Chemical investigations of male and female leaf extracts from *Schinus molle* L.

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Abstract

The pepper-tree Schinus molle is an evergreen ornamental plant with various and diversified list of medical uses. In this article we analysed the chemical composition of male and female leaves of this plant during the off-flowering and flowering seasons. The leaf extracts were obtained by using a sequential extraction with solvents of different polarities and the chemical composition was investigated by GC-MS. **The results showed a total of twenty-three components, in which elemol is the most abundant constituent followed by bicyclogermacrene, γ-eudesmol, α-eudesmol, β-eudesmol and isocalamendiol.** The petroleum ether and diethyl ether extracts from male and female flowering and off-flowering leaves consisted of sesquiterpene hydrocarbons as a major constituent followed by monoterpane hydrocarbons, while the acetone extracts showed a different composition. The obtained results show differences in the chemical composition between male and female and flowering and not flowering
Experimental section

**Plant collection and extracts preparation**

Fresh leaves of male and female *S. molle* plants, included in the internal management system and identified by cod. AS21 (male individual) and cod. AS22 (female individual), were collected at the “Angelo Rambelli” Botanical Garden (Tuscia University, Viterbo, Italy) in October 2017 for the off-flowering samples and in June 2017 for the flowering samples. The plant materials were washed with distilled water, air-dried in a shaded area for 2-4 days and carefully hand-selected to separate the leaves, which were kept in a plastic container and frozen at –80°C for freeze-drying. After lyophilisation, the dried material was chopped and stored at 4°C until use. Dried leaves were extracted using three solvents (100 mL each one) of increasing polarity (petroleum ether 40-60, diethyl ether and acetone) in a Soxhlet apparatus. After 6 extraction cycles for each solvent, the solid extracts were obtained by rotary evaporation (RV 08-VC, IKA, USA).

**GC-MS analysis:**

GC-MS Perkin Elmer Clarus 500 instrument equipped with flame ionization detector (FID) and a Restek Stabilwax fused-silica capillary column (length 60 m x 0.25 mm ID) was employed. Helium was used as the carrier gas with a flow rate of 1 mL/min, and the oven temperature program was as follows: 5 minutes at 60°C then a gradient of 5°C/minute to 220°C. 1 µl of sample was diluted in 1 ml of CH₃OH and 1 µl of the solution was manually injected at 280°C into the GC injector in the splitless mode. All mass spectra were recorded in the electron impact ionization (EI) at 70 eV. The mass spectrometer was scanned from m/z 30-350 amu with scan time 0.2 sec.

Relative percentages for quantification of the components were calculated by electronic integration of the GC-FID peak areas. Identification of the constituents was performed on MS library search (Wiley and Nist). Linear retention indices (LRIs) of each compound were calculated using a mixture of aliphatic hydrocarbons (C8-C30, Ultrasci) injected directly into GC injector at the same
temperature program reported above. Analyses were repeated twice.

| #  | Component2          | Male                  |                       | Female                |                       |
|----|---------------------|-----------------------|-----------------------|-----------------------|-----------------------|
|    |                     | LRI1                  | LRI1lit              | Leaves (flowering)   | Leaves (off-flowering) |
|    |                     | 1037 1038             | -                     | 0.72                  | -                     |
| 1   | α-thujene           | 1133 1133             | 0.70                  | 2.58                  | 0.18                  |
| 2   | sabinene            | 1331 1326             | -                     | -                     | -                     |
| 3   | 2-propanone, 1-hydroxy | 1360 1369       | -                     | -                     | -                     |
| 4   | 2-pentanone, 4-hydroxy-4-methyl | 1510 1514 | 1.71                 | 1.21                  | -                     |
| 5   | elixene             | 1556 1549             | 0.56                  | 0.31                  | 0.91                  |
| 6   | α-gurjunene         | 1608 1598             | 1.27                  | 0.74                  | 2.11                  |
| 7   | β-elemene           | 1610 1621             | 0.31                  | -                     | -                     |
| 8   | 4-terpineol         | 1637 1634             | 1.80                  | 0.68                  | 2.18                  |
| 9   | β-caryophyllene     | 1705 1693             | 0.63                  | 0.32                  | 0.97                  |
| 10  | humulene            | 1732 1726             | 9.63                  | 6.55                  | 15.23                 |
| 11  | germacrene D        | 1774 1769             | 11.59                 | 9.73                  | 0.58                  |
| 12  | bicyclogermacrene   | 1788 1788             | 1.16                  | 0.64                  | 1.76                  |
| 13  | δ-cadinene          | 2077 2069             | -                     | 5.20                  | -                     |
| 14  | germacrene D-4-ol   | 2093 2090             | 53.68                 | 65.83                 | 69.53                 |
| 15  | elemol              | 2117 2110             | -                     | -                     | 4.85                  |
| 16  | viridiflorol        | 2141 *                | -                     | 0.87                  | 2.02                  |
| 17  | dehydroxyisocalamendiol | 2142 2136 | 2.22                 | 0.64                  | -                     |
| 18  | spathulenol         | 2191 2185             | 2.00                  | 0.49                  | -                     |
| 19  | γ-eudesmol          | 2240 2232             | 3.94                  | 1.36                  | 1.93                  |
| 20  | α-eudesmol          | 2258 2249             | 6.69                  | 1.79                  | 1.95                  |
| 21  | β-eudesmol          | 2510 2500             | 2.11                  | -                     | 0.65                  |
| 22  | isocalamendiol      | 2887 2900             | -                     | 0.34                  | -                     |
| 23  | dodecanoic acid     | 10.33 9.85            | 15.41                 | 18.76                 |                       |

1# indicates the compound identification number; 2 the components are reported according their eluition order on polar column; 3 Linear Retention Indices measured on polar column; 4 Linear Retention Indices from literature; LRIslit not available

Table S1. Chemical composition (%) of petroleum ether extract from male and female S. molle
| #  | Component                     | Male | Female |
|----|-------------------------------|------|--------|
|    |                               | LRI  |        | Leaves    | Leaves  | Leaves    | Leaves  |
|    |                               |      |        | (flowering) | (off-flowering) | (flowering) | (off-flowering) |
| 1  | α-thujene                     | 1037 | 1038   | -         | 0.67    | -         | 0.72    |
| 2  | sabinene                      | 1133 | 1133   | 1.46      | 1.84    | 2.42      | 1.66    |
| 3  | 2-propanone, 1-hydroxy        | 1331 | 1326   | -         | -       | -         | -       |
| 4  | 2-pentanone, 4-hydroxy-4-methyl | 1360 | 1369   | -         | -       | -         | -       |
| 5  | elixene                       | 1510 | 1514   | 1.46      | 1.11    | -         | -       |
| 6  | α-gurjunene                   | 1556 | 1549   | 0.67      | 0.22    | 0.90      | 0.54    |
| 7  | β-elemene                     | 1608 | 1598   | 0.95      | 0.61    | 1.32      | 1.22    |
| 8  | 4-terpineol                   | 1610 | 1621   | 0.37      | -       | -         | -       |
| 9  | β-caryophyllene               | 1637 | 1634   | 1.73      | 0.55    | 1.92      | 2.08    |
| 10 | humulene                      | 1705 | 1693   | 0.56      | 0.25    | 0.77      | 0.60    |
| 11 | germacrene D                  | 1732 | 1726   | 7.61      | 5.14    | 11.39     | 10.86   |
| 12 | bicyclogermaclere             | 1774 | 1769   | 9.56      | 7.76    | 0.38      | 0.41    |
| 13 | δ-cadinene                    | 1788 | 1788   | 1.05      | 0.52    | 1.28      | 1.07    |
| 14 | germacrene D-4-ol             | 2077 | 2069   | -         | 1.65    | -         | 3.83    |
| 15 | elemol                        | 2093 | 2090   | 57.57     | 72.58   | 71.37     | 60.80   |
| 16 | viridiflorol                  | 2117 | 2110   | -         | -       | -         | -       |
| 17 | dehydroxyisocalamendiol        | 2141 | *      | -         | 1.83    | 1.65      | 1.26    |
| 18 | spathulenol                   | 2142 | 2136   | 3.22      | 0.65    | -         | -       |
| 19 | γ-eudesmol                    | 2191 | 2185   | 1.83      | 0.46    | -         | 0.64    |
| 20 | α-eudesmol                    | 2240 | 2232   | 3.13      | 1.45    | 2.57      | 1.73    |
| 21 | β-eudesmol                    | 2258 | 2249   | 4.95      | 1.82    | 2.60      | 2.17    |
| 22 | isocalamendiol                | 2510 | 2500   | 3.88      | -       | 1.43      | -       |
| 23 | dodecanoic acid               | 2887 | 2900   | -         | 0.89    | -         | 10.41   |

|                     | Male | Female |
|---------------------|------|--------|
| Monoterpane hydrocarbons | 9.07 | 7.65   |
| Monoterpene alcohol   | 0.37 |        |
| Sesquiterpene hydrocarbons | 76.05 | 79.02 |
| Oxygenated sesquiterpenes | 1.83 | 1.65 |
| Tricyclic sesquiterpenes | 3.22 | 0.65 |
| Sesquiterpene alcohol  | 1.65 | 3.83   |
| Bicyclic sesquiterpenes | 11.29 | 8.31 |

Others | 0.89 | 10.41 |

*1# indicates the compound identification number; 2*the components are reported according their elution order on polar column. Linear Retention Indices measured on polar column; 3Linear Retention Indices from literature; 4LRIs not available.

**Table S2.** Chemical composition (%) of diethyl ether extract from male and female *S. molle*
| #  | Component² | LRI¹ | LRI² | Male Leaves (flowering) | Male Leaves (off-flowering) | Female Leaves (flowering) | Female Leaves (off-flowering) |
|----|------------|------|------|-------------------------|---------------------------|--------------------------|-----------------------------|
| 1  | α-thujene   | 1037 | 1038 | -                       | -                         | -                        | -                           |
| 2  | sabinene    | 1133 | 1133 | -                       | -                         | 0.75                     | 0.39                        |
| 3  | 2-propanone, 1-hydroxy | 1331 | 1326 | -                       | -                         | 1.04                     | 0.32                        |
| 4  | 2-pentanone, 4-hydroxy-4-methyl | 1360 | 1369 | -                       | 57.47                     | 17.86                    | 59.92                       |
| 5  | elixene     | 1510 | 1514 | -                       | -                         | -                        | -                           |
| 6  | α-gurjunene | 1556 | 1549 | -                       | -                         | -                        | -                           |
| 7  | β-elemene   | 1608 | 1598 | -                       | -                         | -                        | -                           |
| 8  | 4-terpineol | 1610 | 1621 | -                       | -                         | -                        | -                           |
| 9  | β-caryophyllene | 1637 | 1634 | 1.57                  | 1.46                     | 3.33                     | 1.54                        |
| 10 | humulene    | 1705 | 1693 | -                       | -                         | 1.07                     | 0.30                        |
| 11 | germacrene D| 1732 | 1726 | 1.34                   | 6.52                     | 9.21                     | 3.70                        |
| 12 | bicyclogermacone | 1774 | 1769 | 1.14                  | 11.07                    | 0.66                     | 0.34                        |
| 13 | δ-cadinene  | 1788 | 1788 | -                       | -                         | 0.96                     | 0.84                        |
| 14 | germacrene D-4-ol | 2077 | 2069 | -                       | 2.07                     | -                        | 1.58                        |
| 15 | elemol      | 2093 | 2090 | 17.42                  | 9.98                     | 51.89                    | 25.72                       |
| 16 | viridiflorol | 2117 | 2110 | -                       | -                         | -                        | -                           |
| 17 | dehydroxyisocalamendiol | 2141 | * | -                       | 1.02                     | 2.23                     | -                           |
| 18 | spathulenol | 2142 | 2136 | 0.06                  | 0.22                     | -                        | -                           |
| 19 | γ-eudesmol  | 2191 | 2185 | 0.08                  | 0.98                     | -                        | 0.37                        |
| 20 | α-eudesmol  | 2240 | 2232 | 0.16                  | 3.69                     | 1.92                     | 1.34                        |
| 21 | β-eudesmol  | 2258 | 2249 | 24.68                 | 3.78                     | 2.33                     | 1.56                        |
| 22 | isocalamendiol | 2510 | 2500 | 3.78                  | -                        | 6.75                     | -                           |
| 23 | n-hexadecanoic acid | 2887 | 2900 | 49.77                | 1.74                     | -                        | 2.08                        |

|         | Monoterpenes hydrocarbons | 1.34 | 6.52 | 9.96 | 4.09 |
|         | Monoterpenes alcohol       | -     | -    | -    | -    |
|         | Sesquiterpene hydrocarbons | 46.12 | 18.43 | 64.92 | 30.13 |
|         | Oxygenated sesquiterpenes  | -     | 1.02 | 2.23 | -    |
|         | Tricyclic sesquiterpenes   | 0.06  | 0.22 | -    | -    |
|         | Sesquiterpene alcohol      | -     | 2.07 | -    | 1.58 |
|         | Bicyclic sesquiterpenes    | 2.71  | 12.53 | 3.99 | 1.88 |
|         | Others                     | 49.77 | 59.21 | 18.9 | 62.32 |

¹# indicates the compound identification number; ²the components are reported according their elution order on polar column; ³Linear Retention Indices measured on polar column; ⁴Linear Retention Indices from literature; *LRIs is not available.

Table S3. Chemical composition (%) of acetone extract from male and female S. molle