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 hydrodistillation, 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, respectively, 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 number of monoterpenes such as 1,8-cineole, apinene, 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...
Callistemon and there are many authors 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 volatile 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 °C 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 HP5MS 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 °C for 2 min, and increased at 3°C/min to 140 °C, then ramped from 140 to 250 °C at the rate of 10 °C/min. The injection temperature was maintained at 250 °C. 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°C. Retention indices were calculated for all essential oil constituents using a homologous series of n-alkanes C₈–C₂₀ (Sigma-Aldrich, USA) on the HP5MS 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%), α-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. camaldulensis essential oil grown in other countries [19]-[21]. On the other hand, the abundance of 1,8cineole 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>
<thead>
<tr>
<th>No.</th>
<th>Chemical Compound</th>
<th>RI</th>
<th>E. camaldulensis</th>
<th>C. viminalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>α-Pinene</td>
<td>931</td>
<td>0.54</td>
<td>21.53</td>
</tr>
<tr>
<td>2</td>
<td>α-Phellandrene</td>
<td>1003</td>
<td>0.02</td>
<td>1.38</td>
</tr>
<tr>
<td>3</td>
<td>α-Terpinene</td>
<td>1015</td>
<td>0.19</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>α-Cymene</td>
<td>1024</td>
<td>17.63</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Limonene</td>
<td>1027</td>
<td>0.48</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>1,8-Cineole</td>
<td>1029</td>
<td>0.46</td>
<td>61.51</td>
</tr>
<tr>
<td>7</td>
<td>γ-Terpinene</td>
<td>1062</td>
<td>71.36</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Terpinolene</td>
<td>1087</td>
<td>1.10</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Linalool</td>
<td>1101</td>
<td>0.02</td>
<td>0.29</td>
</tr>
<tr>
<td>10</td>
<td>cis-Sabinol</td>
<td>1131</td>
<td>-</td>
<td>0.367</td>
</tr>
<tr>
<td>11</td>
<td>Terpinen-4-ol</td>
<td>1178</td>
<td>7.01</td>
<td>0.54</td>
</tr>
<tr>
<td>12</td>
<td>α-Terpineol</td>
<td>1191</td>
<td>0.10</td>
<td>12.47</td>
</tr>
<tr>
<td>13</td>
<td>Piperitone</td>
<td>1253</td>
<td>0.01</td>
<td>-</td>
</tr>
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<td>14</td>
<td>Thymol</td>
<td>1291</td>
<td>0.07</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>Carvacrol</td>
<td>1294</td>
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<td>-</td>
</tr>
<tr>
<td>16</td>
<td>Globulol</td>
<td>1587</td>
<td>0.13</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>γ-Eudesmol</td>
<td>1634</td>
<td>0.05</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>β-Eudesmol</td>
<td>1653</td>
<td>0.05</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td>α-Eudesmol</td>
<td>1657</td>
<td>0.05</td>
<td>-</td>
</tr>
</tbody>
</table>

RI = Retention index relative to C₆-C₂₀ n-alkanes on HP-5MS column; - : Not detected

The most abundant compounds in essential oils of E. camaldulensis and C. viminalis are showed in GCMS 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.

4. Acknowledgments

The authors are grateful to the Institute of Research Management and Monitoring, Universiti of Malaya (IPPP) for the funding (Grant No. PV025/2011B). The authors are also grateful to Institute of Biological Sciences, University of Malaya for research facilities.
Fig. 1: A and B. Essential oil chromatograms of leaves of *E. camaldulensis* and *C. viminalis*, respectively. 1-6 represented the most identified compounds. 1. α-pinene, 2. α-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.

![Chromatograms](image)

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

5. References


