermacrene	1490	-	-	1.4	-	MS, RI
35	spathulenol	1572	1.1	0.3	6.7	t	MS, RI
36	globulol	1578	5.2	2.7	2.4	1.6	MS, RI
37	viridiflorol	1585	1.1	0.9	-	-	MS, RI
38	α-cadinol	1650	0.5	-	-	-	MS, RI
39	Total	-	98.1	98.7	98.4	98.8	

the oil of *E. spathulata* was the highest (more than 70%) and in the oil of *E. microtheca* the lowest (34.0%). In addition, there are some other differences and similarity between oil compositions of these *Eucalyptus* species. The percentage of α-pinene in the oils of *E.*

largiflorens was 2.4%, while in other oils it was more than 10%. The oil of *E. largiflorens* contained limonene (6.5%), neo-isoverbenl (9.1%) and spathulenol (6.7%) as well, while these compounds were not found in other oils or were found at lower amounts. The contents of p-cymene in the oils of *E. mictotheca* (12.4%) and *E. largiflorens* (17.4%) were higher than two other oils

Comparing these results with those of five Eucalyptus oils studied previously (14), showed the 1,8-cineol percentage of *E. spathulata* oil (72.5%) is comparable with 1,8-cinele in the oil of *E. intertexta* (81.5%)*, E. leucoxylon* (85.5%) and *E. sargentii* (77.2%).

It can be concluded that the highest oil yield was obtained for *E. spathulata* (1.88% w/w) and the lowest for *E. microtheca* (0.38%). Statistical data showed no significant difference between oil yields of *E. spathulata* and *E. torquata*. 22, 21, 26 and 16 compounds were identified in the oils of *E. microtheca*, *E. spathulata*, *E. largiflorens* and *E. torquata*, respectively that approximately constitute 99.1%, 98.7%, 98.4% and 98.8% of the oils, in the mentioned order.

The high amounts of 1, 8-cineole in the oils of *E. torquata* and *E. spathulata* is remarkable. 1,8-Cineole, which is a terpenoid oxide present in many plant essential oils, displays an inhibitory effect on some types of experimental inflammation in rats, i.e. paw oedema induced by carrageenan and cotton pellet-induced granuloma. It has anti-microbial, anti-inflammatory and anti-nociceptive effects (7-8). So it can be concluded that the oils of these two *Eucalyptus* species could have the medicinal properties, which should be investigated in other studies.

Eucalyptus spathulata with 1.88% oil and 72.5% 1, 8-cineole is suggested as a good source for medicinal uses. According to the European Pharmacopoeia, it has the most desirable specifications, i.e. 72.5% of 1,8-cineole (not less than 70%), α-phellandrene (0.1%, less than 1.5%), β-pinene (0.4%, less than 0.5%), camphor (0.0%; less than 0.1%). Of course, the percentage of α-pinene (12.7%) is high and limonene (1.1%) is low. So, it needs minor rectification or fractional distillation. In addition, the time of sampling was before flowering. The authors will investigate the oils in other seasons to find the best time of harvesting for obtaining the best quantity and quality of the oils. In addition, the oil of *E. torquata* could also be used for medicinal uses with minor rectification.

The essential oils of other *Eucalyptus* species studied contained low amounts of 1,8-cineole according to the European Pharmacopoeia.

References

(1) Kaspar P, Repges R, Dethlefsen U and Petro W. Sekretolytika im vergleich. anderung der ziliarfrequenz und lungen function nach thrapie mit cineol und ambroxol. *Atemw Lungenkrkh*. (1994) 20: 605-14

(2) Wittman M, Petro W, Kaspar P, Repges R and Dethlesten U. Zur thrapie chronisch obstruktiver atemwegser-krankungen mit sekretolytika. doppelblinder, randomisierter cross-over-vergleich zwischen cineol und ambroxol. *Atemw Lungenkrkh.* (1998) 24: 67-74

(3) Mahlo DH. Obstruktive atemwegserkrankungen mit cineol die lungenfunktionsparameter verbesern. *Therapiewoche* (1990) 40: 3157-62

(4) Juergens UR, Stober M and Vetter H. Steroidartige hemmung des monozytaren arachidonsaure metabolismus und der 1l-1β-produktion durch 1,8-cineole. *Atemw Lungenkrkh* (1998) 24: 3-11

(5) Reynolds JEF and Prasad AB. (Eds.) *Martindale-The Extra Pharmacopoiea*. 28th ed. Pharmaceutical Press, London (1982) 1017-8

(6) Grassmann J, Hippeli S, Dornisch K, Rohnert U, Beuscher N and Elstner EF. Antioxidant properties of essential oils. Possible explanations for their anti-inflammatory effects. *Arzneim Forsch/Drug Res*. (2000) 50: 135-139

(7) Juergens UR, Dethlefsen U, Steinkamp G, Gillissen A, Repges R and Vetter H. Anti-inflammatory activity of 1,8-cineole (eucalyptol) in bronchial asthma: a double blind placebo-controlled trial. *Respiratory Med*. (2003) 97: 250-6

(8) Santos FA and Rao VS. Anti-inflammatory and antinociceptive effects of 1,8-cineole, a terpenoid oxide present in many plant essential oils. *Phytother Res.* (2000) 14: 240-244

(9) Council of Europe (COE)- European Directorate for the quality of Medicine (EDQM) *European Pharmacopeia*, 5th ed. Supplement 5.5,Council of Europe, london, 270

(10) Brand N. *Eucalyptus*. In: Drogen EO. (Ed.) *Hagers Handbuch der Pharmazeutischen Praxis*. 5th ed. Volume 5, Springer-Verlag, Berlin (1993) 115-130

(11) Harkenthal M, Reichling J, Geiss HK and Saller R. Comparative study on the *in vitro* antibacterial activity of Australian tea tree oil, cajuput oil, niaouli oil, manuka oil, kanuka oil and eucalyptus oil. *Pharmazie* (1999) 54: 460-3

(12) Wilson ND, Watt RA and Moffat AC. A near-infrared method for the assay of cineole in eucalyptus oil as an alternative to the official BP method. *J. Pharm.*

Pharmacol. (2001) 33: 95-102

(13) Renedo J, Otero JA and Mira JR. Huile essentielle d'*Eucalyptus globules* L. de cantabrie (Espange). Variation au cours de la distillation. *Planets Med. Phytother.* (1990) 24: 31-5

(14) Sefidkon F, Assareh M. H., Abravesh Z and Mirza M. Chemical composition of the essential oil of five cultivated *Eucalyptus* species in Iran (*E. intertextra, E. platypus*, *E. leucoxylon*, *E. sargentii* and *E. camaldulensis*). J. Essent. Oil Bearing Plants (2006) 9(3) : 245-250

(15) Zrira S, Bessiere JM, Menut C, Elamrani A and Benjilali B. Chemical composition of the essential oil of nine *Eucalyptus* species growing in Morocco. *Flav. Fragr. J*. (2004) 19: 172-175

(16) Bignell CM, Dunlop PJ, Brophy JJ and Jackson JF. Volatile leaf oils of some south-western and southern australian species of the genus *Eucalyptus*. Part X. Subgenus symphyomyrtus, section bisectaria. (a) Unpublished series erectae, (b) Series contortae and (c) Series decurvae. *Flav. Fragr. J*. (1996) 11: 101-106

(17) Nicolle D, Dunlop PJ and Bignell CM. A study of the variation with time of the compositions of the essential leaf oils of 16 *Eucalyptus* species. *Flav. Fragr. J*. (1998) 13: 324-328

(18) Adams RP. *Identification of Essential Oil Components by Gas Chromatography/ Mass Spectroscopy.* Allured Publishing Corp., Carol Stream, (1995)

(19) Shibamoto T. Retention indices in essential oil analysis. In: Sandra P and Bicchi C. (Eds.) *Capillary Gas Chromatography in Essential Oil Analysis*. Huethig Verlag, New York (1987) 259-274

This article is available online at http://www.ijpr-online.com