GC-MS ANALYSIS OF THE *EUCALYPTUS OBLIQUA* L'HÉR. (MYRTACEAE) CHEMICAL COMPOSITION

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Abstract

Since there are few studies regarding *Eucalyptus obliqua* and that it is just starting to receive the attention in terms of many potential effects and properties, the aim of this study was to analyse its essential oil by GC-MS. The major detected compound in the studied essential oil was 1,8-cineole (eucalyptol) with 64.7% in content, followed by *α*-pinene (12.6% in content), *γ*-terpinene (7.4% in content), limonene (3.9% in content) and *p*-cymene (3.2% in content). Considering the fact that *Eucalyptus* species in great extent owe their medicinal value to the main constituent of their essential oil → 1,8-cineole (eucalyptol) it was expected that eucalyptol would be the most abundant component. Beside the mentioned components there were 20 constituents which in total make less than 10.00% of the studied essential oil.

Introduction

Due to their biologically active components essential oils are gathering remarkable attention which is undeniably growing from year to year. As the by-products of plant metabolism they are regarded as evaporable secondary metabolites of plants which are the mixture of mono and sesquiterpenes [1]. A large number of isolated allelochemicals show their bioactivity in low (10^{-5}–10^{-6} mol/dm3) or extremely low concentrations (10^{-10} mol/dm3) [2].

Genus *Eucalyptus* has been described by a French botanist L' Heritier. Until now circa 800 species have been described all over the world. Originary *Eucalyptus sp.* come from Australia and Tasmania, but now they can be found in almost all tropical and subtropical areas, while being cultivated in many other climates. *Eucalyptus* species have been known for long time in terms of their antimicrobial, antiseptic, antioxidant, anti-inflammatory, astringent and anticancer effects, as well as for their wound healing, disinfectant and expectorant properties, but *Eucalyptus obliqua* is just starting to receive the attention in this regard. *Eucalyptus sp.* have been traditionally used as a treatment for various health conditions, such as: respiratory diseases, common cold, influenza and sinus congestion. Aboriginals used many *Eucalyptus* species in order to deal with gastrointestinal symptoms, open wounds, muscles and joints pains, toothache, fever, enteric infections such as diarrhea and dysentery, constipation among other stomach problems, asthma, bronchitis and athletes foot [3]. *Eucalyptus sp.* also show great antidiabetic and hypoglycemic potentials [4]. In great extent *Eucalyptus* species owe their medicinal value to the main constituent of their essential oil → 1,8-cineole (eucalyptol). Essential oil of eucalyptus has been marked as safe and non-toxic by the United States Food and Drug Administration (FDA). However, that comes with the substantial risk whenever the essential oil is used pure or in high concentrations, with allergic contact dermatitis as the most common observed adverse effect. Considering that the more detailed risk assessment of potential toxicity is of great importance [3].

The main source of eucalyptus oil in the world is *E. globullus*, also known as the Blue Gum. Except for the essential oil, *Eucalyptus* species have been used in the production of timber,

fuel, paper pulp, as well as like water and wind erosion control measure in environmental plantings [3].
Eucalyptus obliqua is a tall fast growing evergreen tree, with the species name derived from the feature of bearing oblique leaves. It is known for being used to drain swamps, while from its leaves the potent antimalarial bioactive components have been extracted [4].

Experimental

The eucalyptus essential oil has been extracted from commercially available dried plant material by supercritical fluid extraction (SFE).
Conventional semi-continuous method was applied, using supercritical CO_2. The CO_2 with increased purity was heated through preheating coil. Forty grams of sample together with glass beads were put into the extraction cell with volume of 100 mL. As to prevent solid samples to enter the system, cotton wool was placed at the end of the cell. Heated CO_2 was injected into the extraction cell, after which it was allowed to dissolve the essential oil by constant static extraction time. The essential oil left the cell by the constant flow rate of the CO_2, while being captured and harvested with 10 mL of ethanol solvent. Essential oil was kept in the refrigerator until the GC and GC-MS analysis.
Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analysis, as well as identification of essential oil components, were done as described in our previous researches [5,6].
Gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS) analyses were performed using an Agilent 7890A GC equipped with an inert 5975C XL EI/CI mass spectrometer detector (MSD) and flame ionisation detector (FID) connected by capillary flow technology 2-way splitter with make-up. The HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) was used. The GC oven temperature was programmed from 60 to 300 °C at a rate of 3 °C min−1 and held for 15 min. Helium was used as the carrier gas at 16.255 psi (constant pressure mode). An auto-injection system (Agilent 7683B Series Injector) was employed to inject 1 μL of sample. The sample was analysed in the splitless mode. The injector temperature was 300 °C, while the detector temperature was 300 °C. MS data were acquired in the EI mode with scan range of 30–550 m/z, source temperature of 230 °C and quadruple temperature of 150 °C; the solvent delay was 3 min.
Identification of all compounds in the analyses was matched by comparison of their linear retention indices (relative to C8-C36 *n*-alkanes on the HP-5MSI column) and MS spectra with those of authentic standards from NIST (2011) and homemade MS library databases.

Results and discussion

The chromatogram obtained by the GC-MS analysis of the studied essential oil can be seen in Figure 1.

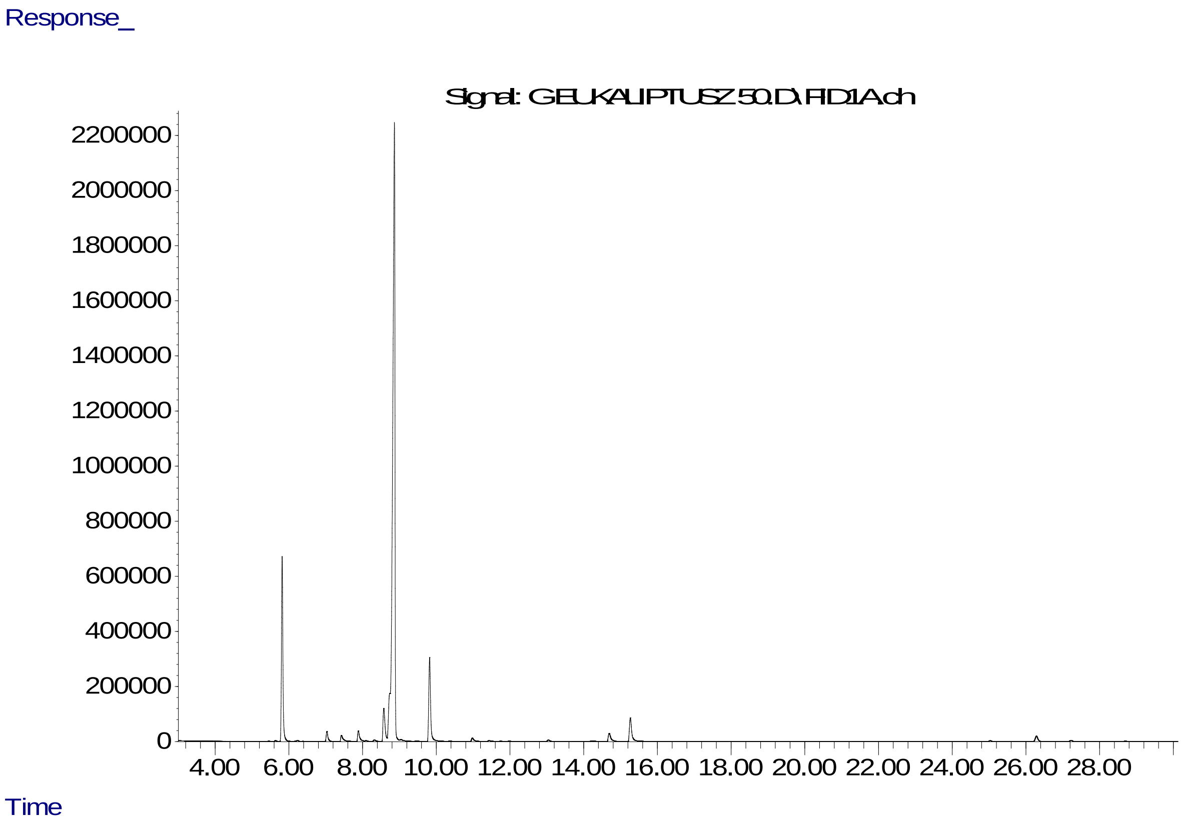

Figure 1. Chromatogram of the *E. obliqua* essential oil

From the obtained results it could be concluded that the main constituent of the *E. obliqua* essential oil is 1,8-cineole (eucalyptol) with 64.7% in content. Other constituents detected in the significant ammount were *α*-pinene (12.6% in content), *γ*-terpinene (7.4% in content), limonene (3.9% in content) and *p*-cymene (3.2% in content). Beside the mentioned components there were 20 constituents which in total make less than 10.00% of the studied essential oil (Table 1).

Table 1. Constituents of the *E. obliqua* essential oil

peak				R.T.	Start	peak	%
#		RI	RI NIST	min	min	area	max.
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1	Thujene<alpha->	923	924	5.632	5.586	88640	0.1%
2	Pinene<alpha->	930	932	5.817	5.74	17974244	12.6%
3	Camphene	944	946	6.228	6.137	147857	0.1%
4	Thuja-2,4(10)-diene	950	952	6.378	6.334	20228	0.0%
5	Pinene<beta->	974	974	7.031	6.984	1200970	0.8%
6	Myrcene	988	988	7.421	7.385	829040	0.6%
7	Phellandrene<alpha->	1004	1002	7.884	7.84	1347632	0.9%
8	Carene<delta-3->	1009	1008	8.09	8.038	70844	0.0%
9	Terpinene<alpha->	1015	1014	8.309	8.254	217585	0.2%
10	Cymene<para->	1022	1020	8.574	8.525	4617134	3.2%
11	Limonene	1027	1024	8.75	8.665	5523423	3.9%
12	Cineole<1,8->	1033	1026	8.864	8.753	92303398	64.7%
13	Ocimene<(Z)-beta->	1035	1032	9.035	8.994	400005	0.3%
14	Ocimene<(E)-beta->	1046		9.45	9.427	27069	0.0%

15	Terpinene<gamma->	1057	1054	9.819	9.759	10511232	7.4%
16	Terpinolene	1088	1086	10.973	10.922	501673	0.4%
17	Linalool	1099	1095	11.423	11.372	135751	0.1%
18	Sabinol<trans-> (trans for OH vs. IPP)	1137	1137	13.036	12.986	203070	0.1%
19	NI	1165		14.257	14.21	96761	0.1%
20	Terpinen-4-ol	1175	1174	14.688	14.639	1360864	1.0%
21	Terpineol<alpha->	1189	1186	15.264	15.2	3566940	2.5%
22	Gurjunene<alpha->	1409	1409	25.023	24.963	166526	0.1%
23	Aromadendrene	1439	1439	26.282	26.179	984478	0.7%
24	Caryophyllene<9-epi-(E)->	1462	1464	27.225	27.054	238351	0.2%
25	Viridiflorene	1497	1496	28.693	28.62	103837	0.1%

Similar results were obtained by [4] who identified 1,8-cineole and α-pinene as being in the top five components in terms of their content in the studied essential oil of *E. obliqua*.

Conclusion

The major detected compound in the studied essential oil of *E. oblique* was 1,8-cineole (eucalyptol) with 64.7% in content, followed by α-pinene (12.6% in content), γ-terpinene (7.4% in content), limonene (3.9% in content) and *p*-cymene (3.2% in content). Considering the fact that *Eucalyptus* species in great extent owe their medicinal value to the main constituent of their essential oil → 1,8-cineole (eucalyptol) it was expected that eucalyptol would be the most abundant component. Beside the mentioned components there were 20 constituents which in total make less than 10.00% of the studied essential oil.

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