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Essential Oil Compositions from Leaves of *Eucalyptus camaldulensis* Dehn. and *Callistemon viminalis* Originated from Malaysia

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Abstract: Leaves of *Eucalyptus camaldulensis* and *Callistemon viminalis* on hydrodistilation, gave 1.40 % and 0.80% w/w an oil dried weight basis, respectively. GC-MS analysis of the oils resulted in the identification of 18 and 7 constituents, repectively, representing 99.31% and 98.07%, respectively, of the oil. *γ*-Terpinene (71.36%) and *o*-cymene (17.63%) were the major components of *E. camaldulensis*. While 1,8-cineole (61.51%) and α-pinene (21.53%) were the major components of *C. viminalis.* From the results; *E. camaldulensis* and *C. viminalis* leaf oils from Malaysia have great potential and can be utilized as cheap sources for the commercial isolation of *γ*-terpinene and 1,8-cineole.

Keywords: *E. camaldulensis, C. viminalis,* Essential oil composition, Malaysia, *γ*-terpinene, 1,8-cineole, α-pinene, *o*-cymene

1. Introduction

Among the families of plants investigated to date, the one that shows enormous potential is Myrtaceae family. Myrtaceae family or the myrtle family, consists of more than 3,800 species of trees and shrubs that occur in temperate, subtropical, and tropical regions of the world. The main genera are *Eucalyptus, Eugenia, Malaleuca, Leptospermum, Myrtus, Pimenta, Psidium and Syzygium*. Species of the Myrtaceae family provide many valuable products, including timber and essential oils and contains a number of economically important species. It is also, rich sources of essential oils containing bioactive constituents [1]. Volatile compounds of great economic importance can be found in the species of the Myrtaceae family. The leaves and the stems of several species are sources of essential oils used for medicinal purposes, food, perfume, cosmetic and pharmaceutical industries [2].

One of the important Myrtaceous genera is *Eucalyptus. Eucalyptus* species contain volatile oils, occur in many parts of the plant, depending on the species, but in the leaves the oils are most plentiful. The volatile oil of the *Eucalyptus* has a number of constituents (terpenes) such as cineole, phellandrene and globulol, which occur in different proportions depending on the species, and can vary within species depending on many factors including subspecies and specific environmental conditions. Of the more than 700 species of *Eucalyptus* less than 20 appear to have been used for the commercial extraction of oil [3]. Another important genus in Myrtaceae family is *Callistemon* which are used for many purposes such as forestry, essential oil production and ornamental horticulture, among other applications [4]. Previous investigations on the essential oil of the members of *Callistemon* genus identified a nunber of monoterpines such as 1,8-cineole, *α*-pinene, *α*-Phellandrene, *α*-terpineol as main constituents [5]-[8].

Yield and composition of essential oils in plants, can be affected by a number of factors, included physiological variations, environmental conditions, geographic variations, genetic factors and evolution [9]. Many studies have been performed on the essential oil composition from different species of *Eucalyptus* and

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Callistemon and there are many authers have reported the chemical composition of the essential oils of *E. camaldulensis* and *C. Viminalis* [10]-[15], but to the best of our knowledge, no

previous reports on the chemistry of the essential oils of *E. camaldulensis* and *C. Viminalis* originated from Malaysia have been carried out. This motivated us to carry out detailed investigation on *E. camaldulensis* and *C. Viminalis* leaf essential oils from plants grown in Malaysia

2. Experimental

2.1. Plant materials

Fresh leaf samples of *E. camaldulensis* and *C. Viminalis* were collected in the month of November, 2012 from the University of Malaya main campus, Kuala Lumpur. After a proper identification of the plants by the scientist of Institute of Biological Sciences (IBS), voucher specimens of the plants have been deposited in the University of Malaya Herbarium.

2.2. Isolation of voltile components

The fresh leaves of *E. camaldulensis* and *C. viminalis* were subjected to hydrodistillation in a conventional Cleavenger-type apparatus for 4 hours. The oil was dried over Sodium Sulfate powdered and stored at - 20 ℃ until analysis.

2.3. Gas chromatography & gas chromatography-mass spectrometry (GC and GC-MS)

GC and GC-MS data were obtained on a HP-5MS Agilent Mass spectrometer instrument using a HP-5MS column (30 m X 0.25 mm i.d., 0.25 μm film thickness). Carrier gas was Helium at a flow rate of 1 ml/min. Temperature programming, initial oven temperature was set at 40 ℃ for 2 min, and increased at 3 ℃/min to 140 ℃, then ramped from 140 to 250 ℃ at the rate of 10 ℃/min. The injection temperature was maintained at 250 ℃. 1 μl of each sample (1%) was injected separately, with a split ratio of 1:10. The ionization energy of 70 eV and ion source temperature at 230 ℃. Retention indices were calculated for all essential oil constituents using a homologous series of n-alkanes C_8–C_{20} (Sigma-Aldrich, USA) on the HP-5MS column.

2.4. Identification of compounds

The identification of chemical constituents was done using their mass spectra, relative retention indices, Wiley/NBS registry of mass spectral database, NIST MS Search, using authentic reference compounds and published mass spectra and retention indices [16]-[18].

3. Results and Discussion

Although the chemical composition of *E. camaldulensis* and *C. Viminalis* leaf oils from other countries have been investigated, however, the chemical composition of both plant species essential oils originated from Malaysia have not been studied so far, thus in this study, we investigated the chemical composition of *E. camaldulensis* and *C. Viminalis* leaf oil from Malaysia.

The total yield of leaf essential oils of the two Myrtaceous species; *E. camaldulensis* and *C. viminalis* were 1.40 %, 0.80% w/w, respectively, based on the dried weight. It can be suggested that the differences in the yield and constituents of the oils could be attributed to the differences in genetic and geographical and environmental conditions.

The chemical composition of the leaf essential oils was assessed using GC-MS technique described above resulted in the identification of 19 compounds of essential oil components from *E. camaldulensis* representing 99.31% of the essential oil and 7 compounds in *C. viminalis* oil representing 98.07% of its oil. *E. camaldulensis* oil main components are *γ*-terpinene (71.36%), *o*-cymene (17.63%) and terpinen-4-ol (7.01%). While, *C. viminalis* essential oils main components are 1,8-cineole (61.51%), *α*-pinene (21.53%) and *α*-terpineol (12.47%). In addition, components such as *α*-phellandrene and linalool were identified in trace amounts in both *E. camaldulensis* and *C. viminalis* essential oils in this study (Table 1).

Our results differed from those authors who reported 1,8-cineole (Eucalyptol) as the main constituent of *E. camaldulesis* essential oil grown in other countries [19]-[21]. On the other hand, the abundance of 1,8-

cineole in the essential oil of *C. viminalis* makes it similar to those obtained in the previous studies from other countries such as India, Australia and South Africa, but a key difference in the oils lies in the relative quantities of 1,8-cineole and α-pinene [6]-[11]. Resembling to the report concerning the essential oil of *C. viminalis* grown in India [11], our study of Malaysian species showed quantitative similarity. In the oil of *C. viminalis* from India, 1,8-cineole and α-pinene represented 61.7% and 24.2 % respectively, of the total oil, while in the Malaysian species, it was 61.51% for 1,8-cineole and 21.5 % for α-pinene which appeared as major constituents in both species. So, our results showed that *C. viminalis* can be considered as a good source for 1,8-cineole (61.51%) and this finding is in agreement with those reported before in other parts of the world.

Table 1 shows the main identified compounds, their percentage of composition and their retention indices values (RI) listed in order of elution from the HP-5MS capillary column.

Table 1. Essential oil composition of leaves of *E. camaldulensis* and *C. viminalis*

No.	**Chemical Compound**	**RI**	**Relative percentage (%)**	
			E. camaldulensis	*C. viminalis*
1	*α*-Pinene	931	0.54	21.53
2	*α*-Phellandrene	1003	0.02	1.38
3	*α*-Terpinene	1015	0.19	-
4	*o*-Cymene	1024	17.63	-
5	Limonene	1027	0.48	-
6	1,8-Cineole	1029	0.46	61.51
7	*γ*-Terpinene	1062	71.36	-
8	Terpinolene	1087	1.10	-
9	Linalool	1101	0.02	0.29
10	*cis*-Sabinol	1131	-	0.367
11	Terpinen-4-ol	1178	7.01	0.54
12	*α*-Terpineol	1191	0.10	12.47
13	Piperitone	1253	0.01	-
14	Thymol	1291	0.07	-
15	Carvacrol	1294	0.04	-
16	Globulol	1587	0.13	-
17	*γ*-Eudesmol	1634	0.05	-
18	*β*-Eudesmol	1653	0.05	-
19	*α*-Eudesmol	1657	0.05	-

RI = Retention index relative to C_8-C_{20} n-alkanes on HP-5MS column; - : Not detected

The most abundant compounds in essential oils of *E. camaldulensis* and *C. viminalis* are showed in GC-MS chromatograms (Figure 1).

The higher percentage of *γ*-terpinene (71.36%) in *E. camaldulensis* and 1,8-cineole (61.51%) in *C. viminalis* leaf oils from Malaysia have great potential for these two oils to be more valuable and long-lasting, hence, the leaves of these two plant species could be utilized as cheap sources for the commercial isolation of *γ*-terpinene and 1,8-cineole in future.

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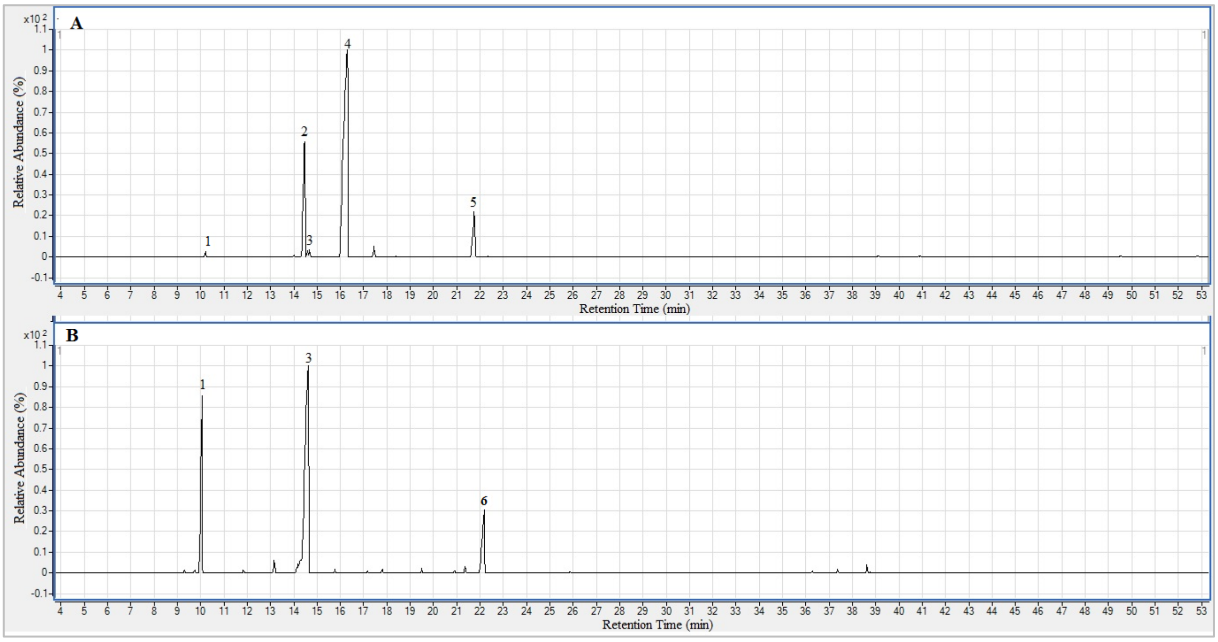

Fig. 1: A and B. Eessential oil chromatograms of leaves of *E. camaldulensis* and *C. viminalis*, respectively. 1- 6 represented the most identified compounds. 1. *α*-pinene, 2. *o*-cymene, 3. 1,8-cineole, 4. *γ*-terpinene, 5. terpinen-4-ol and 6. *α*-Terpineol. The chromatograms were obtained by Agilent GC–MS equipped with an HP- 5MS column

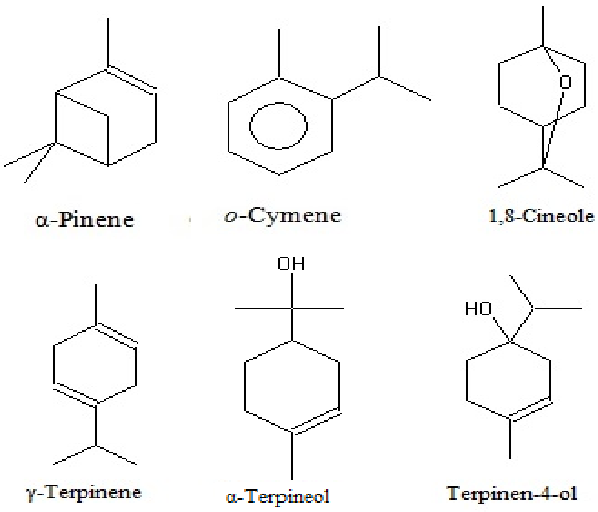

Fig. 2: Structures of the most abundant compounds identified in *E. camaldulensis* and *C. viminalis* leaf essential oils (Source: NIST MS library).

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