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Short Communication

Chemical composition of essential oils of two cultivated *Eucalyptus* species from the central Iran

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The chemical composition of essential oils of leaves of two cultivated species, *Eucalyptus loxophleba* Benth. and *Eucalyptus leocoxylon* F. Muell. in the central of Iran were obtained by hydrodistillation and analyzed by gas chromatography, using flame ionization and mass spectrometric detection. A total of thirty nine compounds representing 98.0% of total oil have been identified in the oil of *E. loxophleba*. 1,8-cineole (39.4%), methyl amyl acetate (19.8%), aromadendrene (10.0%), viridiflorol (6.0%) and *α*-pinene (5.4%) were the major constituents in the volatile oil of *E. loxophleba* oil. Forty three components were identified in the *E. leocoxylon* oil where 1,8-cineole (59.1%), cryptone (15.1%), *α*-pinene (9.9%), *α*-terpineol (5.1%) and globulol (5.0%) are considered as the main components. The amount of 1,8-cineole as the main component was higher in the oil of *E. leocoxylon*.

Key words: *Eucalyptus loxophleba, Eucalyptus leocoxylon*, Myrtaceae, essential oil, 1,8-cineole.

INTRODUCTION

Over the last decades, there is an increasing interest in the study of essential oils and various extracts of plants. They have been investigated for their potential uses as alternative remedies for the treatment of many diseases as food perseveres, and pharmaceutical uses due to their antimicrobial, antioxidant and many other activities (Gholivand et al., 2010; Rahimi-Nasrabadi et al., 2011; Gholivand et al., 2011). Essential oils of various plants contain about 20 to 80 components with quiet different concentration from which 2 to 4 components are the main components with higher concentrations than other components of the oil. Generally, these major compounds determine the biological properties of the essential oils (Bakkali et al., 2008). Also, the formation of essential oil in the plant, and consequently the yield and composition of the produced oil depends on many factors. Genetic differences among plants of the same species that are otherwise indistinguishable (chemotypes) can result in widely different oil types (Rahimi-Nasrabadi et al., 2009). Geographic location and agricultural factors also influence the oil production. This implies the possibility of different medicinal uses of a plant species grown in different regions. Therefore, the study on chemical composition of essential oils of plants is an important filed. *Eucalyptus* species belonging to Myrtaceae family are a native from Australia. This genus includes more than 700 species. Essential oils are one of the most important products of *Eucalyptus*. The essential oils of various species belonging to this genus are important for their pharmaceutical, industrial and perfume uses and as fragrance additive (Goodger and Woodrow 2005; Rahimi-Nasrabadi et al., 2011).

One of the most important compound in the essential oils from the *Eucalyptus* genus is the 1,8-cineole and the value of *Eucalyptus* oil for medicinal uses depend on the 1,8-cinole content which varies from 25 to 90% (Goodger and Woodrow, 2005). The aim of this work is to evaluate chemical composition of essential oil of two *Eucalyptus* species, *Eucalyptus loxophleba* Benth and *Eucalyptus leocoxylon* F. Muell.

EXPERIMENTAL (MATERIALS AND METHODS)

The leaves of *E. loxophleba* and *E. leocoxylon* were cultivated in September 2009 from Kashan (Isfahan province, Iran). The leaves were dried in the shade (at room temperature). A voucher specimen of the plant was deposited at the Herbarium of Kashan botanical garden (KBG 1495 and KBG 1500 respectively). The essential oils were isolated by hydrodistillation of dried plant material (flowering aerial parts of *E. loxophleba* and *E. leocoxylon*) for 2 h (60 g of sample in 500 ml of distilled water) using a Clevenger-type

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Table 1. Essential oil composition of *Eucalyptus species*.

KI[a]	Constituent[b]	*E. leucoxylon*	*E. loxophleba*	KI	Constituent	*E. leucoxylon*	*E.* loxophleba
736	3-methyl-Butanal	0.9	0.3	1302	Exo-2-Hydroxycineole acetate	0.4	-
752	2,2-Dimethyl-3-heptanol	-	0.5	1376	Isoledene	0.2	0.3
767	1,2-Dimethylpropyl acetate	-	0.3	1377	α-Copaene	0.1	-
790	Isoamyl acetate	-	0.9	1410	α-Gurjunene	0.3	1.0
870	Methyl amyl acetate	-	19.8	1421	Calarene	0.5	-
939	α-Pinene	9.9	5.4	1439	Alloaromadendrene	4.4	-
979	β-Pinene	-	0.1	1440	Aromadendrene	0.9	10.0
987	β -Myrcene	-	0.1	1465	Neoalloocimene	-	1.8
1003	α-Phellandrene	-	0.2	1490	β-Selinene	0.1	0.5
1027	p-Cymene	-	1.1	1517	Naphthalene, 1,2,3,4-tetrahydro-6,7-dimethyl-	0.1	-
1031	1,8-Cineole	59.1	39.4	1520	Ledene	0.2	1.3
1060	γ-Terpinene	-	0.1	1523	Δ-Cadinene	-	0.2
1102	Iso-Amyl isovalerate	-	0.2	1554	Epiglobulol	1.1	1.1
1139	Trans-Pinocarveol	1.0	2.0	1569	Ledol	-	0.4
1165	Pinocarvone	0.5	0.6	1585	Globulol	5.0	-
1169	Borneol	0.3	-	1593	Viridiflorol	1.2	6.0
1177	4-Terpineol	1.4	0.4	1651	β-Eudesmol	-	0.5
1182	Cis-p-Mentha-1(7),8-dien-2-ol	-	0.2	1654	α-Eudesmol	-	0.4
1189	α-Terpineol	5.1	0.2		Grouped components		
1191	Trans-p-Mentha-1(7),8-dien-2-ol	-	0.1		Monoterpene hydrocarbons	10.6	8.0
1196	Myrtenol	0.1	-		Oxygenated monoterpenes	68.7	52.5
1199	δ-Terpineol	0.4	-		Sesquiterpene hydrocarbons	6.8	1.4
1217	Trans-carveol	0.2	-		Oxygenated sesquiterpenes	7.3	2.7
1232	Trans-p-Mentha-1(7),8-dien-2-ol	0.4	-		Other	2.8	32.3
1253	Geraniol	0.1	-		Total	96.2	96.9

[a]Retention indices and [b]order of elution on HP5-MS.

apparatus (British Pharmacopeia). The oils were dried over anhydrous sodium sulphate and stored in sealed glass vials at 4 to 5°C prior to analysis. Yield based on dry weight of the plant material was calculated. The analytical GC was carried out on Varian (Walnut Creek, CA, USA) Saturn 3400 GC system equipped with flame ionization detectors (FID) and a DB-5 capillary fused silica column (30 m × 0.25 mm ID, film thickness of 0.25 µm). The oven temperature was held at 40°C for 1 min then programmed at a rate of 3°C/min to 250°C and held isothermal for 10 min. The carrier gas was nitrogen at a flow rate of 1.1 ml/min; injector temperature: 260°C and detector: 280°C. GC-MS analysis of the essential oils were performed using an HP-6890 GC system coupled with a 5973 network mass selective detector and equipped with a HP5-MS capillary fused silica column (60 m, 0.25 mm i.d.; 0.25 µm film thickness). Essential oil solution (1 µl) in hexane (HPLC grade) was injected and analyzed with the column held initially at 40°C for 1 min and then increased to 250°C with a 3°C/min heating ramp and subsequently kept at 250°C for 20 min. Other operating conditions were as follows: carrier gas, He (99.999%); flow rate 1 ml/min; injector temperature, 250°C; split ratio, 1:50. Mass spectra were taken at 70 eV. Mass range was from m/z 20 to 500 amu.

The relative percentage amounts of the separated compounds were calculated from total ion chromatograms by a computerized integrator. 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. Oil constituents were identified

by comparing linear retention indices based on a homologous series of even numbered n-alkanes (C_8 to C_{24}) (Niles, Illinois, USA) with those of standard compounds and by comparison with literature data and MS data with those of reference compounds (Sigma–Aldrich and Acros Organics) and by MS data obtained from Wiley and NIST libraries (Sandra and Bicchi, 1987; Adams, 2001).

RESULTS AND DISCUSSION

Chemical composition of essential oil

The hydrodistillation of the flowering aerial parts of *E. loxophleba* and *E. leocoxylon* gave colorless to light yellowish oils with yield of 7.1 and 5.2% (v/w), respectively. The identified constituents of two *eucalyptus* oils, their retention indices and their percentage compositions are summarized in Table 1 in order of their elution from the HP5-MS column. A total of thirty nine compounds have been identified in the oil of *E. loxophleba*, representing around 97.0% of the oil totality. 1,8-cineole (39.4%), methyl amyl acetate (19.8%) and aromadendrene (10.0%) were the major constituents in the *E. loxophleba* oil with other compounds such us viridiflorol (6.0%) and α-pinene (5.4%). Forty two components were identified in the *E. leucoxylon* oil. The major components were 1,8-cineole (59.1%), α-pinene (9.9%), cryptone (15.1%), α-terpineol (5.1 %) and globulol (5.0%). The results shows that 1,8-cineole was the main component in the essential oils of the two species studied but its relative amount of essential oil of *E. leucoxylon* was higher (59.1%) than *E. loxophleba* (39.4%). The amount of monoterpens in the essential oil of two species is high in respect to other grouped component. Our results show that the oil of *E. loxophleba* contains about 8.0% monoterpene hydrocarbons and 52.5% oxygenated monoterpenes. Also the oil of *E. leucoxylon* contains about 10.6% monoterpene hydrocarbons and 68.7% oxygenated monoterpenes.

The concentration of sesquiterpene hydrocarbons and oxygenated sesquiterpenes is relatively low in the oils of two species (1.4 and 2.7% in the oil of *E. loxophleba*, and 6.8 and 7.3% respectively, in the oil of *E. leucoxylon*). A report from Sefidkon et al. (2006) indicated that 1,8-cineole (85.5%) was the main constituent of the oil of *E. leucoxylon*. These authors identified only seven components in their oil while fourty two components have been identified in the oil analysed in the present work. In another work, Sefidkon et al. (2009) investigated seasonal variation in volatile oil of *Eucalyptus* Species in Iran and identified thirty-three compounds in the oil of *E. leucoxylon* with 1,8-cineole (42.1 to 89.8%) as the main constituent. A report from Assareh et al. (2007) indicated that 1,8-cineole (41.9%), α-pinene (13.7%) and aromadendrene (3.7%) were the main constituents of the oil of *E. loxophleba* from Iran. The major components of *E. loxophleba* essential oil in our study were 1,8-cineole (39.4%), methyl amyl acetate (19.8%) and aromadendrene (10.0%). These differences might have been derived both from harvest time and local, climatic and seasonal factors or we may hypothesize that these sample belongs to a different chemotype (Rahimi-Nasrabadi et al., 2011). However, further investigations are needed to elucidate this hypothesis. According to different activities of various compounds, these differences among constituents of the essential oils will be important in nutritional and medicinal uses.

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