**SUPPLEMENTARY MATERIAL**

# Studies on essential oil from rose-scented geranium, *Pelargonium graveolens* L'Hérit. (Geraniaceae)

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**Abstract**

Rose-scented geranium, *Pelargonium graveolens* L'Hérit. (Geraniaceae), is an economically important plant. GC-MS analysis of the essential oil, prepared by hydro-distillation from this plant species, showed the presence of iso-menthone (15.71%), *epi*-*α*-cadinol (15.49%), *iso*-menthol (6.46%), geranyl formate (6.22%), geraniol (6.16%) and citronellol (5.53%). The composition of the absolute prepared by solvent extraction was compared to that of the essential oil. Change in citronellol to geraniol ratio in the absolute was monitored during leaf development. Estimation of the ratio of the two compounds was carried out using $^{1}$H NMR spectroscopy. Geraniol content was highest in young leaves and citronellol content increased with increase in leaf age. Meta-analysis of the essential oil constituents reported from different countries was carried out. Menthone and isomenthone as well as citronellol and geraniol were negatively correlated. A significantly positive correlation was found between geraniol and linalool.

**Key Words:** Geranium, essential oil, absolute, citronellol, geraniol, $^{1}$H NMR, PCA.

## 1. Experimental

### *1.1. Plant material*

Rose-scented geranium, *Pelargonium graveolens* L'Hérit. (Geraniaceae) was grown in the experimental station of the pharmacognosy department, faculty of pharmacy, Beni-Suef University, Egypt, following standard agricultural practices. The leaves of the plants were harvested in May 2018 during the flowering period. The plant was taxonomically authenticated by Dr. Abdelhalim Mohamed, Flora & Phyto Taxonomy Researches Department – Horticultural Research Institute, Agricultural Research Centre, Cairo,

Egypt. A voucher specimen (CAIM 132) was deposited in the herbarium of Flora and Phyto Taxonomy Researches Department (CAIM).

### *1.2. Hydrodistillation of essential oil*

One hundred gram of fresh aerial parts of the plants was subjected to hydro-distilled for 2 h using a Clevenger apparatus. The obtained essential oil was treated with anhydrous sodium sulphate to make it moisture free. The essential oil was stored at +4°C till analysis.

### *1.3. Solvent Extraction of the plant material*

The absolute was prepared from the leaves of rose-scented geranium using solvent extraction. The fresh plant material (100 g) was extracted with *n*-hexane for 12 h. The extract was then filtered and concentrated by a rotary evaporator under reduced pressure at 30°C. The residue obtained (concrete) was extracted with absolute ethanol and filtered under reduced pressure (absolute). The absolutes prepared from the hydroponically- and field grown plants were stored in glass vials and kept in a freezer till their analysis. Yield was calculated as the mass of the absolute obtained divided by the fresh weight of the leaves.

### *1.4. Gas chromatography/mass spectrometry (GC/MS) analysis*

The analysis of geranium absolute was performed using a Thermo Scientific, Trace GC / ISQ Single Quadrupole mass spectrometer, TG-5MS fused silica capillary column (30 m, 0.25 mm i.d., 0.25 µm film thickness). For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas was used as the carrier gas at a flow rate of 1 ml/min. The injector (MS transfer line) temperature was set at 280°C. The oven temperature was programmed at an initial temperature 40°C (3 min) to 280°C as a final temperature at a gradual increase rate of 5°C/min (5 min). Identification of the compounds was performed based on the comparison of their relative retention time and mass spectra with data published in the literature (Adams 2001) and by matching their recorded mass spectra with those of the NIST, Willy library data of the GC/MS system. The quantification of the identified compounds was investigated using a percent relative peak area.

### *1.5. $^{1}$H NMR analysis of the absolute*

The NMR spectrometer used was a Bruker model AVANCE III HD (Fällanden, Switzerland) equipped with a BBFO Smart Probe and Bruker 400 AEON Nitrogen-Free Magnet, and operating at a $^{1}$H frequency of 400.13 MHz (O1). The following conditions were used for acquisition of the $^{1}$H NMR spectra: 30 degrees pulse experiment, excitation pulse 10 µs corresponding to an angle of xyz; acquisition time of 4.1 s; relaxation delay 0.1 s; sweep width 15.1 ppm (8012 Hz); temperature 295.1 K. Thirty two scans were recorded. Deuterated chloroform ($CDCl_3$) was used for sample preparation.

### *1.6. Meta Analyses*

Essential oil constituents of rose-scented geranium from different parts of the world were studied. References to these reports are provided in Table S2. In these reports, the essential oils were prepared by hydro-distillation and the contents of the essential oil constituents were measured using gas chromatography. Samples shown in Table 2 are named according to their country of origins, arranged in alphabetical order, where geranium plants were collected and processed for their essential oils (Algeria, Bosnia and Herzegovina, China, Egypt, India, Iran, Portugal, Reunion Island, Serbia, Tajikistan, Tunisia and USA).

### *1.7. Statistical analysis*

Principal component analysis was used to visualize the correlation between rose-scented geranium essential oil constituents growing in different countries using Microsoft Office XLSTAT 2019.3.1 (www.xlstat.com). The Pearson test was used to calculate the linear correlation coefficients between the variables.

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**Table S1**

Essential oil composition of rose-scented geranium prepared by hydro-distillation and solvent extraction.

| No. | Compound | Hydro-distillation Area (%) | Solvent extraction Area (%) |
|---|---|---|---|
| 1. | Linalool | 6.18 | - |
| 2. | *cis*-Rose oxide | 2.10 | 0.67 |
| 3. | *trans*-Rose oxide | 0.71 | 0.27 |
| 4. | Citronellal | - | 0.50 |
| 5. | Menthone | - | 0.11 |
| 6. | *iso*-Menthone | 15.71 | 9.87 |
| 7. | *neo*-Menthol | 0.23 | - |
| 8. | Menthol | 5.45 | - |
| 9. | *iso*-Menthol | 6.46 | - |
| 10. | Citronellol | 5.53 | 29.30 |

| | | | |
|---|---|---|---|
| 11. | Nerol | - | 0.13 |
| 12. | Geraniol | 6.16 | 7.54 |
| 13. | Geranial | - | 12.90 |
| 14. | Geranyl formate | 6.22 | 4.80 |
| 15. | Citronellyl formate | 4.76 | - |
| 16. | Citronellyl acetate | 0.27 | 0.3 |
| 17. | *β*-Bourbonene | 0.68 | 1.38 |
| 18. | *trans*-Caryophyllene | 0.72 | 0.64 |
| 19. | Citronellyl propionate | 0.25 | 0.35 |
| 20. | Geranyl propanoate | 0.45 | 0.50 |
| 21. | Germacrene-D | 0.11 | 2.65 |
| 22. | α-Muurolene | 0.99 | 0.17 |
| 23. | Cubebol | 0.10 | 0.44 |
| 24. | Citronellyl butyrate | - | 2.41 |
| 25. | Geranyl butyrate | 0.47 | 1.11 |
| 26. | Caryophyllene oxide | 1.60 | 2..24 |
| 27. | epi-*α*-Cadinol | 15.49 | 8.79 |
| 28. | Geranyl pentanoate | 0.69 | 0.14 |
| 29. | Citronellyl tiglate | 1.12 | 0.62 |
| 30. | Geranyl tiglate | 4.85 | 2.30 |

## Table S2

Contents of the major constituents present in the essential oil of rose-scented geranium from different countries of the world.

| Country | Linalool | *trans*-Rose oxide | *cis*-Rose oxide | Menthone | Isomenthone | Citronellol | Geraniol | Geranial | Citronellyl formate | Geranyl formate | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Algeria | 3.2 | 2.2 | 1.3 | 3.6 | 4.1 | 30.2 | 7.6 | 2.6 | 9.3 | 1.5 | Boukhatem et al., 2013 |
| Bosnia and Herzegovina | 3.6 | 0.1 | 0.1 | 5.2 | 0.0 | 19.0 | 27.5 | 0.0 | 0.0 | 0.1 | Ćavar and Maksimović, 2012 |
| China | 10.9 | 0.4 | 0.9 | 1.0 | 7.9 | 29.7 | 14.7 | 1.4 | 10.5 | 4.8 | Ali et al., 2013 |
| Egypt | 5.1 | 0.3 | 0.0 | 1.5 | 5.4 | 29.9 | 18.0 | 0.0 | 0.0 | 0.0 | Fayed, 2009 |
| India | 6.7 | 0.2 | 0.5 | 0.2 | 5.3 | 28.2 | 22.1 | 0.8 | 6.3 | 4.1 | Rao et al., 2002 |
| Iran | 3.1 | 1.7 | 0.6 | 2.1 | 4.3 | 47.5 | 8.7 | 0.0 | 11.0 | 1.7 | Jalali-Heravi et al., 2006 |
| Portugal | 2.7 | 0.5 | 0.0 | 0.0 | 5.6 | 26.9 | 8.1 | 0.0 | 13.2 | 5.5 | Gomes et al., 2004 |
| Reunion Island | 16.0 | 0.1 | 0.4 | 0.1 | 7.6 | 21.9 | 18.3 | 1.3 | 11.6 | 4.0 | Gauvin et al., 2004 |
| Serbia | 9.8 | 0.4 | 1.2 | 4.3 | 2.9 | 24.5 | 15.3 | 0.1 | 10.7 | 5.6 | Džamić et al., 2014 |
| Tajikistan | 3.0 | 0.8 | 1.9 | 3.1 | 2.1 | 37.5 | 6.0 | 0.0 | 0.0 | 2.0 | Sharopov et al., 2014 |
| Tunisia | 5.6 | 2.0 | 0.0 | 0.3 | 4.4 | 21.9 | 11.1 | 0.0 | 13.2 | 6.2 | Boukhris et al., 2013 |
| USA | 0.1 | 0.3 | 0.7 | 1.0 | 5.5 | 50.9 | 0.1 | 0.2 | 13.3 | 0.0 | Ali et al., 2013 |

**Table S3**

Correlation matrix between the main essential oil constituents reported in rose-scented geranium from different countries.

| Geranyl formate | Citronellyl formate | Geranial | Geraniol | Citronellol | Isomenthone | Menthone | *cis*-Rose oxide | *trans*-Rose oxide | Linalool | Variables |
|---|---|---|---|---|---|---|---|---|---|---|
| 0.510 | 0.173 | 0.327 | 0.480 | -0.537 | 0.478 | -0.215 | -0.029 | -0.325 | **1** | **Linalool** |
| 0.078 | 0.277 | 0.263 | -0.451 | 0.172 | -0.117 | 0.075 | 0.170 | **1** | -0.325 | ***trans*-Rose oxide** |
| -0.071 | -0.136 | 0.317 | -0.405 | 0.344 | -0.197 | 0.424 | **1** | 0.170 | -0.029 | ***cis*-Rose oxide** |
| -0.400 | -0.494 | -0.023 | 0.201 | -0.094 | **-0.820*** | **1** | 0.424 | 0.075 | -0.215 | **Menthone** |
| 0.331 | 0.546 | 0.387 | -0.180 | 0.111 | **1** | **-0.820** | -0.197 | -0.117 | 0.478 | **Isomenthone** |
| -0.466 | 0.155 | -0.135 | **-0.717** | **1** | 0.111 | -0.094 | 0.344 | 0.172 | -0.537 | **Citronellol** |
| 0.042 | -0.468 | -0.011 | **1** | **-0.717*** | -0.180 | 0.201 | -0.405 | -0.451 | 0.480 | **Geraniol** |
| 0.028 | 0.199 | **1** | -0.011 | -0.135 | 0.387 | -0.023 | 0.317 | 0.263 | 0.327 | **Geranial** |
| 0.558 | **1** | 0.199 | -0.468 | 0.155 | 0.546 | -0.494 | -0.136 | 0.277 | 0.173 | **Citronellyl formate** |
| **1** | 0.558 | 0.028 | 0.042 | -0.466 | 0.331 | -0.400 | -0.071 | 0.078 | 0.510 | **Geranyl formate** |

*Values in bold are different from 0 with a significance level alpha = 0.05.

**Table S4**

Dimensions, yield and citronellol : geraniol ratio in rose-scented geranium leaves at different developmental stages.

| Stages | Length | Width | Yield | C/G[a] |
|---|---|---|---|---|
| **1** | 1.0 ± 0.2 | 1.1 ± 0.2 | 0.25 | 0.33 |
| **2** | 2.2 ± 0.1 | 2.8 ± 0.3 | 0.31 | 0.60 |
| **3** | 3.3 ± 0.3 | 4.3 ± 0.9 | 0.22 | 1.38 |
| **4** | 4.9 ± 0.4 | 5.8 ± 0.4 | 0.78 | 1.34 |
| **5** | 6.9 ± 0.5 | 9.4 ± 0.5 | 0.07 | -[b] |

[a] Citronellol to geraniol ratio.

[b] Geraniol was absent in the leaves at this developmental stage.

**Figure captions**

Figure S1. The leaves of rose-scented geranium at different developmental stages.

Figure S2. An expanded region of $^{1}$H NMR spectrum (3.40 – 4.50 ppm) for absolute obtained from rose-scneted geranium leaves (stage 2 according to Fig. 1). Proton signals corresponding to citronellol ($CH_2$(1), 3.64-3.68 (*m*)) and geraniol ($CH_2$(1), 4.14 (*d*, *J* = 6.8)) were used to calculate the ratio between these two compounds.

Figure S3. Correlations circle showing correlation between essential oil constituents reported in rose-scented geranium from different countries.

Figure S4. Two-dimensional map showing correlations between essential oil of rose-scented geranium from different countries.

Figure S1

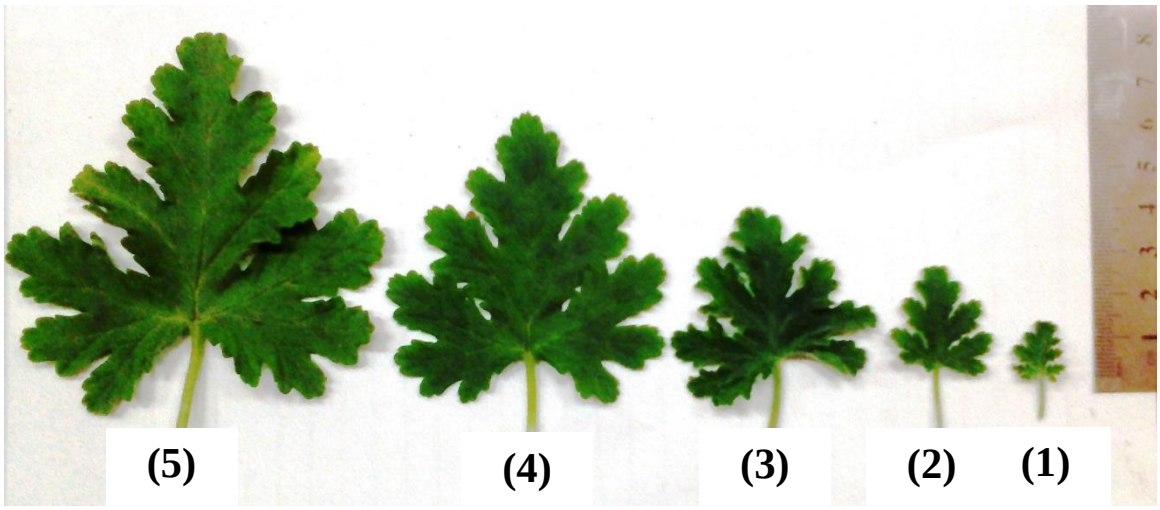


Figure S2

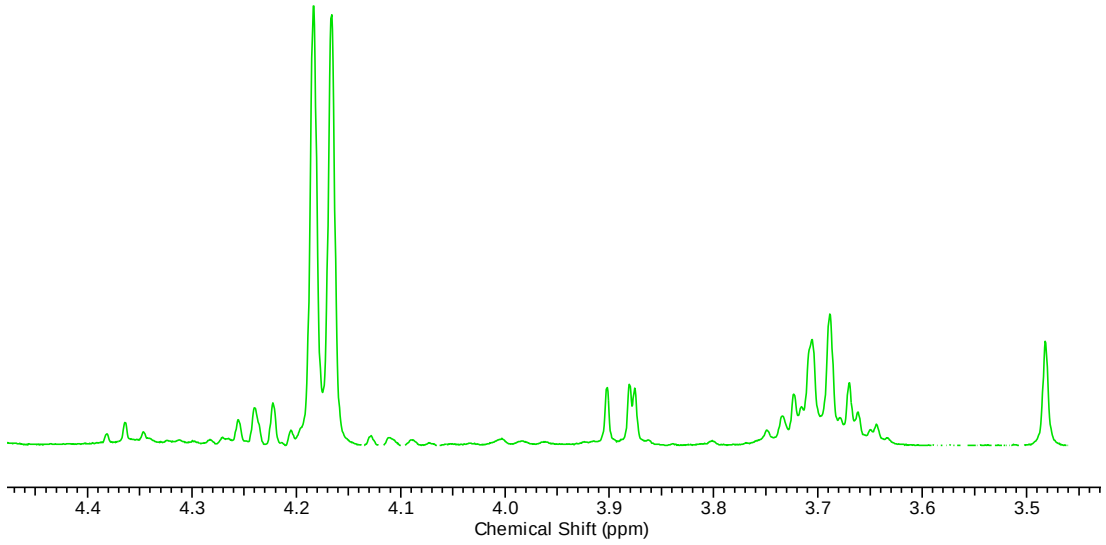


Figure S3

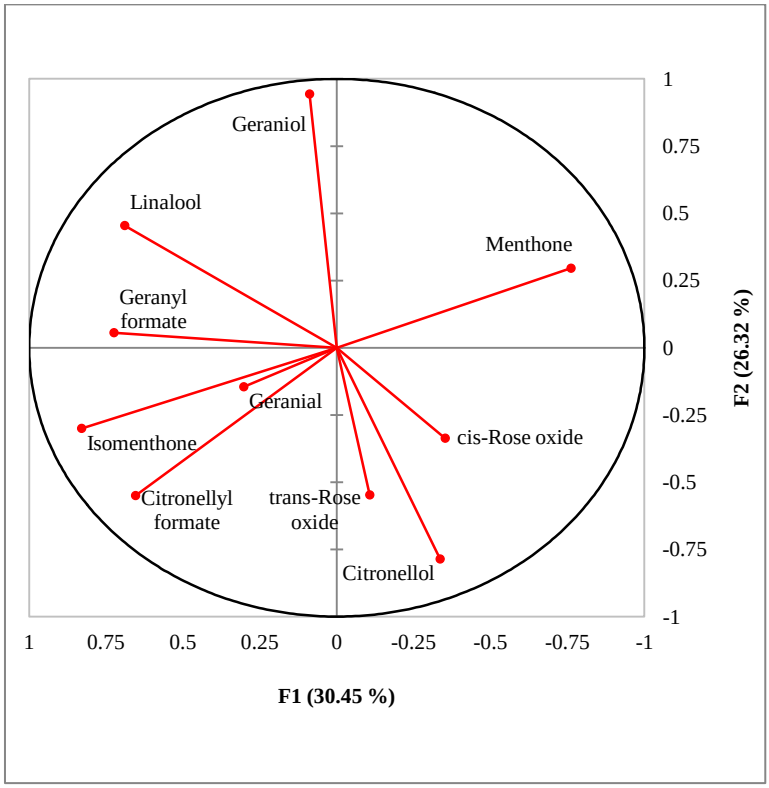
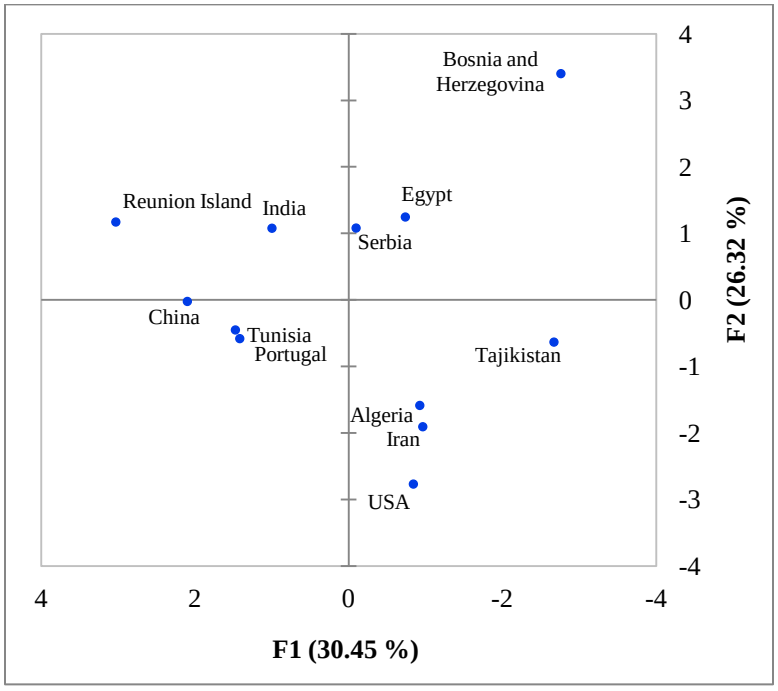

Geraniol
Linalool
Menthone
Geranyl formate
Geranial
Isomenthone
cis-Rose oxide
Citronellyl formate
trans-Rose oxide
Citronellol
1
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
-1
F2 (26.32 %)
F1 (30.45 %)

Figure S4


Bosnia and Herzegovina
Reunion Island
India
Egypt
Serbia
China
Tunisia
Portugal
Tajikistan
Algeria
Iran
USA
4
3
2
1
0
-1
-2
-3
-4
F2 (26.32 %)
4
2
0
-2
-4
F1 (30.45 %)