

**1Phytochemical data parallel morpho-colorimetric variation in *Polygala flavescens* DC.**

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## 27 **Abstract**

28 Phytochemical data, integrated with other sources of information, represent a valuable tool helping  
29 to solve different kinds of taxonomic problems in plant systematics. In the present study, a  
30 comparative investigation, in order to clarify the systematic relationships of the three subspecies  
31 currently recognized within the Italian endemic *Polygala flavescens*, was carried out. Preliminarily,  
32 a morphometric and colorimetric analysis, in order to test the degree of distinctiveness among the  
33 taxa, was performed. Then, a phytochemical analysis based both on volatile and non-volatile  
34 compounds was obtained. Concerning the morpho-colorimetric analysis, our results confirm most  
35 of the characters as useful to discriminate the three subspecies. In addition, some volatile and non-  
36 volatile compounds are good taxonomic markers. Morpho-colorimetric variation is clearly  
37 paralleled by phytochemical results, confirming the value of this kind of data to infer relationships  
38 in plant systematics. Based on these results, we support a taxonomic treatment at subspecific level  
39 for the involved taxa. Finally, based on the most significant morphological characters, a revision of  
40 herbarium specimens allowed to redefine the distribution of the three subspecies. Accordingly, the  
41 range of *P. flavescens* subsp. *maremmana* is limited to Mt. Argentario (southern Tuscany) only.  
42 **Finally, a key is reported for the identification of the three subspecies.**

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44 **Keywords:** *Polygala flavescens* subsp. *flavescens*, *Polygala flavescens* subsp. *maremmana*,  
45 *Polygala flavescens* subsp. *pisaurensis*, Polygalaceae, morphometrics, volatiles, saponins,  
46 flavonoids, oligosaccharides, Italy, **identification key**.

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## 48 **1. Introduction**

49 Phytochemical data are valuable sources of comparative information, helping to solve different  
50 kinds of taxonomic problems in plant systematics (Stuessy 2009). However, as stressed also for  
51 karyology (Astuti et al. 2017), it is fundamental to integrate phytochemistry with other sources of  
52 information, to infer systematic relationships. Several recent studies highlighted congruence

53between phytochemical data and other sources of information, concerning for instance *Juniperus*  
54*oxycedrus* L. group - Cupressaceae (Roma-Marzio et al. 2017), *Crocus* L. ser. *Verni* B.Mathew -  
55Iridaceae (Carta et al. 2015), *Lavandula* L. sect. *Lavandula* (Passalacqua et al. 2017) and *Salvia*  
56*fruticosa* Mill. - Lamiaceae (Tundis et al. 2016). Recently, a phytochemical study on *Polygala*  
57*flavescens* DC. subsp. *flavescens* (Polygalaceae), based on plants collected in Tuscany (De Leo et  
58al. 2017), led to the isolation and structural characterization of 14 compounds, including six  
59flavonol glycosides, four oligosaccharides, an apocarotenoid, and three triterpenoid saponins.  
60Consequently, in order to clarify the systematic relationships of the three subspecies currently  
61recognized within *P. flavescens* (Bartolucci et al. 2018), a comparative integrated phytochemical  
62and morpho-colorimetric study was carried out.

63The genus *Polygala* L. is the largest of the family Polygalaceae, comprising between 325 (Heywood  
64et al. 2007) and 725 (Paiva 1998) species. This genus shows a high diversity of life forms and  
65adaptive strategies, occupying a wide range of ecological niches and showing a nearly cosmopolitan  
66distribution, with the exception of the Arctic, Antarctica, and New Zealand (Paiva 1998). The only  
67comprehensive taxonomic treatment of *Polygala* was published by Chodat (1893), whereas there  
68have been numerous regional treatments, suggesting various morphological traits for taxonomic use  
69(e.g. Marques 1979; Paiva 1998; Bernardi 2000; Peruzzi et al. 2005; Arrigoni 2014). According to  
70Conti et al. (2005), in Italy 28 taxa (including species and subspecies) occur, 14 of which are  
71endemic to the country (Peruzzi et al. 2014). In a recent taxonomic revision of this genus in Italy  
72(Arrigoni 2014), the number of taxa was raised to 35. One of these species, within *P.* subg.  
73*Polygala* (McNeill 1968, is *Polygala flavescens* DC., which includes three taxonomically doubtful  
74infraspecific taxa. *Polygala flavescens* is an Italian endemic species, originally described from  
75Central Italy (Roma-Marzio and Peruzzi 2017), which is actually recorded all along the Italian  
76peninsula, from Emilia-Romagna to Basilicata (Peruzzi et al. 2014). Most of the authors (Zangheri  
771976; Pignatti 1982; Conti et al. 2005; Arrigoni 2014) treated this taxon at specific rank, with the  
78exception of Fiori (1925), who considered it as a variety of *Polygala vulgaris* L. The latter author

79also recorded *P. vulgaris* var. *flavescens* (DC.) Fiori f. *maremmana* (Fiori) Fiori, originally  
80described as *P. flavescens* var. *maremmana* Fiori (Fiori et al. 1908). The latter taxon is currently  
81recognized by Arrigoni (2014) as *P. flavescens* subsp. *maremmana* (Fiori) Arrigoni, a subspecies  
82with a range putatively limited to the coasts of southern Tuscany, from the southern part of the  
83Leghorn province to Mt. Argentario (Arrigoni 2014). *Polygala flavescens* subsp. *maremmana* is  
84still recognised at varietal rank by Pignatti (2017), whereas Bartolucci et al. (2018) consider it as a  
85taxonomically doubtful subspecies. Another species, *P. pisauensis* Caldesi, was described based on  
86plants collected in Marche near Pesaro, and it has always been considered very closely related to *P.*  
87*flavescens* (McNeill 1968; Zangheri 1976; Pignatti 1982; 2017). Recently, Arrigoni (2014) and  
88Bartolucci et al. (2018) treated *P. pisauensis* as a subspecies of *P. flavescens*, i.e. *P. flavescens*  
89subsp. *pisauensis* (Caldesi) Arcang., while Pignatti (2017) is still considering it as a distinct  
90species. However, these taxonomic changes were made in the absence of any quantitative  
91observation. In addition, more recently, the three subspecies were shown to share the same  
92chromosome number, i.e.  $2n = 22$  (Peruzzi et al. 2017).

93In order to quantitatively test the degree of morphological distinctiveness among the three taxa  
94within the *Polygala flavescens* DC. group, morpho-colorimetric analyses were performed.  
95Furthermore, their phytochemical composition was investigated, in order to test the congruence  
96with morpho-colorimetric results, and to provide a more reliable taxonomic treatment. Finally, to  
97update the distribution of the involved taxa, based on morphometric results, herbarium specimens  
98were critically revised.

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## 1002. Materials & Methods

### 1012.1 Plant material

102Since the range of *Polygala flavescens* subsp. *flavescens* covers a large portion of the Italian  
103Peninsula, we chose to sample three populations for this taxon, selected in order a) to cover a  
104reasonable part of its distribution, b) to include its toptypical area. Concerning *P. flavescens* subsp.

105 *maremmana* and *P. flavescens* subsp. *pisaurensis*, since both these subspecies are taxonomically  
106 doubtful and show very restricted ranges, we decided to limit their sampling to topotypical areas  
107 only, in order to have reliable results to be compared with the autonym subspecies. Type locality  
108 areas of the three taxa were identified based on the information published by Arrigoni (2004) and  
109 Roma-Marzio and Peruzzi (2017). The population sampled in Tuscany for *P. flavescens* subsp.  
110 *flavescens* (Polygalaceae) is the same already studied by De Leo et al. (2017) (Table 1). For each  
111 locality, a herbarium voucher was deposited at PI (herbarium acronyms follow Thiers 2017).  
112 Since all the sampling localities fall outside protected areas, and the studied taxa are not  
113 endangered, no specific permissions were required for our activities.

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#### 115 2.2 Chemicals

116 All solvents are HPLC grade and were purchased from VWR. HPLC grade water (18 M $\Omega$ ) was  
117 prepared by a Mill- $\Omega$ 50 purification system (Millipore Corp.). Standard flavonoids (5, 8, 10, 12,  
118 14, and 17), saponins (35 and 43), oligosaccharides (3, 6, 7, and 9), and an apocarotenoid (2) were  
119 previously isolated and fully characterized from *P. flavescens* subsp. *flavescens* DC. (Polygalaceae)  
120 (PFF-T) in our laboratory (De Leo et al. 2017).

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#### 122 2.3 Morphometric analyses

123 We sampled 20 individuals for each population. On each individual, we selected one stem, two well  
124 developed flowers, and one middle cauline leaf for the measurement of 10 characters (Table 2). The  
125 measurements obtained from the two flowers were averaged to a single value per individual. In  
126 addition, for each population, we measured 50 fruits and 20 seeds, for a total of 8 characters (Table  
127 2). Entire plants, fruits and seeds were scanned and then measured by means of ImageJ 1.47  
128 software (Rasband 1997). Three data matrices were built (S1.1; S1.2; S1.3 Tables): one for flower,  
129 leaf, and stem characters (dataset 1 in Table 2), one for fruits (dataset 2 in Table 2), and one for  
130 seeds (dataset 3 in Table 2).

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#### 1322.4 *Flower colorimetric analysis*

133 Since the colour of the flowers could change with phenology (Weiss 1995), to quantitatively  
134 evaluate differences of this characters, pictures of 20 flowers at the same developmental stage (from  
135 plants in full blossom and without fruits) for each population were taken under the same light  
136 conditions. Then, using the image analysis software Gimp 2.8.14 (Kimball and Mattis 2014), the  
137 relative contributions of Red, Green and Blue (RGB) of flower wing, fringe and tube were  
138 measured, averaging the values obtained in an area of 300 pixels (S2 Table). While, in systematics,  
139 a RGB quantitative approach was previously used to compare diaspores (Bacchetta et al. 2008;  
140 Grillo et al. 2012), to the best of our knowledge this is the first time that this approach is used to  
141 quantify the differences in the colour of flowers.

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#### 1432.5 *Volatile Organic Compounds analysis*

144 Volatile organic compounds (VOCs) were investigated separately in flowers, leaves, fruits and  
145 seeds from living plants collected in the field and temporarily cultivated in the Botanical Garden of  
146 the University of Pisa.

147 SPME (Solid Phase Micro-extraction) sampling was performed for all the analyses using the same  
148 new fibre, preconditioned according to the manufacturer's instructions. Sampling was performed in  
149 an air-conditioned room ( $23 \pm 1$  °C) to guarantee a stable temperature during sampling. Supelco  
150 SPME devices coated with polydimethylsiloxane (PDMS, 100  $\mu\text{m}$ ) were used to collect the  
151 volatiles emitted by flowers, leaves, fruits and seeds inserted into a 12 ml glass septum vial, and  
152 allowed to equilibrate for 20 min. Subsequently, the fibre was exposed to the headspace for 25 min.  
153 Once sampling was finished, the fibre was withdrawn into the needle and transferred to the injection  
154 port of a GC/MS (Gas Chromatography-Mass Spectrometry) system.

155 GC/Electron Impact (EI)-MS analyses were performed with a Varian CP-3800 gas-chromatograph  
156 equipped with a DB-5 capillary column (30 m  $\times$  0.25 mm; coating thickness 0.25  $\mu\text{m}$ ) linked to a

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157Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line  
158temperatures 250 and 240 °C, respectively; oven temperature programmed from 60 to 240 °C at 3  
159°C/min; carrier gas helium at 1 ml/min; splitless injection. Identification of the constituents was  
160based on comparison of the retention times with those of authentic samples, comparing their linear  
161retention indices relative to the series of *n*-alkanes, and on computer matching against commercial  
162(NIST 14 and ADAMS) and home-made library mass spectra built up from pure substances and  
163components of known mixtures and MS literature data (Stenhagen et al. 1974, Masada 1976,  
164Jennings and Shibamoto 1980, Swigar et al. 1981, Davies 1990, Adams 2007). SPME sampling and  
165desorption conditions were identical for all samples. Furthermore, blanks were performed before  
166each first SPME extraction and randomly repeated during each series. Quantitative comparisons of  
167relative peak areas were performed between the same chemicals in different samples. All analyses  
168were performed at least in triplicate.

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#### 1702.6 *Non-volatile compounds analysis*

171Non-volatile compounds were obtained from dried and powdered aerial parts (5 mesh) of flowering  
172plants from each population. Plants (367.4 g of PFF-T, 313.4 g of PFF-A, 311.3 g of PFF-M, 152.9  
173g of PFP, and 184.6 g of PFM) were first defatted with *n*-hexane and successively extracted at room  
174temperature with methanol (1 g of dried drug in 5 ml of solvent for three times, every 24 h). The  
175obtained extracts were dried under vacuum at 38 °C to give 5.7 and 118.4 g (PFF-T), 5.0 and 93.9 g  
176(PFF-A), 4.7 and 103.5 g (PFF-M), 1.8 and 40.3 g (PFP), and 1.8 and 40.4 g (PFM) of *n*-hexane  
177and methanol residues, respectively. The dried methanol extracts (5 g each) were partitioned  
178between ethyl acetate and *n*-butanol, and water. The obtained *n*-butanol extracts were dried and  
179dissolved in methanol (2.0 mg/ml) and centrifugated. Finally, 20 µl of each supernatant solution  
180were injected in the HPLC-PDA/UV-ESI-MS system.

181HPLC-PDA/UV-ESI-MS/MS analyses were performed using a Surveyor LC pump, a Surveyor  
182autosampler, coupled with a Surveyor PDA detector, and a LCQ Advantage ion trap mass

183spectrometer (ThermoFinnigan) equipped with Xcalibur 3.1 software. Analyses were performed  
184using a 4.6 × 250 mm, 4 μm, Synergi Fusion-RP column (Phenomenex). The eluent was a mixture  
185of methanol (solvent A) and a 0.1% aqueous solution of formic acid (solvent B). The solvent  
186gradient was as follows: 0–20 min, 35% A isocratic mode; 20–35 min, 35-50% A; 35–48 min, 50%  
187A isocratic mode; 48–108 min, 50-80% A; 108–109 min, 80–100% A. The column was  
188successively washed for 15 min with methanol and equilibrated with 35% A for 10 min. Elution  
189was performed at a flow rate of 0.8 ml/min with a splitting system of 2:8 to MS detector (160  
190μl/min) and PDA detector (640 μl/min), respectively. The volume of the injected methanol solution  
191was 20 μl. Analyses were performed with an ESI interface in the negative mode. The ionization  
192parameters used were optimized as previously reported by Abdallah et al. (2017). N<sub>2</sub> was used as  
193the sheath and auxiliary gas. PDA data were recorded with 200-600 nm range, with preferential  
194channels 254, 280, and 325 nm as the detection wavelengths. The identification of compounds was  
195performed comparing their HPLC retention times (*t<sub>R</sub>*), ESI-MS data, and UV with authentic  
196reference compounds (0.5 mg/ml methanol solution).

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## 1982.7 Statistical analyses

199The three matrices obtained for the morphometric studies (S1.1; S1.2; S1.3 Tables), and those  
200obtained for the flower colorimetric study (S2 Table), were subjected to multivariate Discriminant  
201Analysis (DA), an identification optimization procedure based on the probability of identification  
202using *a priori* classification (Peruzzi et al. 2015, Tundis et al. 2016, Roma-Marzio et al. 2017).  
203Furthermore, each morphological character, as well as the relative R, G, and B contribution was  
204also subjected to univariate analysis (non parametric Kruskal-Wallis test with Bonferroni correction  
205for multiple comparisons). Only P values < 0.01 have been considered significant. Concerning  
206phytochemical data, we built a single matrix (S3 Table) including both volatile and non-volatile  
207compounds. For this purpose, since for non-volatile compounds only relative comparisons among  
208the same chemicals in the different samples (obtained by measuring the peaks area) were available,



209we followed the same approach also for volatile compounds. We assigned a default value of 1 to  
210that population where a certain compound was detected in the highest amount whereas, in the other  
211populations, the relative amount of the same compound was scaled proportionally. To evaluate the  
212phytochemical relationships among the five populations, the matrix was subjected to Principal  
213Component Analysis (PCA). All the statistical analyses have been carried out by means of the  
214PAST version 3.15 (Hammer et al. 2001; Hammer 2017) and R version 3.3.1 (R Core Team 2016)  
215software.

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#### 2172.8 Morphological investigation (updating the geographic distribution)

218Based on the morphometric results, we selected those morphological characters showing less  
219overlapping values among taxa, for identification purposes on herbarium material. Then, using  
220these characters, we performed a morphological analysis on specimens preserved in the following  
221herbaria: APP, FI, HLUC, PI, RO, SIENA, UTV (see S4). Finally, using QGIS 2.18 software, we  
222georeferenced all the specimens in order to draw an updated distribution map of the three taxa.

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### 2243. Results

#### 2253.1 Morphometric analyses

226Results of univariate analysis of morphological characters are summarized in Table 3. The states of  
227five characters (LW25, CL, StL, SL, and SW) showed significant differences among the three taxa  
228( $P < 0.01$ ). The states of eight characters (WL, WW, BL, LW50, LW75, CW, CmA, and CA)  
229resulted significantly different between *P. flavescens* subsp. *maremmana* and the other two taxa,  
230whereas *P. flavescens* subsp. *pisarenensis* showed significant differences from other taxa concerning  
231the character-states of SL and EL ( $P < 0.01$ ). No significant difference among the three taxa in BW  
232and L was found. Among the statistically significant characters, those with less overlapping among  
233the three taxa were WL, BL, CL, and EL (Figure 1).

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235 **Figure 1.** Boxplots showing the morphological character-states less overlapping among the three  
236 subspecies of *Polygala flavescens*. A = Length of flower wing (WL); B = Length of flower  
237 bracteole (BL); C = Length of the capsule stipe (StL); D = Length of the elaiosome (EL).

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239 Discriminant Analysis (DA) based on the three datasets of morphological characters, resulted  
240 respectively in 85.0% (dataset 1), 67.6% (dataset 2), and 89.0% (dataset 3) of jackknifed correct  
241 classification of individuals, *a priori* attributed to the three subspecies (Figure 2).

242

243 **Figure 2.** Discriminant Analysis based on quantitative continuous morphological characters of  
244 dataset 1 (A) and dataset 2 (B). PFF = *Polygala flavescens* subsp. *flavescens* (squares = PFF-A;  
245 circles = PFF-M; triangles = PFF-T), PFM = *P. flavescens* subsp. *maremmana*, PFP = *P. flavescens*  
246 subsp. *pisaurensis*. In blue, the relative contribution of each variable is reported.

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248 Concerning the dataset 1, the characters most contributing to the discriminant function (loading  
249 values higher than |0.2|) are WL, BL, LW25, and SL. In particular, high values of WL and BL  
250 contribute to neatly separate *Polygala flavescens* subsp. *maremmana* from the other two taxa, while  
251 high values of LW25 and low values of SL contribute to separate *P. flavescens* subsp. *pisaurensis*  
252 from *P. flavescens* subsp. *flavescens*. A very small overlapping among *P. flavescens* subsp.

253 *maremmana* and *P. flavescens* subsp. *flavescens* was outlined (1 out of 120 individuals not correctly  
254 classified), whereas a certain degree of possible confusion between *P. flavescens* subsp. *pisaurensis*  
255 and *P. flavescens* subsp. *flavescens* was found (11 out of 120 individuals not correctly classified).

256 Concerning the dataset 2, the characters most contributing to the discriminant function (loading  
257 values higher than |0.5|) are CmA and CA. Contrarily to what obtained for dataset 1, a higher  
258 overlapping degree among individuals of the three taxa could be observed. In particular, *P.*

259 *flavescens* subsp. *flavescens* can be confused with the other two subspecies (21 out of 150

260 individuals wrongly attributed to *P. flavescens* subsp. *maremmana*, and 38 out of 150 individuals  
261 wrongly attributed to *P. flavescens* subsp. *pisaurensis*).  
262 Finally, based on the dataset 3, the character most contributing to the discriminant function (loading  
263 value |0.2|) is EL: high values of this character contribute to separate *P. flavescens* subsp.  
264 *pisaurensis* from the other two taxa. Based on dataset 3, we found a small overlap among *P.*  
265 *flavescens* subsp. *flavescens* and *P. flavescens* subsp. *maremmana* (7 out of 80 individuals not  
266 correctly classified) and among *P. flavescens* subsp. *flavescens* and *P.* subsp. *pisaurensis* (4 out 80  
267 individuals not correctly classified), whereas no overlap was found among *P. flavescens* subsp.  
268 *maremmana* and *P. flavescens* subsp. *pisaurensis*.

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### 270 3.2 Flower colorimetric analysis

271 Results of univariate analysis of relative contribution of Red, Green and Blue are summarized in  
272 Table 4. Our results highlighted that *P. flavescens* subsp. *maremmana* is characterized by wings and  
273 fringed keel with significantly higher contribution of Red and lower contribution of Blue, resulting  
274 in flowers more markedly yellow-orange, whereas in *P. flavescens* s.str. and *P. flavescens* subsp.  
275 *pisaurensis* the flowers range from yellow-whitish to yellow (Figure 3).

276 DA based on the colorimetric characters, resulted in 91% of jackknifed correct classification of  
277 individuals, *a priori* attributed to the three subspecies (Figure 4).

278 Accordingly, *P. flavescens* subsp. *maremmana* was clearly separated from both *P. flavescens* s.str.  
279 and *P. flavescens* subsp. *pisaurensis*. On the contrary, a certain overlap degree (7 out of 80  
280 individuals not correctly classified) can be observed among the latter two taxa.

281

282 **Figure 3.** Colour of wings, fringes keel and flower tube of *P. flavescens* subsp. *flavescens*, *P.*  
283 *flavescens* subsp. *maremmana*, and *P. flavescens* subsp. *pisaurensis*. For each photo, a pie chart  
284 with the relative mean contribution of R (Red), G (Green), and B (Blue) is reported.

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286**Figure 4.** Discriminant Analysis based colorimetric characters. In blue, the relative contribution of  
287each R (Red), G (Green), and B (Blue) variable for fringed keels, wings and tube flowers. PFF =  
288*Polygala flavescens* subsp. *flavescens* (squares = PFF-A; circles = PFF-M; triangles = PFF-T), PFM  
289= *P. flavescens* subsp. *maremmana*, PFP = *P. flavescens* subsp. *pisaurensis*. In blue, the relative  
290contribution of each variable is reported.

291

### 2923.3 Phytochemical analysis

293Concerning VOCs, 58, 76, 75, and 89 compounds have been identified in leaves, flowers, fruits and  
294seeds, respectively (Tables 5–8), representing from 88.7% to 99.9% of the total emission.

295A comparison of the volatile profiles among the three populations of *Polygala flavescens* subsp.  
296*flavescens* revealed that non-terpene derivatives represent the main class of compounds emitted by  
297leaves of all populations, ranging from 95.7% to 83.7% (Tables 5). In flowers (Table 6),  
298apocarotenes are the most abundant class in PFF-T (64.0%), oxygenated monoterpenes in PFF-A  
299(44.1%), and monoterpene hydrocarbons in PFF-M (82.4%). In fruits (Table 7) and seeds (Table 8),  
300non-terpene derivatives resulted the most abundant class of compound emitted by PFF-T (66.5%) and  
301PFF-M (65.0%), whereas in PFF-A mostly oxygenated monoterpenes are emitted by fruits (64.9%),  
302and monoterpenes hydrocarbons by seeds (40.8%). In PFF-A (*E*)-3-hexen-1-ol (compound 1) is the  
303most abundant compound emitted by the leaves of all the investigated populations, ranging from  
30462.8% in PFF-A to 85.6% in PFF-M. In flowers, *cis*- $\alpha$ -ambrinol (compound 97) prevails in PFF-T  
305(62.2%), limonene (compound 16) in PFF-A (21.7%), and myrcene (compound 12) in PFF-M  
306(77.6%); in fruits, nonanal (compound 29) prevails in PFF-T (18.1%), carvone (compound 63) in  
307PFF-A (43.8%) and decanal (compound 55) in PFF-M (22.5%), whereas the most abundant  
308compounds emitted by seeds are nonanal (compound 29) in PFF-T (29.8%), PFF-M (12.0%) and  $\alpha$ -  
309pinene (compound 3) in PFF-A (31.4%).

310Comparing the three subspecies, non-terpene derivatives emitted by leaves (Table 5) are the most  
311abundant class of compound also in PFM and PFP. In *P. flavescens* subsp. *maremmana* (PFM),

312 sesquiterpene hydrocarbons (49.9%) prevail in flowers (Table 6), oxygenated monoterpenes  
313 (53.3%) in fruits (Table 7) and monoterpene hydrocarbons (73.9%) in seeds (Table 8), whereas in  
314 *P. flavescens* subsp. *pisaurensis* (PFP) the most abundant classes of compounds are monoterpene  
315 hydrocarbons (47.0%), sesquiterpene hydrocarbons (47.0%), and non-terpene derivatives (52.0 %)  
316 in flowers (Table 6), fruits (Table 7), and seeds (Table 8), respectively.

317 Some VOCs are unique to only one of the three subspecies. Particularly, the following compounds  
318 were detected only in *P. flavescens* subsp. *pisaurensis*: methyl 4-nonenoate (compound 58, 1.7%)  
319 (Table 5); octanoic acid (compound 46, 1.8%) and 6-methyltridecane (compound 77, 0.6%) (Table  
320 6);  $\beta$ -chamigrene (compound 109, 1.5%) and (*Z*)- $\gamma$ -bisabolene (compound 128, 1.4%) (Table 7);  
321 (*E*)-2-octenal (compound 21, 1.6%) and *cis*-thujopsene (compound 94, 5.2%) (Table 8);  
322 aromadendrene (compound 99, 13.5%, Table 7; 7%, Table 8). In *P. flavescens* subsp. *maremmana*,  
323 the following unique compounds are found:  $\alpha$ -longipinene (compound 79, 1.3%) (Table 6); *cis*-  
324 dihydrocarvone (compound 50, 1.6%) and *trans*-dihydrocarvone (compound 54, 5.7%) (Table 7);  $\beta$ -  
325 pinene (compound 10, 1.2%), 6-camphenone (compound 26, 0.7%) and pinocarvone (compound  
326 41, 1.1%) (Table 8). Only  $\beta$ -Elemene (compound 85) is unique to *P. flavescens* subsp. *flavescens*  
327 (Table 8). In addition to unique compounds, compared to the other two subspecies *P. flavescens*  
328 subsp. *maremmana* shows high levels of  $\beta$ -caryophyllene (compound 92, 31.1%) and  $\alpha$ -pinene  
329 (compound 3, 70.8%) in flowers (Table 6) and seeds (Table 8), respectively.

330 Concerning non-volatile compounds, 75 different constituents have been detected by HPLC-  
331 photodiode array (PDA)/UV-electrospray ionization (ESI)-MS/MS (Figure 5 and Table 9).

332

333 **Figure 5.** Comparison of the LC-ESI-MS profiles in the sampled populations. PFF-T = Cerbaie  
334 Hills (Pisa, Tuscany); PFF-A = Torano (Rieti, Lazio); PFF-M = Vallerotonda (Frosinone, Lazio);  
335 PFM = Monte Argentario (Grosseto, Tuscany); PFP = Fano (Pesaro e Urbino, Marche). For peak  
336 characteristics, see Table 9.

337

338Compounds 2, 3, 5–10, 12, 14, 17, 35, and 43 were identified by comparison with retention times,  
339ESI-MS data, and UV of authentic reference compounds, previously isolated from *P. flavescens*  
340subsp. *flavescens* (De Leo et al. 2017). Compound 2 ( $[M-HCOO]^-$  at  $m/z$  565), was the only  
341apocarotenoid identified in all analysed extracts, characterized by an  $\alpha$ -ionol aglycon and a  
342saccharide portion constituted by a glucose and an apiose units, as deduced by product ions at  $m/z$   
343403 and 271, respectively. As revealed by ESI-MS/MS, compounds 5 ( $[M-H]^-$  at  $m/z$  741), 10 ( $[M-$   
344 $H]^-$  at  $m/z$  947), 12 ( $[M-H]^-$  at  $m/z$  917), 14 ( $[M-H]^-$  at  $m/z$  887), and 17 ( $[M-H]^-$  at  $m/z$  845), were  
345flavonol triglycosides having quercetin as aglycon, as deduced from the presence of the product ion  
346at  $m/z$  301. ESI-MS/MS of all flavonols showed product ions due to the subsequent losses of apiose  
347( $[M-H-132]^-$ ) and rhamnose-glucose ( $[M-H-132-146-162]^-$ ), leading to characterize the  
348trisaccharide chain. In four compounds, the C-5 of the apiose unit is linked to an aromatic acid  
349identified as synapic acid ( $[M-H-206]^-$ ), ferulic acid ( $[M-H-176]^-$ ), coumaric acid ( $[M-H-146]^-$ ),  
350and benzoic acid ( $[M-H-122]^-$ ) for compounds 10, 12, 14, and 17, respectively. Complete names of  
351detected flavonoids are supplied in Table 9. The product ions at  $m/z$  463 and 301 generated by ESI-  
352MS/MS experiment of compound 8 ( $[M-H]^-$  at  $m/z$  609), showed the presence of one glucose and  
353one rhamnose moiety linked to a quercetin skeleton. Thus, compound 5 was identified as rutin.  
354Full MS of compound 9 showed a deprotonated molecule  $[M-H]^-$  at  $m/z$  915 and product ions at  $m/z$   
355709, 503, and 341 corresponding to the subsequent losses of two sinapoyl and one hexose moieties,  
356according with the structure of the oligosaccharide reiniose F. Other oligosaccharides detected in all  
357extracts had a different behaviour in the ESI-MS, due to the formation of anionized molecules  
358 $[M+HCOO]^-$  at  $m/z$  653 (compound 3), 695 (compound 6), and 799 (compound 7). All  
359oligosaccharides were identified by comparison with reference standards (Table 9).  
360In addition to previous metabolites, two bidesmodic triterpenoid saponins, compounds 35 ( $[M-H]^-$   
361at  $m/z$  1469) and 43 ( $[M-H]^-$  at  $m/z$  1411) were identified in all extracts, excluding *P. flascensces*  
362subsp. *pisaurensis* lacking compound 43. The fragmentation pathways of both compounds,  
363registered in negative mode, are in agreement with data reported by De Leo et al. (2017). The

364injection of reference standards led to establish both chemical structures (Table 9), characterized by  
365six sugar units linked to the aglycons presegenin (compound 35) and medicagenic acid (compound  
36643).

367Other 62 compounds remained unidentified, although 37 were tentatively characterized as saponins,  
368due to the observation of diagnostic ion fragments in the ESI-MS/MS spectra of the parent ions,  
369such as sugar fragments and product ions due to the losses of sugar portions from the aglycon. ESI-  
370MS/MS of compounds 24, 25, 27, 28, 31–34, 37–42, 44–48, 50, 53, 54, and 57 showed a very  
371similar fragmentation pattern of compound 35, with a base peak due to the loss of  $-\text{CH}_2\text{OH}$  unit  
372from the aglycon presegenin and characteristic product ions at  $m/z$  937, 747, 455, 439, and 423 due  
373to sugar product ions. On the contrary, MS/MS experiments for compounds 49, 51, 58–62, 66, 68,  
374and 70–72 are similar to that of compound 43 with a base peak generated by the scission of one or  
375two sugar portions and sugar fragments such as  $m/z$  585, 455, 439, and 423. The exact identification  
376of detected triterpenoid saponins was not achieved for the lack of reference standards.

377Totally, the class of 48 compounds has been identified, resulting in 37 saponins, 4 oligosaccharides,  
3786 flavonoids, and 1 apocarotenoid. No compound is unique to *P. flavescens* subsp. *flavescens*.

379Contrarily, the compounds corresponding to the peak 73 (unidentified) and to the peak 57 (a  
380saponin) are unique to *P. flavescens* subsp. *maremmana* and to *P. flavescens* subsp. *pisaurensis*,  
381respectively. Furthermore, the compounds corresponding to the peak 3 ( $\beta$ -D-(6-*O*-benzoyl)-  
382fructofuranosyl-(2→1)-[ $\beta$ -D-glucopyranosyl-(1→3)]- $\alpha$ -D-glucopyranoside) and to the peak 40 (a  
383saponin) are more abundant in *P. flavescens* subsp. *maremmana*, compared to the other two  
384subspecies (Table 9). Twelve compounds, all identified as saponins (peaks 35, 37, 39, 44, 48, 50,  
38553, 54, 66, 68, 71, and 72) are more abundant in *P. flavescens* subsp. *pisaurensis*, with respect to  
386other subspecies (Table 9). According to the PCA results (Figure 6; 80.5% of variance explained by  
387the first three axes), both *P. flavescens* subsp. *maremmana* and *P. flavescens* subsp. *pisaurensis* fall  
388outside the overall (both volatile and non-volatile) chemical variability of *P. flavescens* subsp.

389*flavescens*.

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391**Figure 6.** PCA 3D scatter plot based on phytochemical data (Component 1: 32.2%, Component 2:  
39224.4%, Component 3: 23.8% of the observed variance). Yellow bubbles: *Polygala flavescens* subsp.  
393*flavescens* (PFF-A; PFF-M; PFF-T); orange bubble: *P. flavescens* subsp. *maremmana* (PFM); green  
394bubble: *P. flavescens* subsp. *pisaurensis* (PFP).

395

3963.4 *Morphological investigation (updating the geographic distribution)*

397By measuring WL, BL, StL, and EL on herbarium specimens, we were able to revise their  
398identification. Consequently, the distribution of the three subspecies of *P. flavescens* in Italy was  
399updated (Figure 7). *Polygala flavescens* subsp. *flavescens* is confirmed to occur in peninsular Italy  
400from Emilia-Romagna northwards, to Puglia and Basilicata southwards. *Polygala flavescens* subsp.  
401*maremmana* occurs only in Mt. Argentario (Tuscany), whereas *P. flavescens* subsp. *pisaurensis* can  
402be found along the east side of central Italy in Emilia-Romagna and Marche, but it has never been  
403recorded to co-occur at the same sites with *P. flavescens* subsp. *flavescens*.

404

405*Identification key to the three subspecies*

- 4061a. Length of the elaiosome 2.53 (± 0.31) mm; flowers of plants in full blossom yellow-  
407whitish.....*P. flavescens* subsp. *pisaurensis*
- 4081b. Length of the elaiosome < 2.1 mm; flowers of plants in full blossom yellow to yellow-  
409orange.....2
- 4102a. Flowers in full blossom yellow, showing wings 8.22 ± 0.83 mm long, bracteoles 3.88 ± 0.49  
411mm long.....*P. flavescens* subsp. *flavescens*
- 4122b. Flowers in full blossom yellow-orange, showing wings 10.86 ± 0.94 mm long, bracteoles 5.10 ±  
4130.46 mm long.....*P. flavescens* subsp. *maremmana*

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416**Figure 7.** Distribution of *Polygala flavescens* subsp. *flavescens* (in yellow), *P. flavescens* subsp.  
417*maremmana* (in orange), and *P. flavescens* subsp. *pisaurensis* (in green), as resulted by the revision  
418of herbarium specimens. The map is implemented by dots in light colours, which are derived from  
419literature (Fenaroli 1970; Gubellini et al. 2014; Del Guacchio 2010).

420

## 4214. Discussion

### 4224.1 Integrative systematics

423Our morphometric analyses confirm that most of the characters reported in the literature (Fiori et al.  
4241908; Zangheri 1976; Pignatti 1982; Arrigoni 2014) are useful to discriminate the three taxa.

425Particularly, *P. flavescens* subsp. *maremmana* shows bracteoles, flower wings and capsule stipes  
426longer than the other two subspecies, whereas the length of the elaiosome is the most reliable  
427morphological character to discriminate *P. flavescens* subsp. *pisaurensis*. However, concerning the  
428length of flower wings, considered the most important character to identify *P. flavescens* subsp.  
429*maremmana*, we never found wings shorter than 9.9 mm, contrarily to the range values reported by  
430Arrigoni (2004) for this subspecies (9–11.5 mm). In addition, we did not find significant differences  
431in the angle formed by the apex of flower wings, which was also considered as a character useful to  
432discriminate *P. flavescens* subsp. *pisaurensis* (apex putatively obtuse) from the other two subspecies  
433(apex putatively acute) (Arrigoni 2004).

434A quantitative colorimetric approach to flowers was never tried before in *Polygala*. However,  
435according to Arrigoni (2004), flower wings vary from yellow-greenish in *P. flavescens* subsp.  
436*flavescens*, to pale-yellow in *P. flavescens* subsp. *pisaurensis*, whereas in *P. flavescens* subsp.  
437*maremmana* wings are generally purplish-tinged. Although we noticed that purplish wings can be  
438observed in all the three subspecies at fruiting stage, we confirm a differentiation in colour profiles  
439at flowering stage. Indeed, when evaluated at the same phenological stage (full blossom and without  
440fruits), flowers of *P. flavescens* subsp. *maremmana* show higher Red and lower Blue contributions,

441resulting in flowers more markedly yellow-orange, whereas *P. flavescens* subsp. *pisaurensis* shows  
442flowers ranging from yellow-whitish to yellow.

443Our phytochemical study revealed the occurrence of unique compounds, both VOCs and non-  
444volatiles, that can be considered as molecular markers useful to discriminate the three subspecies of  
445*Polygala flavescens*. This phytochemical differentiation is also confirmed by the results of  
446multivariate analysis based on the overall phytochemical screening, showing a clear separation of  
447the three subspecies, and pointing out the importance of phytochemical studies in plant systematics  
448(Stuessy 2009; Astuti et al. 2017; and literature cited therein).

449As far the geographic distribution is concerned, after herbarium revision we confirmed that the  
450range of *P. flavescens* subsp. *flavescens* goes from Emilia-Romagna, in Northern Italy, to Apulia  
451and Basilicata, in Southern Italy. On the other hand, *P. flavescens* subsp. *pisaurensis* is restricted to  
452the east coast in Marche and Emilia-Romagna, while *P. flavescens* subsp. *maremmana* is limited to  
453Mt. Argentario, despite Arrigoni (2004) quoted this subspecies for a larger area, i.e. from the  
454southern part of the province of Leghorn (San Vincenzo) to the southern part of the province of  
455Grosseto (Capalbio). In our opinion, this discrepancy is putatively due to differences in the  
456phenological stage of the flowers measured by previous authors. Indeed, we noticed a marked  
457elongation of wings from flower stage to fruit.

458Considering that the three taxa show significant morphological and phytochemical differences, they  
459are allopatric, and share the same chromosome number (Peruzzi et al. 2017), we deem appropriate  
460their taxonomic treatment at subspecific level.

461

#### 4624.2 Possible ecological role of phytochemical and morpho-colorimetric variation

463Among the unique compounds, the occurrence of (*E*)-2-octenal (compound 21) in seeds of *P.*  
464*flavescens* subsp. *pisaurensis* could be discussed in the light of myrmecochory, the ant-mediated  
465seed dispersal mechanism, whose occurrence in *P. flavescens* is suggested by the occurrence of  
466elaiosomes. Since seed VOCs elicit ant-carrying behaviour of elaiosomes (Brew et al.1989;

467 Youngsteadt et al. 2008), the occurrence of unique compounds may deserve further studies  
468 concerning the ecological role of VOCs, particularly in plant-ant interactions (Willmer 2009).  
469 Incidentally, (*E*)-2-octenal was also identified in gland abdominal extract of two ant species  
470 (*Eurydema ventrale* and *E. oleraceum*) collected in Central Italy, suggesting that this compound  
471 plays a pheromonal role in ant communication (Aldrich et al. 2017). Interestingly, *P. flavescens*  
472 subsp. *pisauensis* is also the subspecies showing the largest elaiosomes.

473 The ecological role of VOCs is also particularly relevant in floral scent that, in synergy with floral  
474 colour and shape, can act as signals for attraction of pollinators (Schiestl et al. 2013). Typically,  
475 floral scent is determined by volatile compounds and represents an important mode of  
476 communication among flowering plants, pollinators, and enemies (Knudsen et al. 2006; Raguso  
477 2008). It has been observed that emissions rich in benzenoids or in linalool (and its oxides) seem to  
478 be an adaptation to butterflies or to generalist pollinators (Andersson et al. 2002). On the other  
479 hand, when the floral bouquet is dominated by a sole volatile in relatively large percentages, the  
480 pollination is often bee-mediated (Borg-Karlson et al 1996). The latter situation is experienced for  
481 the studied taxa, which emitted 1-2 main compounds in their floral bouquet. In particular, PFF-T  
482 mainly emitted *cis*- $\alpha$ -ambrinol (compound 97, 62.2%), while PFF-M and PFP emitted myrcene  
483 (compound 12) as their main volatile (77.6 and 46.6%, respectively). *Polygala flavescens* subsp.  
484 *maremmana* and the remaining population of *P. flavescens* subsp. *flavescens* (PFF-A) have two  
485 main volatiles in their flower emission: limonene (compound 16, 21.7%) and  $\alpha$ -terpineol  
486 (compound 49, 20.5%) for PFF-A and  $\beta$ -caryophyllene (compound 92, 31.1%) and 1,8-cineole  
487 (compound 17, 15.3%) in the case of PFM. The hypothesis of bee-attraction is also in good  
488 agreement with the morphological requirements for such pollination (Faegry and van der Pijl 1979;  
489 Westerkamp 1997). Also the changes in flower colour quantified in this study, paralleled by change  
490 in floral scent, could reflect a change in pollinators, possibly leading to reproductive isolation of the  
491 three subspecies, as demonstrated for example in the two closely related species *Mimulus*  
492 *verbenaceus* Greene and *M. cardinalis* Douglas ex Benth. (Phrymaceae) (Vickery 1992). In

493 addition, genes controlling the flower colour might influence plant resistance to herbivory (Irwin et  
494 al. 2003), causing a synergism that may have a positive effect on reproductive fitness.

495 The flavonoid biosynthetic pathway, culminating in the production of anthocyanins, with  
496 carotenoids and betalains, are the main pigments responsible for the flower colour (Weiss 1995;  
497 Irwin et al. 2003; Borghi et al. 2017). In our study, we failed to find relevant differences in the  
498 overall flavonoid composition among the three subspecies, but further studies aimed to investigate  
499 specifically the non-volatile compounds occurring in flowers, as well as gene expression and  
500 biosynthetic pathways, could clarify the phytochemical basis of the documented differences in  
501 flower colour among the three taxa.

502

#### 503 4.3 Potential pharmacological implications of our study

504 Besides their systematic value, our results concerning non-volatile compounds confirm that  
505 *Polygala* is a genus rich in flavonoids, saponins, and oligosaccharides (Clegg and Durbin 2000),  
506 supporting a pharmacological potential for all the three subspecies within *P. flavescens*. For  
507 example, due to the expectorant and anti-inflammatory effects, vaccine adjuvants or neurotrophic  
508 activity of the saponins (Klein et al. 2012 and literature therein), it is noteworthy that in *P.*  
509 *flavescens* subsp. *pisauensis* we highlighted a unique saponin, and that further 12 saponins were  
510 more abundant than in other taxa. In addition, the two oligosaccharides (3,6'-di-*O*-sinapoylsucrose  
511 and reiniose F) isolated by De Leo et al.(2017) in *P. flavescens* subsp. *flavescens* as potential  
512 hLDH5 inhibitors, were also found in the other two subspecies, confirming their interest for the  
513 potential development of new anticancer agents (De Leo et al. 2017).

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516 **Table 1.** Sampled localities used for the phytochemical, morphometric and colorimetric investigations.

<b>Taxon</b>	<b>Acronym</b>	<b>Locality</b>	<b>Coordinate (WGS84)</b>	<b>Altitude m a.s.l.</b>	<b>Voucher numbers</b>
<i>P. flavescens</i> subsp. <i>flavescens</i>	PFF-T	Cerbaie Hills (Pisa, Tuscany)	43.751228 N, 10.719234 E	55	PI n. 000455–58
<i>P. flavescens</i> subsp. <i>flavescens</i>	PFF-A	Torano (Rieti, Lazio)	42.157098 N, 13.270760 E	760	PI n. 000453–54
<i>P. flavescens</i> subsp. <i>flavescens</i>	PFF-M	Vallerotonda (Frosinone, Lazio)	41.588942 N, 14.007237 E	765	PI n. 000459–60
<i>P. flavescens</i> subsp. <i>maremmana</i>	PFM	Monte Argentario (Grosseto, Tuscany)	42.421952 N, 11.140779 E	130	PI n. 000466–69
<i>P. flavescens</i> subsp. <i>pisauensis</i>	PFP	Fano (Pesaro e Urbino, Marche)	43.864231 N, 12.984113 E	25	PI n. 000461–62

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519 **Table 2.** Measured morphological characters.

<b>Part of the plant</b>	<b>ID</b>	<b>Character</b>
<i>Dataset 1</i>		
Flower	WL	Length of flower wings (mm)
Flower	WW	Width of flower wings (mm)
Flower	WA	Angle formed by the apex of flower wings (rad)
Flower	BL	Length of flower bracteole (mm)
Flower	BW	Width of flower bracteole (mm)
Leaf	LL	Leaf length (cm)
Leaf	LW25	Leaf width on 25% of leaf's length from base up (mm)
Leaf	LW50	Leaf width on 50% of leaf's length from base up (mm)
Leaf	LW75	Leaf width on 75% of leaf's length from base up (mm)
Stem	SL	Stem length (dm)
<i>Dataset 2</i>		
Fruit	CL	Capsule length (mm)
Fruit	CW	Capsule width (mm)
Fruit	StL	Length of the capsule stipe (mm)
Fruit	CmA	Area of the capsule membranous marginal part (mm <sup>2</sup> )
Fruit	CA	Capsule area (mm <sup>2</sup> )
<i>Dataset 3</i>		
Seed	SL	Seed length (mm)
Seed	SW	Seed width (mm)
Seed	EL	Length of the elaiosome (mm)

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533 **Table 3.** Comparison of morphological features among the three subspecies of *Polygala flavescens*.

534 Quantitative numerical values are expressed as mean  $\pm$  SD. Character-states marked by different

535 superscript letters are significantly different ( $P < 0.01$ ). Characters in bold are also shown in Figure

536 1. For the meaning of the character acronyms, see Table 2.

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<b>ID character</b>	<i>P. flavescens</i> subsp. <i>flavescens</i>	<i>P. flavescens</i> subsp. <i>maremmana</i>	<i>P. flavescens</i> subsp. <i>pisauensis</i>
<i>Dataset 1</i>			
<b>WL (mm)</b>	<b>8.22 <math>\pm</math> 0.83<sup>a</sup></b>	<b>10.86 <math>\pm</math> 0.94<sup>b</sup></b>	<b>8.12 <math>\pm</math> 0.70<sup>a</sup></b>
WW (mm)	3.07 $\pm$ 0.40 <sup>a</sup>	3.49 $\pm$ 0.51 <sup>b</sup>	3.31 $\pm$ 0.27 <sup>a</sup>
WA (rad)	1.17 $\pm$ 0.21 <sup>a</sup>	0.90 $\pm$ 0.12 <sup>b</sup>	1.08 $\pm$ 0.12 <sup>b</sup>
<b>BL (mm)</b>	<b>3.88 <math>\pm</math> 0.49<sup>a</sup></b>	<b>5.10 <math>\pm</math> 0.46<sup>b</sup></b>	<b>4.13 <math>\pm</math> 0.43<sup>a</sup></b>
BW (mm)	0.48 $\pm$ 0.08 <sup>a</sup>	0.47 $\pm$ 0.00 <sup>a</sup>	0.55 $\pm$ 0.16 <sup>a</sup>
LL (cm)	2.23 $\pm$ 0.43 <sup>a</sup>	2.31 $\pm$ 0.39 <sup>a</sup>	2.17 $\pm$ 0.34 <sup>a</sup>
LW25 (mm)	1.91 $\pm$ 0.49 <sup>a</sup>	1.33 $\pm$ 0.42 <sup>b</sup>	2.38 $\pm$ 0.51 <sup>c</sup>
LW50 (mm)	2.31 $\pm$ 0.57 <sup>a</sup>	1.60 $\pm$ 0.48 <sup>b</sup>	2.57 $\pm$ 0.53 <sup>a</sup>
LW75 (mm)	1.82 $\pm$ 0.41 <sup>a</sup>	1.39 $\pm$ 0.36 <sup>b</sup>	1.93 $\pm$ 0.45 <sup>a</sup>
SL (dm)	2.00 $\pm$ 0.55 <sup>a</sup>	2.04 $\pm$ 0.46 <sup>a</sup>	1.52 $\pm$ 0.21 <sup>b</sup>
<i>Dataset 2</i>			
CL (mm)	5.55 $\pm$ 0.65 <sup>a</sup>	6.63 $\pm$ 0.66 <sup>b</sup>	5.21 $\pm$ 0.48 <sup>c</sup>
CW (mm)	4.09 $\pm$ 0.56 <sup>a</sup>	4.56 $\pm$ 0.54 <sup>b</sup>	4.15 $\pm$ 0.47 <sup>a</sup>
<b>StL (mm)</b>	<b>1.11 <math>\pm</math> 0.20<sup>a</sup></b>	<b>1.52 <math>\pm</math> 0.26<sup>b</sup></b>	<b>0.89 <math>\pm</math> 0.17<sup>c</sup></b>
CmA (mm <sup>2</sup> )	5.58 $\pm$ 1.78 <sup>a</sup>	7.04 $\pm$ 1.73 <sup>b</sup>	5.74 $\pm$ 1.30 <sup>a</sup>
CA (mm <sup>2</sup> )	16.97 $\pm$ 4.36 <sup>a</sup>	21.16 $\pm$ 4.15 <sup>b</sup>	16.83 $\pm$ 3.44 <sup>a</sup>
<i>Dataset 3</i>			
SL (mm)	2.62 $\pm$ 0.19 <sup>a</sup>	3.13 $\pm$ 0.17 <sup>b</sup>	2.43 $\pm$ 0.14 <sup>c</sup>
SW (mm)	1.33 $\pm$ 0.09 <sup>a</sup>	1.48 $\pm$ 0.09 <sup>b</sup>	1.24 $\pm$ 0.12 <sup>c</sup>
<b>EL (mm)</b>	<b>1.83 <math>\pm</math> 0.24<sup>a</sup></b>	<b>1.89 <math>\pm</math> 0.16<sup>a</sup></b>	<b>2.53 <math>\pm</math> 0.31<sup>b</sup></b>

550 **Table 4.** Comparison of colorimetric features among the three subspecies of *Polygala flavescens*.

551 RGB (Red, Green, and Blue) values, expressed as mean  $\pm$  SD, represent the coordinates in the RGB

552 colour-space, where each value ranges from 0 to 255. Character states marked by different

553 superscript letters are significantly different ( $P < 0.01$ ).

554	<b>ID character</b>	<i>P. flavescens</i>	<i>P. flavescens</i>	<i>P. flavescens</i>
555		<b>subsp. <i>flavescens</i></b>	<b>subsp. <i>maremmana</i></b>	<b>subsp. <i>pisauensis</i></b>
	<i>Wings</i>			
556	R	126 $\pm$ 9 <sup>a</sup>	144 $\pm$ 9 <sup>b</sup>	128 $\pm$ 9 <sup>a</sup>
	G	114 $\pm$ 9 <sup>a</sup>	116 $\pm$ 8 <sup>a</sup>	119 $\pm$ 9 <sup>a</sup>
557	B	64 $\pm$ 13 <sup>a</sup>	32 $\pm$ 4 <sup>b</sup>	74 $\pm$ 11 <sup>c</sup>
	<i>Fringes</i>			
558	R	153 $\pm$ 14 <sup>a</sup>	172 $\pm$ 19 <sup>b</sup>	162 $\pm$ 20 <sup>a</sup>
	G	126 $\pm$ 13 <sup>a</sup>	123 $\pm$ 14 <sup>a</sup>	145 $\pm$ 17 <sup>b</sup>
559	B	38 $\pm$ 14 <sup>a</sup>	22 $\pm$ 3 <sup>b</sup>	71 $\pm$ 17 <sup>c</sup>
	<i>Tube</i>			
560	R	138 $\pm$ 10 <sup>a</sup>	160 $\pm$ 13 <sup>b</sup>	152 $\pm$ 13 <sup>b</sup>
	G	127 $\pm$ 10 <sup>a</sup>	138 $\pm$ 11 <sup>b</sup>	140 $\pm$ 13 <sup>b</sup>
561	B	63 $\pm$ 9 <sup>a</sup>	54 $\pm$ 11 <sup>b</sup>	82 $\pm$ 14 <sup>c</sup>



562**Table 5. Comparison of the chemical composition (% of VOCs) emitted by the leaves of**  
563**sampled populations. PFF-T = Cerbaie Hills (Pisa, Tuscany); PFF-A = Torano (Rieti, Lazio);**  
564**PFF-M = Vallerotonda (Frosinone, Lazio); PFM = Monte Argentario (Grosseto, Tuscany);**  
565**PFP = Fano (Pesaro e Urbino, Marche). ap: apocarotenes, mh: monoterpene hydrocarbons,**  
566**ntp: non-terpene derivatives, om: oxygenated monoterpenes, os: oxygenated sesquiterpenes,**  
567**ph: phenylpropanoids, sh: sesquiterpene hydrocarbons. Yellow columns = *P. flavescens* subsp.**  
568***flavescens*, orange column = *P. flavescens* subsp. *maremmana*, green column = *P. flavescens***  
569**subsp. *pisauensis*.**

ID compound	Compound	Class	LRI	PFF-T	PFF-A	PFF-M	PFM	PFP
1	( <i>E</i> )-3-Hexen-1-ol	ntp	850	83.1	62.8	85.6	70.1	43.9
6	Benzaldehyde	ntp	962	1.1	0.2	0	tr	0
7	1-Ethyl-3-methylbenzene	ntp	967	0	0	0.2	0	0
8	1-Octen-3-ol	ntp	976	0.2	0	0	0	0
11	6-Methyl-5-hepten-2-one	ntp	986	0	0.3	0	0	0
14	( <i>Z</i> )-3- Hexenyl acetate	ntp	1007	3.0	2.4	4.1	0	1.3
17	1,8-Cineole	om	1034	0	0.2	0	0	2.9
29	Nonanal	ntp	1104	0.8	0.5	0.5	1.0	1.9
38	Isobutyl hexanoate	ntp	1150	0	0	0	0	2.0
44	Menthol	om	1174	0	0.2	0	tr	0
45	4-terpineol	om	1179	0	0	0	0	3.3
47	( <i>Z</i> )-3- Hexenyl butyrate	ntp	1188	0	0.5	0	0	1.7
48	Methyl salicylate	ntp	1191	3.9	3.2	3.7	13.1	8.7
55	Decanal	ntp	1206	1.7	2.9	0.6	2.6	2.3
58	Methyl 4-nonenoate	ntp	1216	0	0	0	0	1.7
59	$\beta$ -Cyclocitral	ap	1217	0	0	0	0.2	0
61	( <i>Z</i> )-3-Hexenyl isovalerate	ntp	1238	0	0	0	0	1.4
65	Ethyl salicylate	ntp	1267	0	0	0	0.2	0
67	Citronellyl formate	om	1275	0	0.1	0	0.3	0
70	Isobornyl acetate	om	1285	0.4	0	0	0.3	0
74	Undecanal	ntp	1306	0.3	0.2	0.1	0.3	0.6
80	Eugenol	ph	1358	0	0.9	0.4	0	1.5
85	$\beta$ -Elemene	sh	1391	0	0.2	0	0	0
86	( <i>E</i> )-Jasmone	ntp	1392	0	0	0	0.5	0
88	<i>n</i> -Tetradecane	ntp	1399	0.1	0.2	0.2	0.4	1.8
89	Methyl eugenol	ph	1401	0	1.4	0	0	0
91	Dodecanal	ntp	1407	0.3	0	0.1	0.3	0.8
92	$\beta$ -Caryophyllene	sh	1419	0	tr	0	0.2	0.6

95	$\beta$ -Gurjunene	sh	1432	0	0	0	0.7	0
98	<i>trans</i> - $\alpha$ -Bergamotene	sh	1439	0	2.0	0	0	0
103	( <i>E</i> )-Geranylacetone	ap	1453	1.4	1.9	0.7	0.8	3.2
106	( <i>E</i> )- $\beta$ -Farnesene	sh	1459	0	1.6	0	0	0
107	<i>cis</i> -Muurolo-4(14),5-diene	sh	1460	0	0.2	0	0	0
111	Germacrene D	sh	1482	0	0.3	0	0	0
116	<i>n</i> -Pentadecane	ntp	1500	0	0.2	0	0.9	0
123	Tridecanal	ntp	1509	0.3	0	0	0	0
125	<i>trans</i> - $\gamma$ -Cadinene	sh	1514	0	0.2	0	0	0
126	Geranyl isobutyrate	mh	1515	0.3	0	0	0	0
129	Benzoic acid, 4-ethoxyethyl ester	ntp	1522	0.5	0	0	0	3.8
133	( <i>Z</i> )-3-Hexenyl benzoate	ntp	1570	0	0	0.2	0	4.6
135	Caryophyllene oxide	os	1582	0	0	0	0	1.6
138	<i>n</i> -Hexadecane	ntp	1600	0	0.9	0	0	0
139	Tetradecanal	ntp	1613	0.2	0	0	0	0
140	1,10-di- <i>epi</i> -cubenol	os	1614	0	0.2	0	0	0
143	$\alpha$ -Muurolol	os	1645	0	2.5	0	0	0
144	$\beta$ -Eudesmol	os	1649	0	0.4	0	0	0
147	<i>n</i> -Heptadecane	ntp	1700	0.2	tr	0.2	0	0
148	Benzyl benzoate	ntp	1762	0	tr	0	0	0
149	<i>n</i> -Octadecane	ntp	1800	0	0.8	0	0	0
150	2-Ethylhexyl salicylate	ntp	1807	0	7.6	0	0.3	0
152	Isopropyl tetradecanoate	ntp	1830	0	1.0	0	0	0
-	<b>Apocarotenes</b>	<b>ap</b>		<b>1.4</b>	<b>1.9</b>	<b>0.7</b>	<b>1.0</b>	<b>3.2</b>
-	<b>Monoterpene hydrocarbons</b>	<b>mh</b>		<b>0.3</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>	<b>0.0</b>
-	<b>Non-terpene derivatives</b>	<b>ntp</b>		<b>95.7</b>	<b>83.7</b>	<b>95.5</b>	<b>89.7</b>	<b>77</b>
-	<b>Oxygenated monoterpenes</b>	<b>om</b>		<b>0.4</b>	<b>0.5</b>	<b>0.0</b>	<b>0.6</b>	<b>6.2</b>
-	<b>Oxygenated sesquiterpenes</b>	<b>os</b>		<b>0.0</b>	<b>3.1</b>	<b>0.0</b>	<b>0.0</b>	<b>1.6</b>
-	<b>Phenylpropanoids</b>	<b>ph</b>		<b>0.0</b>	<b>2.3</b>	<b>0.4</b>	<b>0.0</b>	<b>1.5</b>
-	<b>Sesquiterpene hydrocarbons</b>	<b>sh</b>		<b>0.0</b>	<b>4.5</b>	<b>0.0</b>	<b>0.9</b>	<b>0.6</b>
-	<b>Total</b>			<b>97.8</b>	<b>96.0</b>	<b>96.6</b>	<b>92.2</b>	<b>90.1</b>

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571 **Table 6.** Comparison of the chemical composition (% of VOCs) emitted by the flowers of sampled  
572 populations. PFF-T = Cerbaie Hills (Pisa, Tuscany); PFF-A = Torano (Rieti, Lazio); PFF-M =  
573 Vallerotonda (Frosinone, Lazio); PFM = Monte Argentario (Grosseto, Tuscany); PFP = Fano  
574 (Pesaro e Urbino, Marche). Ap: apocarotenes, mh: monoterpene hydrocarbons, ntp: non-terpene  
575 derivatives, om: oxygenated monoterpenes, os: oxygenated sesquiterpenes, sh: sesquiterpene  
576 hydrocarbons. Yellow columns = *P. flavescens* subsp. *flavescens*, orange column = *P. flavescens*  
577 subsp. *maremmana*, green column = *P. flavescens* subsp. *pisaurensis*.

ID compound	Compound	Class	LRI	PFF-T	PFF-A	PFF-M	PFM	PFP
3	$\alpha$ -Pinene	mh	941	0	6.0	0.3	0	0
4	Camphene	mh	955	0	1.1	tr	0	0
9	Sabinene	mh	977	0	0.8	tr	0	0
12	Myrcene	mh	993	0	4.6	77.6	0.7	46.6
16	Limonene	mh	1032	0	21.7	4.1	0	0
17	1,8-Cineole	om	1034	0	0	0	15.3	3.3
23	<i>cis</i> -Sabinene hydrate	om	1070	0	0.9	0	0	0
24	Fenchone	om	1088	0	0	0	0	1.0
25	Terpinolene	mh	1090	0	1.2	0.4	0	0
28	Linalool	om	1101	0	8.3	2.8	0	1.1
29	Nonanal	ntp	1104	2.3	0	0	6.4	2.6
30	( <i>Z</i> )-2-Undecene	ntp	1114	0	2.8	0	0	0
32	Nerol	om	1127	0	1.7	0	0	0
38	Isobutyl hexanoate	ntp	1150	0	0	0	0.5	0
35	Camphor	om	1145	0	0.6	0	0	0.4
45	4-Terpineol	om	1179	0	0.5	tr	0	0.5
46	Octanoic acid	ntp	1180	0	0	0	0	1.8
48	Methyl salicylate	ntp	1191	2.7	0	0	4	0
49	$\alpha$ -Terpineol	om	1192	0	20.5	0.9	0	0.7
55	Decanal	ntp	1206	1.9	3.3	0.5	6.1	2.9
61	( <i>Z</i> )-3-Hexenyl isovalerate	ntp	1238	0	0.5	0	0	0
63	Carvone	om	1244	0	0.9	0	0	0
64	( <i>E</i> )-2-Decenal	ntp	1263	0	tr	0	0.3	0.3
68	Methyl nerolate	om	1282	4.1	9	5.5	0	2.4
70	Isobornyl acetate	om	1285	0	0	0	0	3.3
72	<i>n</i> -Tridecane	ntp	1300	0	tr	0	0	6.2
74	Undecanal	ntp	1306	0	0.5	tr	1.0	0.6
76	Methyl geranate	om	1325	0	1.7	2.4	0	1.7
77	6-Methyltridecane	ntp	1346	0	0	0	0	0.6
79	$\alpha$ -Longipinene	sh	1352	0	0	0	1.3	0

82	$\alpha$ -Copaene	sh	1377	0	0	0.1	1.7	0.4
83	$\beta$ -Bourbonene	sh	1385	0	0	0	0.6	0
84	$\beta$ -Cubebene	sh	1391	0.8	tr	0	3.3	tr
87	( <i>Z</i> )-Jasmone	ntp	1395	0	0.9	0	0.5	0
88	<i>n</i> -Tetradecane	ntp	1400	0.9	0	0	0	0.5
91	Dodecanal	ntp	1407	0	0.7	0.2	1.3	1.3
92	$\beta$ -Caryophyllene	sh	1419	8.4	2	0.6	31.1	6.1
96	$\gamma$ -Elemene	sh	1434	0	0	0	2.4	0.2
97	<i>cis</i> - $\alpha$ -ambrinol	ap	1437	62.2	tr	0	1.9	0.2
102	5-Methyltetradecane	ntp	1452	0	1.3	0	0	0
103	( <i>E</i> )-Geranylacetone	ap	1456	1.8	5.3	1.3	4.6	2
104	$\alpha$ -Humulene	sh	1455	0.7	0	0.3	2.1	0
105	4-Methyltetradecane	ntp	1457	1.9	0	0	0	0
106	( <i>E</i> )- $\beta$ -Farnesene	sh	1459	1.3	0	1.1	0	1.5
108	2-Methyltetradecane	ntp	1462	0	0	0	1.7	0.7
110	$\gamma$ -Muurolene	sh	1478	0	0.5	0	6.3	0
111	Germacrene D	sh	1482	0.9	0	0.1	0	0.8
112	( <i>E</i> )- $\beta$ -Ionone	ap	1486	0	0	0	0.9	0
113	$\beta$ -Selinene	sh	1487	0	0	0	0	0.8
114	<i>cis</i> - $\beta$ -Guaiene	sh	1491	0	0	0.2	0	0
117	Pentadecane	ntp	1500	0	0	0.3	0	0
118	$\delta$ -Decalactone	ntp	1501	0	0	0	0	0.8
121	( <i>E,E</i> )- $\alpha$ -Farnesene	sh	1508	0	0	0	0.6	0.4
123	Tridecanal	ntp	1510	0	0	0	0.5	0
125	<i>trans</i> - $\gamma$ -Cadinene	sh	1514	0	0	0	0	0.7
127	10- <i>Epi</i> -italicene ether	os	1516	0.7	0	0	0	0
129	Benzoic acid 4-ethoxyethyl ester	ntp	1522	0	1.2	0	0	0
130	$\delta$ -Cadinene	sh	1524	0	0	0	0.5	0.8
133	( <i>Z</i> )-3-Hexenyl benzoate	ntp	1570	0	tr	0	0	0
134	Dendrolasin	os	1580	2.0	0	0	0	0
135	Caryophyllene oxide	os	1582	2.0	0	0	0.4	0
139	Tetradecanal	ntp	1613	0	tr	0	0.5	0.4
145	<i>Cis</i> -Methyl dihydrojasmonate	ntp	1655	0	0	0	0	0.5
147	<i>n</i> -Heptadecane	ntp	1700	0	0.4	0	0	0.7
149	<i>n</i> -Octadecane	ntp	1800	0	0	0	0	0.5
150	2-Ethylhexyl salicylate	ntp	1807	0	0	0	0	0.7
152	Isopropyl tetradecanoate	ntp	1830	0	0	0	0	0.8
-	<b>Apocarotenes</b>	<b>ap</b>		<b>64.0</b>	<b>5.3</b>	<b>1.3</b>	<b>7.4</b>	<b>2.2</b>
-	<b>Monoterpene hydrocarbons</b>	<b>mh</b>		<b>0.0</b>	<b>35.4</b>	<b>82.4</b>	<b>0.7</b>	<b>47.0</b>
-	<b>Non-terpene derivatives</b>	<b>ntp</b>		<b>9.7</b>	<b>11.6</b>	<b>1.0</b>	<b>22.8</b>	<b>22.0</b>
-	<b>Oxygenated monoterpenes</b>	<b>om</b>		<b>4.1</b>	<b>44.1</b>	<b>11.6</b>	<b>15.3</b>	<b>14.0</b>

-	Oxygenated sesquiterpenes	os	4.7	0.0	0.0	0.4	0.0
-	Phenylpropanoids	ph	0.0	0.0	0.0	0.0	0.0
-	Sesquiterpene hydrocarbons	sh	12.1	2.5	2.4	49.9	12.0
-	Total		94.6	98.9	98.7	96.5	97.2

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580**Table 7.** Comparison of the chemical composition (% of VOCs) emitted by the fruits of sampled  
581populations. PFF-T = Cerbaie Hills (Pisa, Tuscany); PFF-A = Torano (Rieti, Lazio); PFF-M =  
582Vallerotonda (Frosinone, Lazio); PFM = Monte Argentario (Grosseto, Tuscany); PFP = Fano  
583(Pesaro e Urbino, Marche). Ap: apocarotenes, ntp: non-terpene derivatives, om: oxygenated  
584monoterpenes, os: oxygenated sesquiterpenes, ph: phenylpropanoids, sh: sesquiterpene  
585hydrocarbons. Yellow columns = *P. flavescens* subsp. *flavescens*, orange column = *P. flavescens*  
586subsp. *maremmana*, green column = *P. flavescens* subsp. *pisaurensis*.

ID Compound	Compound	Class	LRI	PFF-T	PFF-A	PFF-M	PFM	PFP
15	1-Hexyl acetate	ntp	1010	0	0	3.5	0	tr
17	1,8-Cineole	om	1034	5.7	0.7	0	tr	0
19	Benzyl alcohol	ntp	1044	5.2	0	tr	tr	0
24	Fenchone	om	1088	3.9	tr	0	0	0
29	Nonanal	ntp	1104	18.1	0	12.2	3.5	15.2
35	Camphor	om	1145	1.1	0.3	0.9	tr	0
36	Ethyl 2-heptenoate	ntp	1146	0	0	0	0	0.5
40	(E)-2-nonenal	ntp	1163	1.1	0.2	0	tr	0.9
43	Neo-menthol	om	1167	0	0.2	1.2	0	0
44	Menthol	om	1174	0	0	0	0.6	0
45	4-Terpineol	om	1179	4	11.5	0	18.2	0
48	Methyl salicylate	ntp	1191	0	0	3.8	0	0
49	$\alpha$ -Terpineol	om	1192	4.7	7.4	0	3.7	0
50	cis-Dihydrocarvone	om	1194	0	0	0	1.6	0
52	Dihydrocitronellol	om	1196	0	0.6	0	0	0
54	trans-Dihydrocarvone	om	1204	0	0	0	5.7	0
55	Decanal	ntp	1206	15.5	8.8	22.5	12.7	18.6
57	1-Octyl acetate	ntp	1213	0	0	0.7	0	0.5
60	cis-p-Mentha 1(7)-8-dien-2-ol	om	1231	0	0	0	0	0.6
62	Cumin aldehyde	om	1241	0	0.2	0	0	0
63	Carvone	om	1244	0	43.8	0	23.5	0

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63	( <i>E</i> )-2-decenal	ntp	1263	0.7	0.5	0.3	0.5	0
67	Citronellyl formate	om	1275	0	0	1.5	0	0
69	( <i>E</i> )-Anethole	ph	1284	0	11.1	0	12.5	0
71	10-Undecenal	ntp	1299	0	0	1.2	0	0
72	<i>n</i> -Tridecane	ntp	1300	1.7	0.2	0	tr	0
73	Carvacrol	om	1301	0	0.2	0	0	0
74	Undecanal	ntp	1306	2.3	0.6	3.2	tr	1.7
83	$\beta$ -Bourbonene	sh	1385	0	0	0	0	9.8
88	<i>n</i> -Tetradecane	ntp	1400	2.3	tr	1.5	tr	0
91	Dodecanal	ntp	1407	5.5	0.5	4.3	1.1	0
92	$\beta$ -Caryophyllene	sh	1419	tr	0	4.4	tr	4.6
99	Aromadendrene	sh	1440	0	0	0	0	13.5
101	$\alpha$ -Himachalene	sh	1450	0	0	1.4	0.6	0
103	( <i>E</i> )-Geranylacetone	ap	1453	4.1	2.3	21.9	3	4.8
106	( <i>E</i> )- $\beta$ -Farnesene	sh	1459	0	0	0	0	2.4
109	$\beta$ -Chamigrene	sh	1476	0	0	0	0	1.5
111	Germacrene D	sh	1482	0	0	0	0	1.2
112	( <i>E</i> )- $\beta$ -Ionone	ap	1486	2.6	0.2	0	tr	1
116	<i>n</i> -Pentadecane	ntp	1500	3.4	0	0	0	0
118	$\delta$ -Decalactone	ntp	1501	0	0	2.4	0	0
122	$\beta$ -Bisabolene	sh	1508	0	0	0	0	4.7
123	Tridecanal	ntp	1509	1.7	tr	0	tr	0
124	$\alpha$ -Alaskene	sh	1511	0	0	0	tr	7.7
126	Geranyl isobutyrate	om	1515	0	0	0	0	1.5
128	( <i>Z</i> )- $\gamma$ -Bisabolene	sh	1517	0	0	0	0	1.4
132	Dihydroactinidiolide	ap	1536	4.8	0.6	0	0	3.5
135	Caryophyllene oxide	os	1582	0	6.3	0	8.7	0
136	Viridiflorol	os	1591	0	0.4	0	0	0
137	Carotol	os	1595	0	0	0	0.6	0
138	<i>n</i> -Hexadecane	ntp	1600	0	0	6.6	0	0.5
139	Tetradecanal	ntp	1613	1.7	0	tr	0	0.9
142	Selina-3,11-dien-6- $\alpha$ -ol	os	1644	0	0.7	0	0	0
146	Cadalene	os	1675	0	0	0	1.1	0
147	<i>n</i> -Heptadecane	ntp	1700	2.7	0.2	1.6	0.8	1.2
149	<i>n</i> -Octadecane	ntp	1800	2.4	0.1	1.2	tr	1.6
152	Isopropyl tetradecanoate	ntp	1830	2.2	0	0	0	0
153	( <i>E,E</i> )- $\alpha$ -Farnesyl acetate	os	1843	0	0.4	0	1.1	0
-	<b>Apocarotenes</b>	<b>ap</b>		<b>11.5</b>	<b>3.1</b>	<b>21.9</b>	<b>3.0</b>	<b>9.3</b>
-	<b>Non-terpene derivatives</b>	<b>ntp</b>		<b>66.5</b>	<b>11.1</b>	<b>65.0</b>	<b>18.6</b>	<b>42.0</b>
-	<b>Oxygenated monoterpenes</b>	<b>om</b>		<b>19.4</b>	<b>64.9</b>	<b>3.6</b>	<b>53.3</b>	<b>2.1</b>
-	<b>Oxygenated sesquiterpenes</b>	<b>os</b>		<b>0.0</b>	<b>7.8</b>	<b>0.0</b>	<b>12.5</b>	<b>0.0</b>

-	<b>Phenylpropanoids</b>	<b>ph</b>	<b>0.0</b>	<b>11.1</b>	<b>0.0</b>	<b>12.5</b>	<b>0.0</b>
-	<b>Sesquiterpene hydrocarbons</b>	<b>sh</b>	<b>0.0</b>	<b>0.0</b>	<b>5.8</b>	<b>0.6</b>	<b>47.0</b>
-	<b>Total</b>		<b>97.4</b>	<b>98.0</b>	<b>96.3</b>	<b>100</b>	<b>100</b>

587

588**Table 8.** Comparison of the chemical composition (% of VOCs) emitted by the seeds of sampled  
589populations. PFF-T = Cerbaie Hills (Pisa, Tuscany); PFF-A = Torano (Rieti, Lazio); PFF-M =  
590Vallerotonda (Frosinone, Lazio); PFM = Monte Argentario (Grosseto, Tuscany); PFP = Fano  
591(Pesaro e Urbino, Marche). Ap: apocarotenes, mh: monoterpene hydrocarbons, ntp: non-terpene  
592derivatives, om: oxygenated monoterpenes, os: oxygenated sesquiterpenes, ph: phenylpropanoids,  
593sh: sesquiterpene hydrocarbons. Yellow columns = *P. flavescens* subsp. *flavescens*, orange column  
594= *P. flavescens* subsp. *maremmana*, green column = *P. flavescens* subsp. *pisauensis*.

ID Compound	Compound	Class	LRI	PFF-T	PFF-A	PFF-M	PFM	PFP
2	Heptanal	ntp	901	0	0	0	0	2.1
3	$\alpha$ -Pinene	mh	941	19.1	31.4	1.0	70.8	0
4	Camphene	mh	955	0	0	0	0.9	0
5	Thuja-2,4(10)-diene	mh	959	0.8	0	0	0	0
9	Sabinene	mh	977	0	0.4	0	0	0
10	$\beta$ -Pinene	mh	982	0	0	0	1.2	0
13	Octanal	ntp	1002	0	0	0	0	11.1
15	1-Hexyl acetate	ntp	1010	0	0	tr	0	0.2
16	Limonene	mh	1032	0	0	0	1.0	0
18	3-Octen-2-one	ntp	1043	0	0	0	0	0.8
20	( <i>E</i> )- $\beta$ -Ocimene	mh	1052	0	9	0	0	0
21	( <i>E</i> )-2-Octenal	ntp	1062	0	0	0	0	1.6
22	$\gamma$ -Terpinene	mh	1063	0	0	1.2	0	0
23	<i>cis</i> -Sabinene hydrate	om	1070	0	0	3.7	0	0
26	6-Camphenone	om	1091	0	0	0	0.7	0
27	<i>trans</i> -Sabinene hydrate	om	1099	0	0	3.6	0	0
28	Linalool	om	1101	0	0	0	0	0.6
29	Nonanal	ntp	1104	29.8	11.5	12	0.4	25
31	<i>trans-p</i> -Mentha-2,8-dien-1-ol	om	1126	0	0	0	1.2	0
33	$\alpha$ -Campholenal	om	1127	1.8	0.9	0	1.7	0
34	<i>cis</i> -Verbenol	om	1144	0.8	0	0	0	0
35	Camphor	om	1145	1.1	0	0	1.8	0
37	<i>trans</i> -Verbenol	om	1147	0.2	0	0	3.5	0
39	<i>trans</i> -Pinocamphone	om	1162	0	0	0	0.4	0
41	Pinocarvone	om	1164	0	0	0	1.1	0
42	Benzyl acetate	ntp	1165	0	0	4.4	0	0
48	Methyl salicylate	ntp	1191	0	0	1.3	0	0
51	Myrtenal	om	1194	1.1	2.0	0	0.7	0
53	<i>n</i> -Dodecane	ntp	1200	0.3	0	0	0	0.2
55	Decanal	ntp	1206	6.1	2.4	7.7	0.4	4.3



56	Verbenone	om	1207	7.6	5.1	0	4.9	0
62	Cumin aldehyde	om	1241	0	0	0	0.2	0.1
64	( <i>E</i> )-2-Decenal	ntp	1263	0.6	0	0	0.1	0
66	<i>Neo</i> -menthyl acetate	om	1273	0.5	0	0	0	0
67	Citronellyl formate	om	1275	0.8	0	0	0	0
69	( <i>E</i> )-Anethole	ph	1284	8.6	9.5	11.9	0	tr
70	Isobornyl acetate	om	1285	0	0	0	1.0	0
72	<i>n</i> -Tridecane	ntp	1300	0.8	0	0	0	0
74	Undecanal	ntp	1306	1.5	1.2	2.3	tr	0
75	Methyl 4-decenoate	ntp	1310	0	0	0	0	2.4
78	$\alpha$ -Cubebene	sh	1352	0	0	0	0.5	0
81	2-Methylundecanal	ntp	1368	0	0	0	0	4.2
82	$\alpha$ -Copaene	sh	1377	0.2	0	0	0.1	0.2
83	$\beta$ -Bourbonene	sh	1385	0	0	0	0.2	0
85	$\beta$ -Elemene	sh	1391	1.5	2.9	3.3	0	0
90	( <i>Z</i> )-Caryophyllene	sh	1406	0	0	0	0	1.0
91	Dodecanal	ntp	1407	1.2	0	2.2	0.1	0
92	$\beta$ -Caryophyllene	sh	1419	0.3	1.9	2.0	tr	0.7
93	$\beta$ -Copaene	sh	1430	tr	tr	tr	0.1	0.4
94	<i>cis</i> -Thujopsene	sh	1431	0	0	0	0	5.2
99	Aromadendrene	sh	1441	0	0	0	0	7.0
100	$\alpha$ -Guaiene	sh	1440	0	4.3	0	0	0
101	$\alpha$ -Himachalene	sh	1450	1.9	0	4.8	0.2	0
103	( <i>E</i> )-Geranylacetone	ap	1456	0.8	0	tr	tr	0.8
104	$\alpha$ -Humulene	sh	1455	0	0.5	0.1	0.2	tr
110	$\gamma$ -Muurolene	sh	1478	1.1	1.2	1.5	0.3	1.1
111	Germacrene D	sh	1482	1.1	6.6	11.1	1.1	3.1
115	$\alpha$ -Muurolene	sh	1499	3.2	0	0	0	0
119	( <i>Z</i> )- $\alpha$ -Bisabolene	sh	1504	1.1	0.7	4.2	0	0
120	$\alpha$ -Bulnesene	sh	1507	0	0	2.1	0	7.4
122	$\beta$ -Bisabolene	sh	1508	0	0	0	0	4
125	<i>trans</i> - $\gamma$ -cadinene	sh	1514	1.2	1.1	2.9	0.7	2.5
130	$\delta$ -Cadinene	sh	1524	0.7	1.6	2.1	0.2	0
131	<i>trans</i> -Cadina-1(2),4-diene	sh	1534	0	0	0	0	1.9
139	Tetradecanal	ntp	1613	0	0	0.5	0	0
141	Eremoligenol	os	1630	0	0	0	0	0.5
145	<i>cis</i> -Methyl dihydrojasmonate	ntp	1655	0	0	0.4	0	0
146	Cadalene	os	1675	0.7	1.0	0	tr	1.6
147	<i>n</i> -Heptadecane	ntp	1700	0	0.5	1.4	tr	0
149	<i>n</i> -Octadecane	ntp	1800	0.5	0	1.2	tr	0
151	Hexadecanal	ntp	1817	0	1.5	0	0	0
154	Hexahydrofarnesylacetone	ap	1843	0	0	0.7	0	0
155	Cyclohexadecanolide	ntp	1930	0	0	1.3	0	0
156	Hexadecanoic acid	ntp	1960	0	0	2.6	0	0

-	<b>Apocarotenes</b>	<b>ap</b>	<b>0.8</b>	<b>0.0</b>	<b>0.7</b>	<b>0.0</b>	<b>0.8</b>
-	<b>Monoterpene hydrocarbons</b>	<b>mh</b>	<b>19.9</b>	<b>40.8</b>	<b>2.2</b>	<b>73.9</b>	<b>0.0</b>
-	<b>Non-terpene derivatives</b>	<b>ntp</b>	<b>40.8</b>	<b>17.1</b>	<b>37.3</b>	<b>1.0</b>	<b>52.0</b>
-	<b>Oxygenated monoterpenes</b>	<b>om</b>	<b>13.9</b>	<b>8.0</b>	<b>3.6</b>	<b>17.2</b>	<b>0.7</b>
-	<b>Oxygenated sesquiterpenes</b>	<b>os</b>	<b>0.7</b>	<b>1.0</b>	<b>0.0</b>	<b>0.0</b>	<b>2.1</b>
-	<b>Phenylpropanoids</b>	<b>ph</b>	<b>8.6</b>	<b>9.5</b>	<b>11.9</b>	<b>0.0</b>	<b>0.0</b>
-	<b>Sesquiterpene hydrocarbons</b>	<b>sh</b>	<b>12.3</b>	<b>20.8</b>	<b>34.1</b>	<b>3.6</b>	<b>35.0</b>
-	<b>Total</b>		<b>97.0</b>	<b>97.2</b>	<b>89.8</b>	<b>95.7</b>	<b>90.6</b>

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597**Table 9.** Comparison of the non-volatile compounds (relative amounts) in the sampled populations.

598PFF-T = Cerbaie Hills (Pisa, Tuscany); PFF-A = Torano (Rieti, Lazio); PFF-M = Vallerotonda

599(Frosinone, Lazio); PFM = Monte Argentario (Grosseto, Tuscany); PFP = Fano (Pesaro e Urbino,

600Marche). F: flavonoid, A: apocarotenoid, O: oligosaccharide, S: saponin, un = unidentified. Yellow

601columns = *P. flavescens* subsp. *flavescens*, orange column = *P. flavescens* subsp. *maremmana*,

602green column = *P. flavescens* subsp. *pisaurensis*. Compound numbers correspond to peak numbers

603indicated in Figure 5.

Compound	Class	$t_R$ (min)	Parent ion	Product ions	PFF-T	PFF-A	PFF-M	PFM	PFP
1	un	2.0	m		0.688	0.122	0.874	0.691	1.000
2	A	5.7	565 <sup>b</sup>	519, 403, 337, 261	1.000	0.787	0.773	0.786	0.973
3	O	6.9	653 <sup>b</sup>	635, 585, 517	0.645	0.444	0.409	1.000	0.469
4	un	11.7	213 <sup>a</sup>	169, 125, 11	0.751	0.608	0.577	1.000	0.836
5	F	15.5	741 <sup>a</sup>	723, 609, 591, 475, 343, 301	1.000	0.390	0.610	0.441	0.382
6	O	18.2	695 <sup>b</sup>	635, 529, 491	0.932	0.389	0.371	1.000	0.773
7	O	21.0	799 <sup>b</sup>	783, 731, 623, 551, 371	1.000	0.221	0.018	0.082	0.360
8	F	23.7	609 <sup>a</sup>	591, 463, 343, 301, 271, 179	1.000	0.458	0.518	0.401	0.646
9	O	27.0	915 <sup>a</sup>	900, 723, 709, 691, 503, 341	1.000	0.639	0.511	0.212	0.939
10	F	30.5	947 <sup>a</sup>	741, 723, 609, 591, 475, 301	1.000	0.499	0.479	0.385	0.519
11	un	31.8	783 <sup>a</sup>	661, 607, 485	0.543	0.503	0.809	1.000	0.781
12	F	33.1	917 <sup>a</sup>	899, 741, 723, 609, 591, 475, 301	0.973	0.745	0.613	1.000	0.767
13	un	33.7	669 <sup>b</sup>		0.637	0.347	0.246	0.851	1.000
14	F	34.6	887 <sup>a</sup>	869, 741, 723, 609, 475, 301	1.000	0.390	0.176	0.245	0.546
15	un	35.6	783 <sup>a</sup>	661, 607, 485	1.000	0.453	0.591	0.733	0.691
16	un	36.7	813 <sup>a</sup>	691, 607, 485	1.000	0.137	0.010	0.468	0.710
17	F	37.9	845 <sup>a</sup>	827, 723, 609, 591, 457, 301	1.000	0.318	0.391	0.387	0.233
18	un	38.8	1121 <sup>a</sup>	929, 915, 897, 691, 529	0.463	1.000	0.662	0.284	0.512
19	un	39.5	1121 <sup>a</sup>	929, 915, 897, 691, 529	0.894	1.000	0.800	0.672	0.767
20	un	40.5	825 <sup>a</sup>	783, 703, 649, 631, 527	1.000	0.126	0.347	0.270	0.339
21	un	41.1	855 <sup>a</sup>	733, 649, 631, 527	1.000	0.090	0.168	0.211	0.232
22	un	42.5	1121 <sup>a</sup>	915, 897, 691, 673	0.474	0.858	1.000	0.281	0.660
23	un	43.6	1121 <sup>a</sup>	915, 897, 691, 529	0.316	1.000	0.775	0.447	0.246
24	S	45.3	1589 <sup>a</sup>	1559 <sup>c</sup> , 1427, 747 <sup>d</sup> , 455 <sup>d</sup>	1.000	0.169	0.000	0.133	0.000
25	S	46.2	1427 <sup>a</sup>	1397 <sup>c</sup> , 1203, 937, 747 <sup>d</sup> , 455 <sup>d</sup> , 439 <sup>d</sup>	1.000	0.924	0.000	0.204	0.000
26	un	47.6	1121 <sup>a</sup>	915, 897, 691	0.699	0.987	1.000	0.617	0.992
27	S	49.6	1427 <sup>a</sup>	1397 <sup>c</sup> , 1203, 937, 747 <sup>d</sup> , 455 <sup>d</sup> , 439 <sup>d</sup>	1.000	0.631	0.033	0.731	0.275
28	S	51.1	1265 <sup>a</sup>	1235 <sup>c</sup> , 1011, 937, 455 <sup>d</sup> , 439 <sup>d</sup>	0.608	1.000	0.256	0.397	0.244
29	un	52.8	1695 <sup>b</sup>	1649, 1611, 1044, 949, 683	0.733	0.695	0.774	1.000	0.856

30	un	53.9	1695 <sup>b</sup>	1573, 1259, 1045, 965	0.951	0.278	0.935	1.000	0.949
31	S	56.4	1235 <sup>a</sup>	1205 <sup>c</sup> , 1011, 981, 455 <sup>d</sup> , 423 <sup>d</sup>	1.000	0.570	0.000	0.206	0.646
32	S	57.8	1103 <sup>a</sup>	1073 <sup>c</sup> , 879, 455 <sup>d</sup> , 439 <sup>d</sup>	0.541	1.000	0.223	0.368	0.352
33	S	60.6	1235 <sup>a</sup>	1205 <sup>c</sup> , 1011, 981, 455 <sup>d</sup>	1.000	0.169	0.000	0.638	0.105
34	S	62.3	1631 <sup>a</sup>	1601 <sup>c</sup> , 1471, 747 <sup>d</sup> , 455 <sup>d</sup>	1.000	0.115	0.146	0.794	0.291
35	S	63.5	1469 <sup>a</sup>	1439 <sup>c</sup> , 937 <sup>d</sup> , 747 <sup>d</sup> , 455 <sup>d</sup> , 439 <sup>d</sup>	0.373	0.140	0.300	0.207	1.000
36	un	64.7	1173 <sup>a</sup>	741, 723, 609, 547, 343	1.000	0.387	0.162	0.144	0.000
37	S	64.9	1469 <sup>a</sup>	1439 <sup>c</sup> , 747 <sup>d</sup> , 455 <sup>d</sup> , 423 <sup>d</sup>	0.000	0.000	0.061	0.016	1.000
38	S	66.0	1601 <sup>a</sup>	1571 <sup>c</sup> , 937 <sup>d</sup> , 747 <sup>d</sup> , 455 <sup>d</sup>	0.956	0.620	0.344	1.000	0.663
39	S	67.5	1439 <sup>a</sup>	1409 <sup>c</sup> , 455 <sup>d</sup> , 439 <sup>d</sup>	0.392	0.041	0.070	0.291	1.000
40	S	67.7	1615 <sup>a</sup>	1585 <sup>c</sup> , 1291, 1277, 1173, 747 <sup>d</sup> , 455 <sup>d</sup>	0.407	0.414	0.182	1.000	0.252
41	S	68.8	1453 <sup>a</sup>	1423 <sup>c</sup> , 1291, 937 <sup>d</sup> , 455 <sup>d</sup>	1.000	0.447	0.561	0.532	0.000
42	S	69.8	1307 <sup>a</sup>	1277 <sup>c</sup> , 1175, 1011, 455 <sup>d</sup> ,	0.534	0.000	0.353	0.180	1.000
43	S	70.3	1411 <sup>a</sup>	1249, 1187, 1025, 747 <sup>d</sup> , 585 <sup>d</sup> , 439 <sup>d</sup>	0.532	1.000	0.050	0.252	0.000
44	S	70.8	1439 <sup>a</sup>	1409 <sup>c</sup> , 1215, 1173, 1277, 455 <sup>d</sup> , 423 <sup>d</sup>	0.118	0.000	0.000	0.000	1.000
45	S	71.3	1145 <sup>a</sup>	1115 <sup>c</sup> , 849, 747 <sup>d</sup> , 455 <sup>d</sup>	0.000	0.617	0.491	1.000	0.688
46	S	72.4	1277 <sup>a</sup>	1247 <sup>c</sup> , 1115, 1055, 455 <sup>d</sup>	1.000	0.415	0.198	0.445	0.592
47	S	73.6	1239 <sup>a</sup>	1501 <sup>c</sup> , 1369, 455 <sup>d</sup>	0.000	1.000	0.000	0.000	0.000
48	S	74.0	1511 <sup>a</sup>	1481 <sup>c</sup> , 1349, 937 <sup>d</sup> , 747 <sup>d</sup> , 455 <sup>d</sup>	0.092	0.042	0.419	0.093	1.000
49	S	74.7	1381 <sup>a</sup>	1219, 1157, 995, 585 <sup>d</sup> , 439 <sup>d</sup>	0.851	1.000	0.000	0.352	0.000
50	S	75.1	1349 <sup>a</sup>	1319 <sup>c</sup> , 1187, 937 <sup>d</sup> , 455 <sup>d</sup>	0.050	0.147	0.438	0.067	1.000
51	S	75.8	1395 <sup>a</sup>	1233, 1171, 1025, 585 <sup>d</sup> , 439 <sup>d</sup>	0.000	1.000	0.000	0.192	0.000
52	un	76.8	647 <sup>a</sup>	579	1.000	0.723	0.195	0.000	0.000
53	S	76.4	1319 <sup>a</sup>	1289 <sup>c</sup> , 1187, 1011, 455 <sup>d</sup>	0.000	0.000	0.127	0.000	1.000
54	S	77.1	1481 <sup>a</sup>	1451 <sup>c</sup> , 1319, 1173, 455 <sup>d</sup>	0.000	0.000	0.024	0.000	1.000
55	un	77.9	1087 <sup>a</sup>	863, 759, 627, 423	1.000	0.684	0.000	0.277	0.000
56	un	79.3	566 <sup>a</sup>	581, 543, 499	1.000	0.492	0.211	0.322	0.000
57	S	79.3	1349 <sup>a</sup>	1319 <sup>c</sup> , 1187, 1083, 1041, 455 <sup>d</sup>	0.000	0.000	0.000	0.000	1.000
58	S	80.2	1219 <sup>a</sup>	1087, 995, 863, 439 <sup>d</sup>	0.000	0.000	1.000	0.000	0.000
59	S	81.0	1453 <sup>a</sup>	1291, 1187, 747 <sup>d</sup> , 455 <sup>d</sup> , 439 <sup>d</sup>	0.595	0.746	0.623	0.376	1.000
60	S	81.7	1531 <sup>a</sup>	1291, 1231, 1187, 747 <sup>d</sup> , 439 <sup>d</sup>	1.000	0.096	0.177	0.253	0.262
61	S	82.5	1501 <sup>a</sup>	1471, 1177, 455 <sup>d</sup>	0.414	0.577	0.766	0.318	1.000
62	S	83.5	1423 <sup>a</sup>	1199, 1157, 995, 439 <sup>d</sup> , 1171, 1125, 439 <sup>d</sup>	0.710	0.382	0.491	0.380	1.000
63	un	84.4	668 <sup>a</sup>	436, 396	0.759	0.181	1.000	0.184	0.656
64	un	85.1	602 <sup>a</sup>	579, 549, 273	1.000	0.000	0.000	0.116	0.415
65	un	85.4	675 <sup>a</sup>	652, 631, 355	1.000	0.382	0.162	0.215	0.000
66	S	85.9	1261 <sup>a</sup>	1219, 995, 585 <sup>d</sup> , 423 <sup>d</sup>	0.137	0.070	0.131	0.102	1.000
67	un	86.7	587 <sup>a</sup>	375	0.536	0.150	1.000	0.445	0.607
68	S	87.2	1291 <sup>a</sup>	1025, 863, 523 <sup>d</sup> , 439 <sup>d</sup>	0.000	0.169	0.000	0.000	1.000
69	un	87.8	1275 <sup>a</sup>	1051, 1009, 991, 863, 669	1.000	0.162	0.068	0.263	0.000
70	S	88.1	1333 <sup>a</sup>	1171, 1125, 585 <sup>d</sup> , 439 <sup>d</sup>	0.000	0.000	1.000	0.000	0.000
71	S	88.6	1261 <sup>a</sup>	1219, 995, 585 <sup>d</sup> , 423 <sup>d</sup>	0.308	0.174	0.000	0.000	1.000

72	S	90.3	1303 <sup>a</sup>	995, 977, 439 <sup>d</sup>	0.137	0.106	0.262	0.064	1.000
73	un	93.2	929 <sup>a</sup>	883, 817, 725, 657, 493	0.000	0.000	0.000	1.000	0.000
74	un	99.4	883 <sup>a</sup>	845, 747, 575	0.982	1.000	0.530	0.304	0.222
75	un	102.7	721 <sup>a</sup>	675, 637, 585, 415, 235	0.993	0.769	0.503	0.769	1.000

604 **Compound 2** = 3 $\beta$ -hydroxy-5,6-epoxy- $\beta$ -ionol 9-*O*- $\beta$ -D-apiofuranosyl (1 $\rightarrow$ 6)- $\beta$ -D-glucopyranoside; **compound 3** =  $\beta$ -605D-(6-*O*-benzoyl)-fructofuranosyl-(2 $\rightarrow$ 1)-[ $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)]- $\alpha$ -D-glucopyranoside; **compound 5** = quercetin 6063-*O*- $\beta$ -D-apiofuranosyl(1 $\rightarrow$ 2)-*O*-[ $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 6)]- $\beta$ -D-glucopyranoside]; **compound 6** =  $\beta$ -D-(6-*O*-607benzoyl) fructofuranosyl-(2 $\rightarrow$ 1)-[ $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)]-6-acetyl- $\alpha$ -D-glucopyranoside; **compound 7** = 3,6'-di-*O*-608sinapoylsucrose; **compound 8** = rutin; **compound 9** = reinirose F; **compound 10** = quercetin 3-(5-*O*-sinapoyl)- $\beta$ -D-609apiofuranosyl(1 $\rightarrow$ 2)-*O*-[ $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 6)]- $\beta$ -D-glucopyranoside]; **compound 12** = quercetin 3-(5-*O*-*t*-610feruloyl)- $\beta$ -D-apiofuranosyl(1 $\rightarrow$ 2)-*O*-[ $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 6)]- $\beta$ -D-glucopyranoside]; **compound 14** = quercetin 6113-(5-*O*-coumaroyl)- $\beta$ -D-apiofuranosyl(1 $\rightarrow$ 2)-*O*-[ $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 6)]- $\beta$ -D-glucopyranoside]; **compound 17** = 612quercetin 3-(5-*O*-benzoyl)- $\beta$ -D-apiofuranosyl(1 $\rightarrow$ 2)-*O*-[ $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 6)]- $\beta$ -D-glucopyranoside]; 613**compound 35** = 3-*O*- $\beta$ -D-glucopyranosyl preseggenin 28-*O*-{ $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 4)-614 $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)-[ $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)]-[4-*O*-acetyl]}- $\beta$ -D-fucopyranosyl ester; **compound 43** = 3-615*O*- $\beta$ -D-glucopyranosyl medicagenic acid 28-*O*-{ $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)- $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 4)- $\alpha$ -L-616rhamnopyranosyl-(1 $\rightarrow$ 2)-[ $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)]}- $\beta$ -D-fucopyranosyl ester.

617<sup>a</sup> [M-H]<sup>-</sup>. <sup>b</sup> [M+HCOO]<sup>-</sup>. <sup>c</sup> [M-H-30]<sup>-</sup>. <sup>d</sup> Sugar fragments.

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