

## Composition of Essential Oils from the Leaf-margin of Lemon Balm

Mudasir A. Tantry<sup>1\*</sup>, Gulzar A. Bhat<sup>2</sup>, Bilal Ahmad Wani<sup>1</sup>, Bashir A. Ganai<sup>1</sup>, Azra N. Kamili<sup>1</sup>, Ruqeya Nazir<sup>1</sup> and Abdul S. Shawl<sup>3</sup>

<sup>1</sup>Centre of Research for Development, University of Kashmir Srinagar 190006

<sup>2</sup>Department of Biochemistry, University of Kashmir Srinagar 190006

<sup>3</sup>Indian Institute of Integrative Medicine, Sanatnagar Srinagar 190005

\*Email: mudaek@gmail.com

### ABSTRACT

The composite oil samples of leaf-margin of lemon balm (*Melissa officinalis* L.), on hydro-distillation provided a refreshing white viscous essential oil with characteristic lemon balm smell. The oil was found to be complex mixture of mono- and sesquiterpenes and 50 compounds comprising 98.95 % of the oil were characterized with the help of Gas Chromatography (GC), Gas Chromatography/Mass Spectrometry (GC/MS). Major compounds of the oil were characterized as caryophyllene oxide (12.5 %),  $\beta$ -pinene (11.2 %),  $\gamma$ -terpinene (10.3 %), and terpinene-4-ol (8.7%). This investigation performed on Lemon balm allowed the distinction of first sort of essential oils composition of, as far leaf-margin of Lemon balm is concerned.

**Keywords:** Lemon balm, leaf-margin, essential oils

### INTRODUCTION

Lemon balm (*Melissa officinalis* L.) is an important aromatic plant, a perennial herb cultivated for lemon-scented leaves used as seasoning and in medicine. There are number of reports of literature on the essential oils of Lemon balm (Patora *et al.*, 2003). Mostly the volatile oil, its chemical profile; and its different pharmacological

activities such as antifungal, antibacterial and spasmolytic properties (Mimica-Dukic *et al.*, 2004; Larrondo *et al.*, 1995; Carnat *et al.*, 1998) are well documented. The alcoholic extracts of Lemon balm having antioxidant properties, normally because of high phenolic content such as rosmarinic acid (Mencherini *et al.*, 2007). Biosynthesis of proteins in cancer cells has been reported, by Lemon balm containing substances. Studies on the volatile oils of the balm is extensive but the action on other secondary metabolites is not so much in detail, but as far as leaf-margin there is as such no report of essential oils composition of Lemon balm.

## **MATERIAL AND METHODS**

### *2.1. Plant source*

The leaf of Lemon balm was collected from Bonera Field Station (Kashmir Valley) and identified by the taxonomist Dr Anzar Khuroo at Centre for Biodiversity and Taxonomy Biodiversity (CBT), University of Kashmir, Srinagar India.

### *2.2. Extraction and isolation*

The essential oil was obtained by the hydro-distillation of fresh plant material in a Clevenger type apparatus for four hours. The sample afforded white viscous oil with characteristic lemon flavour (yield 0.04%). The oil was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and was placed at low temperature in refrigerator until analysis.

### *2.3. GC analysis*

The composition of the oil was carried out by GC on a gas chromatograph Perkin Elmer-8500 with Flame Ionization Detector (FID), using a fused-silica column (30 m × 0.32 mm i.d.; 0.25 µm film thickness) coated with 5% diphenyl and 95% polysiloxane (BP-5). Oven temperature programmed from 60-220 °C. "Injector temperature, 240 °C"; "detector temperature, 270 °C". Carrier gas nitrogen at 8 psi, split ratio 1:80. Retention indices (RI) of the sample components and authentic compounds were determined on the basis of homologous *n*-alkanes hydrocarbons under the same

conditions.

#### 2.4. GC/MS

GC/MS data obtained on Varian Mass Spectrometer using VF-5 column (60 m × 0.32 mm i.d.; 0.25 µm film thickness). Column temperature programmed 5 min. at 60 °C, then rising at 2 and 3 °C upto 240 °C. “Injector temperature, 240 °C”; “ion source temperature, 250 °C”, “interface temperature, 270 °C; acquisition mass range 700-40 amu; ionization energy, 70 eV. Helium was used as carrier gas with a flow rate 0.5 ml/min. The identification of peaks was accomplished by comparison of the mass spectra with those reported in the NIST library (Adam, 1989). Identification of the oil components was also done by comparison of their linear RI with those from Mass Finder library.

### RESULTS

The composite oil samples of Leaf-margin of Lemon balm (*Melissa officinalis* L.), on hydro-distillation provided a refreshing white viscous essential oil with characteristic lemon balm smell. The oil was found to be complex mixture of mono- and sesquiterpenes and 50 compounds comprising 98.95 % of the oil were characterized with the help of Gas Chromatography (GC), Gas Chromatography/Mass Spectrometry (GC/MS). Major compounds of the oil were characterized as caryophyllene oxide (12.5 %), β-pinene (11.2 %), γ-terpinene (10.3 %), and terpinene-4-ol (8.7%). The composition of the leaf-margin essential oils of Lemon balm (*Melissa officinalis* L.) is tabulated (Table 1).

Table 1. Composition of the leaf-margin essential oils of Lemon balm (*Melissa officinalis* L.)

| Constituent                    | Retention Index | %age   | Method of Identification |
|--------------------------------|-----------------|--------|--------------------------|
| □-Thujene                      | 926             | 3.4    | MS, RI                   |
| □-Pinene                       | 932             | 2.1    | MS, RI                   |
| Camphene                       | 936             | 0.03   | MS, RI                   |
| Sabinene                       | 942             | 2.3    | MS, RI                   |
| Linalool                       | 947             | 0.02   | MS, RI                   |
| □-Pinene                       | 984             | 11.2   | MS, RI                   |
| Myrcene                        | 1005            | 0.34   | MS, RI                   |
| □-Phellandrene                 | 1009            | 1.2    | MS, RI                   |
| Citronellal                    | 1079            | 4.8    | MS, RI                   |
| Citranellol                    | 1087            | 3.1    | MS, RI                   |
| □-Terpinene                    | 1092            | 1.0    | MS, RI                   |
| <i>p</i> -Cymene               | 1098            | 0.01   | MS, RI                   |
| Limonene                       | 1128            | 0.5    | MS, RI                   |
| Geraniol                       | 1145            | 0.05   | MS, RI                   |
| 1,8-Cineole                    | 1151            | 0.8    | MS, RI                   |
| (Z)-□-Ocimene                  | 1197            | 0.7    | MS, RI                   |
| Neral                          | 1213            | 0.008  | MS, RI                   |
| Benzene acetaldehyde           | 1268            | 0.001  | MS, RI                   |
| □-Terpinene                    | 1392            | 10.3   | MS, RI                   |
| <i>cis</i> -Sabinene hydrate   | 1415            | 1.2    | MS, RI                   |
| Geranic acid                   | 1431            | 1.3    | MS, RI                   |
| Linalool                       | 1447            | 0.04   | MS, RI                   |
| <i>trans</i> -Sabinene         | 1461            | 0.02   | MS, RI                   |
| <i>trans</i> -Caryophyllene    | 1537            | 3.4    | MS, RI                   |
| <i>trans</i> -Pinocarveol      | 1542            | 0.7    | MS, RI                   |
| <i>trans</i> -Verbenol         | 1571            | 0.006  | MS, RI                   |
| Geranylacetate                 | 1627            | 2.03   | MS, RI                   |
| Pinocarvone                    | 1634            | 3.7    | MS, RI                   |
| <i>p</i> -Mentha-1,5-dien-8-ol | 1688            | 0.002  | MS, RI                   |
| Terpinen-4-ol                  | 1722            | 8.7    | MS, RI                   |
| □-Humulene                     | 1743            | 0.2    | MS, RI                   |
| Myrtenal                       | 1779            | 0.001  | MS, RI                   |
| □-Cadinenene                   | 1856            | 0.01   | MS, RI                   |
| Bornyl acetate                 | 1872            | 1.3    | MS, RI                   |
| Thymol                         | 1897            | 2.5    | MS, RI                   |
| Humulene oxide                 | 1899            | 0.002  | MS, RI                   |
| Carvacrol                      | 1910            | 0.06   | MS, RI                   |
| ( <i>E</i> )-□-Damascenone     | 1912            | 0.05   | MS, RI                   |
| □-Bourbonene                   | 1918            | 1.2    | MS, RI                   |
| ( <i>E</i> )-Caryophyllene     | 1951            | 3.1    | MS, RI                   |
| □-Humulene                     | 1967            | 0.2    | MS, RI                   |
| Germacrene D                   | 1989            | 2.7    | MS, RI                   |
| <i>E</i> -□-Ionone             | 2021            | 0.7    | MS, RI                   |
| Bicyclogermacrene              | 2053            | 1.1    | MS, RI                   |
| <i>trans</i> -□-Guaiene        | 2063            | 0.07   | MS, RI                   |
| □-Bisabolene                   | 2113            | 4.9    | MS, RI                   |
| Caryophyllene oxide            | 2178            | 12.5   | MS, RI                   |
| Caryophyllenol II              | 2185            | 1.2    | MS, RI                   |
| Hexahydrofarnesyl              | 2191            | 0.2    | MS, RI                   |
| Allyl Oxide                    | 2229            | 0.002  | MS, RI                   |
| Total                          |                 | 98.952 |                          |

RI = Relative retention indices relative to C9–C23 *n*-alkanes on the BP-5 column. GC-MS identification based on comparison of mass spectra.

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