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)

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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 ailments 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

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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).


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 tunida* Brooker & Hopper; *Eucalyptus histophylla* Brooker & Hopper; *Eucalyptus flavidula* Brooker & Hopper; *Eucalyptus clinicola* 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 aequiporta* 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* Schafer subsp. *lobata* Brooker & Sree. 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
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 time 1 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%), α-pinene (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-pinocarveol (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
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. microtheca* (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-cineole 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 quality and quantity 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

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