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SURVEY OF THE BIOACTIVE AND FRAGRANT CONSTITUENTS SEPARATED BY NANO SCALE INJECTION OF *Eucalyptus camaldulensis* Var. obtusa CULTIVATED IN KASHAN AREA

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The dried leaves and flowers of *Eucalyptus camaldulensis* Var. obtusa which belongs to Myrtaceae family were hydrodistilled to produce oils in the yields of 0.43% and 0.58% (w/w), respectively. The oils were analyzed by GC and GC/MS**.** The amount of the samples injected by nano scale included were 1.0 nL (diluted 1.0 µL of sample in 1000 ml of *n*-pentane, v/v). Eleven and twelve bioactive and flavour molecules were identified, representing 99.50% and 99.25% of the leaves and flowers oil, one by one. The main components were 1,8-cineol (Eucalyptol), trans-pinocarvol, and α–Pinene in leaves and 1,8-cineol, α–Pinene, *δ*-Cadinene, and globulol in flowers oils. The compositions of the oils were mostly quantitatively rather than qualitatively different.

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1. Introduction

Eucalyptus is one of the world’s most important and most widely planted genera. It includes more than 700 species and belongs to the family of Mitraceae [1].

Eucalyptus camaldulensis (common name: River red gum) is one of the species of *Eucalyptus* introduced into Nigeria. The *Eucalyptus* has been used in folklore medicine as a remedy for sore throat and other bacterial infection of the respiratory and urinary tracts. The inhalation of the decoction vapour of the leaves is used for catarrh and nasal congestion [2]. Essential oils of the leaves are used in the treatment of lung diseases while the volatile oils are used as expectorant and cough stimulant. These oils were stated to have antitubercular properties [3]. Poultice of the leaves is applied over ulcers and wounds [4].

In addition, *Eucalyptus camaldulensis* is also known to contain bioactive products that display antibacterial [5], antifungal [6], analgesic and anti-inflammatory effects [7], antioxidative and antiradical [8] activities.

To the best of our knowledge, the essential oils of the leaves and flowers of this plant in Kashan area have not been considered before. The maters on hand of this study were the determination of the percentage bioactive and fragrant molecules by nano scale injection.

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2. Material and methods

Plant material

Leaves and flowrs of *E. camaldulensis* Var. obtusa were collected in May 2008 from Isfahan Research Center of Natural Resources, Kashan station, Kashan, Iran. The voucher specimens of the plant were deposited in the herbarium of Research Institute of Forests and Rangelands, Kashan, Iran.

Isolation of the Essential Oils

Dried and ground leaves (60g) and flowers (28g) of *E. camaldulensis* Var. obtusa were subjected to separate hydrodistillation for 3.5 h using a Clevenger-type apparatus [9]. After decanting and drying over anhydrous sodium sulfate, the corresponding yellowish colored oils were recovered from the leaves and flowers in yields of 0.43% and 0.58% (w/w), respectively.

Gas Chromatography (GC)

GC analysis of the oils was performed on an Agilent HP-6890 gas chromatograph equipped with flame ionization detector (FID) and an HP-5MS capillary column (30 m × 0.25 mm i.d., film thickness, 0.25 μm). The oven temperature was programmed as follows: 50ºC (2 min), 50-130ºC (3ºC min^{-1}), 130ºC (2 min), 130-270ºC (5ºC min^{-1}). Injector and detector temperatures were maintained at 220ºC and 290ºC, respectively. The amount of the sample injected was 1.0 nL (diluted 1.0 μL of sample in 1000 ml of *n*-pentane, v/v) in the splitless mode. Helium was used as carrier gas with a flow rate of 1 mL min^{-1}.

Gas Chromatography-Mass Spectrometry (GC/MS)

GC-MS analysis of the oils was performed on a Agilent HP-5973 mass selective detector coupled with a Agilent HP-6890 gas chromatograph, equipped with a cross-linked 5% PH ME siloxane HP-5MS capillary column (30 m × 0.25 mm i.d, film thickness, 0.25 μm) and operating under the same conditions as above was described. The flow rate of helium as carrier gas was 1 mL min^{-1}. The MS operating parameters were as follows: ionization potential, 70 eV; ionization current, 2 A; ion source temperature, 200ºC; resolution, 1000.

Identification of bioactive and fragrant components

Essential oils was analyzed by GC and GC/MS systems using a non-polar column and identification of components in the oil was based on retention indices (RI) relative to *n*-alkanes and computer matching with the WILEY 275.L library, as well as by comparison of the fragmentation pattern of the mass spectra with data published in the literature [10,11]. The percentage composition of the sample was computed from the GC-FID peak areas without the use of correction factors.

3. Results and discussion

Air-dried leaves and flowers of the plant were subjected to hydrodistillation using a Clevenger-type apparatus and yellowish oils were obtained in the yields of 0.43% and 0.58% (w/w), respectively. Eleven and twelve bioactive, flavour and fragrance molecules, constituting 99.50% and 99.25% of the total components detected, were identified in this plant and listed in Table 1 with their percentage. Constituents are listed in order of their elution from HP-5MS column. 1,8-cineol (34.87%), trans-pinocarvol (30.70%), α–Pinene (13.98%) and 1,8-cineol (40.08%), α–Pinene (14.61%), *δ*-Cadinene (14.55%), and globulol (14.54%) were the major components of leave and flower oils, respectively.

Table 1. Bioactive and fragrance components of leaves (A) and flowers (B) of E. camaldulensis Var. obtusa

Compound[a]	**A, %**	**B, %**	**RI** [b]	**Compound**[a]	**A, %**	**B, %**	**RI** [b]
α-Pinene [M]	13.98	14.61	927	Pinocarvone [M]	12.23	-	1158
Camphene [M]	1.77	-	960	Terpinene-4-ol [M]	0.67	2.78	1173
β Pinene [M]	2.08	3.06	970	Aromadenderene [S]	-	1.36	1439
α-Phellandrene [M]	1.56	2.23	997	Bicyclogermacrene [S]	-	0.26	1492
p-Cymene [M]	0.43	2.27	1021	δ-Cadinene [S]	-	14.55	1513
1,8-Cineol [M]	34.87	40.08	1033	Epiglobulol [S]	0.98	3.30	1546
γ –Terpinene [M]	-	0.21	1056	Globulol [S]	0.23	14.54	1573
trans-pinocarveol [M]	30.70	-	1140	**Total identified**	**99.25**	**99.50**	

[a]Compounds listed in order of their RI.
[b]RI (retention index) measured relative to n-alkanes (c_8-c_{32}) on the non-polar HP-5MS column.
%, Relative percentage obtained from peak area.
[M] Monoterpenes, are natural compounds with ten carbon atoms in their skeleton.
[S] Sesquiterpenes, are natural compounds with fifteen carbon atoms in their skeleton.

α-pinene (22.52%), *p*-cymene (21.69%), α-phellandrene (20.08%), 1,8-cineole (9.48%), γ -terpinene (9.36%), and limonene (4.56%) were also found in the oil of *E. camaldulensis* collected from eastern Taiwan [12].

Lucia et al. [13] reported 1,8-cineole, α-phellandrene, β-phellandrene, and *p*-cymene, as major components in *E. camaldulensis* oils.

Collectively, several authors have performed comparative studies of the chemical composition of essential oils from *E. camaldulensis* [14–19].

The results showed small differences in composition with respect to data in the literature, these differences can be attributed to the effect of geographical and climatic factors.

On the basis of the results obtained, we conclude that the oils of *E. camaldulensis* Var. obtusa from Kashan area contain a sufficient quantity of oil that is rich in 1,8-cineole. Hence, we believe that this oil could be used as medicinal and as a substitute for the imported material.

In this part, we present biological properties and the application of 1,8-cineol as the major components from *E. camaldulensis* Var. obtusa essential oils.

1,8-Cineol (Eucalyptol): is used in flavorings, fragrances, and cosmetics. Cineole based eucalyptus oil is used as a flavouring at low levels (0.002%) in various products, including baked goods, confectionery, meat products and beverages [20]. Also, 1,8-cineol was listed as one of the 599 additives to cigarettes. It is claimed that it is added to improve the flavor.

Eucalyptol is an ingredient in many brands of mouthwash and cough suppressant. It controls airway mucus hypersecretion and asthma via anti-inflammatory cytokine inhibition [21, 22]. Eucalyptol is an effective treatment for nonpurulent rhinosinusitis [23]. Research showed that treated subjects experienced less headache on bending, frontal headache, sensitivity of pressure points of trigeminal nerve, impairment of general condition, nasal obstruction, and rhinological secretion. Side effects from treatment were minimal. Eucalyptol reduces inflammation and pain when applied topically [24]. It kills leukaemia cells *in vitro* [25].

Eucalyptol is used as an insecticide and insect repellent [26, 27].

Conversely, eucalyptol is one of many compounds that is attractive to males of various species of orchid bees, who apparently gather the chemical to synthesize pheromones; it is commonly used as bait to attract and collect these bees for study [28].

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