



# Chemical Composition of the Essential Oils From Leaves and Flowers of *Passiflora sexocellata* and *Passiflora trifasciata*

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## Abstract

The chemical composition of the essential oils of *Passiflora sexocellata* and *Passiflora trifasciata* (Passifloraceae, subgenus *Decaloba*) were studied for the first time. Essential oils were obtained by steam distillation of fresh leaves and flowers. The chemical composition was assessed by using GC/FID and GC/MS. For *P. sexocellata* leaves, the optimized analytical procedure allowed the identification of 33 compounds (75% of the total oil composition) and 29 (74% of the total oil composition) in flowers. Regarding *P. trifasciata*, 35 compounds (76% of the total oil composition) were detected in leaves and 32 (71% of the total oil composition) in flowers. Terpenes and mono unsaturated hydrocarbons were quantified as major constituents of the volatile fraction in flowers (17.0 to 52.6%) and (13.7 to 20.0%). Organic acids were detected in both leaves and flowers with a percentage ranging from 3.3% to 32.0%. Aldehydes were also detected in leaves (12.6 to 41.4%) and in flowers (1.4 to 5.1%). The GC/MS analyzes allowed alcohols to be detected in leaves (20.6 to 42.9%) and in flowers (8.2 to 18.1%). These compounds represent the most important feature of the large *Passiflora* family. Moreover, a critical role in the coevolved mechanisms of pollinators' interaction has been investigated.

## Keywords

*Passiflora sexocellata*, *Passiflora trifasciata*, essential oil, pollinator, steam distillation

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*Passiflora trifasciata* Lem., and *Passiflora sexocellata* Schldtl. are two tropical vines of the subgenus *Decaloba* (DC.) Rchb., family Passifloraceae, with tendrils to aid in climbing and they differ from the most common subgenus *Passiflora* L. because of relatively small flowers, two to 3 series of coronal filaments and a chromosome number of  $2n = 12$ .<sup>1</sup> *Passiflora trifasciata* (Figure 1(A)) is included in the supersection *Decaloba*<sup>2</sup> and it is an endemic species in Ecuador, Perù and Brasil. The tri-color passionvine produces single or coupled white fragrant flowers with green pistils and stamens that stand out against the beautifully marked foliage. This species is cultivated as ornamental. *Passiflora sexocellata* (Figure 1(B)) is included in the supersection *Cieca* (Medic.) J. M. a monophyletic group primarily distributed in the southern United States, México, Central and South America, and the Caribbean. *P. sexocellata* is found from southern Mexico to Nicaragua. It possesses peltate bilobed leaves bearing 4–13 laminar nectaries often with 2–4 of the nectaries occurring proximal to the lateral leaf veins. Flowers are often produced in long inflorescences and the fruits are spherical and possess more than 40 arillate seeds. *Passiflora sexocellata* is pollinated by small to medium guild bees (*Colletes* sp.) as reported for the majority of the species in the supersection *Cieca* (e.g., *P.*

*coriacea*, *P. mcvaughiana*, *P. megacoriacea*, *P. trinifolia*). The flower coronal filaments act as a landing platform for pollinators and have conspicuous areas of yellow and/or purplish coloration to attract them. Moreover, the more advanced species of supersection *Cieca* (*P. xikezodz*, *P. juliana*, *P. viridiflora*, *P. coriacea*, *P. sexocellata*, *P. megacoriacea*) are mainly visited by tropical butterflies. In particular *Dryas julia* and *Heliconius erato* are *P. sexocellata* herbivory in México and Central America.<sup>3</sup> The close association of species in the Heliconiinae and Passifloraceae is

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**Figure 1.** *Passiflora trifasciata* Lem. (A) and *Passiflora sexocellata* Schltld. (B), greenhouse-grown plants cultivated in Sanremo (NW-Italy).

commonly reported as an example of plant-insect coevolution. Scent plays an important role in foraging, sometimes acting synergistically with color. The importance of visual and olfactory signals most likely varies with species, with each species having a unique favored combination of color cues and chemical compounds.<sup>4</sup> GC/FID and GC-MS coupled with steam distillation has been employed to analyze fresh flowers and leaves of *P. mucronata* (subgenus *Passiflora*) in order to assess the role of floral volatiles in plant pollination.<sup>5</sup> Long chain methyl and ethyl esters, accounting for 50.8% of the essential oil from flowers, seems to be key of attraction for bats such as *Carollia perspicillata* and *Glossophaga soricina*, driven by the olfactory stimuli of these compounds when visiting *P. mucronata* for foraging. A high content of odd linear long-chain hydrocarbons was detected in fresh tissues of three bee-pollinated *Passiflora* hybrids, acting as allelochemicals in plant herbivore interactions.<sup>6</sup> In this work we have studied, for the first time, the chemical composition of essential oil obtained by steam distillation of both fresh leaves and flowers of *P. sexocellata* and *P. trifasciata* in order to assess their role in plant pollination and plant association with “passion flower butterfly”.

## Results and Discussion

The yields of *P. sexocellata* and *P. trifasciata* essential oil obtained by steam distillation from fresh leaves and flowers were quantified and reported as a percentage of 0.04% and 0.06%, 0.07% and 0.11% (weight/fresh weight basis), respectively. Table 1 shows the results of qualitative and quantitative oil analyzes on the Elite-5MS column. The compounds are listed in order of

their elution and are reported as percentages of the total essential oil, determined using octyl octanoate as internal standard. For *P. sexocellata* leaves, the total number of peaks was 44 with a number of 33 identified peaks (75% identification), for the flowers was 39 with a number of 29 identified peaks (74% identification). For *P. trifasciata* leaves, the total number of peaks was 46 with a number of 35 identified peaks (76% identification) and, for flowers was 45 with a number 32 identified peaks (71% identification). Differences in the qualitative and quantitative composition of the obtained essential oils have been observed.

### *Passiflora Sexocellata*

**Leaves essential oil.** The main bulk of constituents of the volatile fractions were found to be alcohols (42.9%), detected in the range of 0.2 to 24.7%, from which 4-vinylphenol (24.7%), 4-vinylguaiacol (12.6%) and benzyl alcohol (1.6%) are the most abundant compounds. Organic acids accounting for 18.3% of the total essential oil represent the second largest class. This class is dominated by pentadecanoic acid (9.6%) and dodecanoic acid (8.7%). Aldehydes are present at 12.6%, from which (*Z*)-2-hexenal (7.0%) and hexanal (2.2%) are the most representative compounds. Mono unsaturated hydrocarbons accounting for 9.4% of the total essential oil are represented by 1-icosene (7.0%) followed by 3-tetradecene (1.5%).

**Flowers essential oil.** The main bulk of constituents of the volatile fractions were found to be terpenes (52.6%), detected in the range of 0.2 to 47.5%, and dominated by  $\alpha$ -humulene

**Table 1.** Composition of Essential Oils From Leaves and Flowers of *P. Sexocellata* and *P. Trifasciata*.

#	Compound <sup>a</sup>	RI tab <sup>b</sup>	RI mean <sup>c</sup>	<i>P. sexocellata</i> <i>P. trifasciata</i>			
				leaves %	flowers %	leaves %	flowers %
1	Hexanal	801	800	2.2	0.2	2.4	1.1
2	Diacetone alcohol	844	841	0.5	0.4	0.65	0.3
3	(Z)-2-hexenal	854	853	7.0	-	12.4	-
4	(Z)-2-hexenol	856	857	1.1	0.4	7.06	2.3
5	1-hexanol	868	869	0.1	0.8	1.2	1.7
6	2,4-hexadienal	912	910	0.9	-	0.1	-
7	3-methyl-butenal	929	921	0.4	-	0.3	-
8	2-heptenal	944	944	0.3	-	1.1	-
9	(E)-2-hexenal	957	957	0.3	-	0.1	0.2
10	5-methyl-2-furfural	966	965	-	-	5.3	-
11	1-octen-3-ol	980	980	0.2	0.2	3.1	1.9
12	2,4-heptadienal	1013	1012	0.2	-	0.2	-
13	2-ethyl-hexanol	1029	1029	0.2	0.2	-	0.3
14	Benzyl alcohol	1033	1037	1.6	0.6	1.9	1.0
15	<i>o</i> -cresol	1052	1054	-	0.2	2.0	2.7
16	Unidentified MW = 170	-	1060	0.5	0.3	0.2	0.7
17	1-octanol	1067	1071	-	0.3	-	2.7
18	Guaiacol	1092	1094	-	-	0.6	-
19	Linalool	1097	1097	2.0	0.4	0.2	-
20	Tetrahydro-linalol	1099	1102	0.5	-	1.4	4.9
21	Nonanal	1113	1112	0.6	1.2	19.4	3.9
22	2-phenylethanol	1117	1118	-	-	-	0.3
23	Dimethyl glutarate	1135	1135	0.2	-	-	0.2
24	1,3-bis(1-methylethyl)-benzene	1140	1141	2.7	0.6	-	2.3
25	1,4-dimethoxy-benzene	1143	1143	-	-	12.6	-
26	1-nonanol	1151	1151	1.4	1.7	3.8	2.3
27	Ethyl benzaldehyde	1175	1172	0.8	-	0.2	-
28	Epoxylinolol	1183	1180	-	0.2	-	0.5
29	1-Dodecene	1194	1193	1.0	0.3	0.4	1.6
30	Dodecane	1200	1200	-	0.1	0.1	-
31	Methyl salicylate	1201	1204	-	-	9.6	1.5
32	4-vinylphenol	1215	1217	24.7	0.5	-	-
33	Unidentified MW = 122	-	1256	-	1.8	0.2	0.6
34	Tridecane	1300	1302	-	-	0.2	-
35	4-vinylguaiacol	1321	1323	12.6	2.97	0.4	2.6
36	3-Tetradecene	1393	1393	1.5	1.7	1.6	2.4
37	Tetradecane	1400	1400	-	0.2	-	0.4
38	$\alpha$ -Ionone	1423	1422	0.4	-	0.5	-
39	$\beta$ -Caryophyllene	1428	1428	-	3.6	0.2	9.7
40	$\beta$ -Ionone	1458	1451	1.8	-	1.3	0.2
41	$\alpha$ -Humulene	1463	1460	1.6	47.5	1.2	1.9
42	Dodecanoic acid	1488	1480	8.7	2.7	1.3	7.0
43	6-methyl-coumarin	1555	1556	-	-	3.5	-
44	Unidentified MW = 355	-	1565	0.8	1.1	-	-
45	Pentadecanoic acid	1857	1860	9.6	14.4	2.0	24.9
46	1-Eicosene	1988	1988	7.0	1.4	-	2.0
47	Eicosane	2000	2001	0.6	0.8	-	1.0
48	Phytol	2112	2113	5.3	0.9	-	-
49	Docosane	2200	2201	0.4	1.9	-	1.0
50	9-Tricosene <sup>d</sup>	2279	2273	-	10.4	1.6	13.2

(Continued)

Table 1. Continued

#	Compound <sup>a</sup>	RI tab <sup>b</sup>	RI mean <sup>c</sup>	<i>P. sexocellata</i> <i>P. trifasciata</i>			
				leaves %	flowers %	leaves %	flowers %
51	9-Heptacosene <sup>d</sup>	2676	2676	-	-	-	0.8
	Class of Compounds						
	Alcohols			42.9	8.2	20.6	18.1
	Aldehydes			12.6	1.4	41.4	5.1
	Ketones			2.2	-	1.9	0.2
	Esters			0.2	-	9.6	1.7
	Acids			18.3	17.1	3.3	32.0
	Terpenes			9.3	52.6	2.9	17.0
	Saturated hydrocarbons			1.0	3.1	0.3	2.4
	Mono unsaturated hydrocarbons			9.4	13.7	3.6	20.0
	Unidentified			1.3	3.3	0.3	1.3
	Miscellanea			2.7	0.6	16.1	2.3

<sup>a</sup>Compounds are listed in order of elution from an Elite-5 column.

<sup>b</sup>Kovats Retention Indices according to Adams et al.<sup>7</sup>

<sup>c</sup>Retention index determined on an Elite-5 column using a homologous series of n-alkanes.

<sup>d</sup>sec.<sup>8</sup>

(47.5%) followed by  $\beta$ -caryophyllene (3.6%). Organic acids (17.1%) characterize the second largest class where pentadecanoic acid (14.4%) and dodecanoic acid are the most abundant compounds. Mono unsaturated hydrocarbons (13.7%) are represented by 9-tricosene (10.4%) and 3-tetradecene (1.7%). Alcohols are represented by 4-vinylguaiaicol (3.0%) and 1-nonanol (1.7%).

### *Passiflora Trifasciata*

**Leaves essential oil.** The main bulk of constituents of the volatile fractions were found to be aldehydes (41.4%), detected in the range of 0.1 to 19.4%, from which nonanal (19.4%), (Z)-2-hexenal (12.4%) and 5-methyl-2-furfural (5.3%) are the most abundant constituents. Alcohols (20.6%) represent the second largest class and (Z)-2-hexenol (7.0%), 1-nonanol (3.8%), 1-octen-3-ol (3.1%) and o-cresol (2.0%) are the most representative compounds. Esters class is dominated by methyl salicylate (9.6%). Mono unsaturated hydrocarbons (3.6%) are represented by 9-tricosene (1.6%) and 3-tetradecene (1.6%). Organic acids, accounting for 3.3% of the total essential oil are represented by pentadecanoic acid (2.0%) and dodecanoic acid (1.3%). 1,4-dimethoxy-benzene (12.6%) and 6-methyl-coumarin (3.5%) were also detected.

**Flowers essential oil.** The main bulk of constituents of the volatile fractions were found to be organic acids (32.0%), represented by pentadecanoic acid (24.9%) and dodecanoic acid (7.0%), respectively. The second largest class is represented by mono unsaturated hydrocarbons (20.0%), from which 9-tricosene (13.2%), 3-tetradecene (2.4%), 1-eicosene (2.0%) and 1-dodecene (1.6%) are the most abundant compounds. Alcohols account for 18.1% of the total essential oil are

represented by 1-octanol (2.7%), o-cresol (2.7%), 4-vinylguaiaicol (2.6%), 1-nonanol (2.3%), (Z)-2-hexenol (2.3%), 1-octen-3-ol (1.9%), 1-hexanol (1.7%) and benzyl alcohol (1.0%). Terpenes (17.0%) are featured by  $\beta$ -caryophyllene (9.7%), followed by tetrahydro-linalol (4.9%) and  $\alpha$ -humulene (1.9%). Aldehydes are represented by nonanal (3.9%).

The highest intraspecific differentiation level of subgenus *Decaloba* was studied by Pérez et al., (2017) by using qualitative descriptors of 61 *Passiflora* L. species. This investigation showed morphological variations when subgenus *Decaloba* was compared to the *Passiflora* and *Astrophea* subgenus.<sup>9</sup> In addition, a comparison between cytogenetic and morphological features with pollinator syndrome underlined different evolutionary time scales. To this regard, the supersection *Decaloba* of the subgenus *Decaloba* showed the highest divergence among plant-pollination adaptation mechanisms. Although the whole subgenus is characterized by a reduced flowers size, the pollination by small insects (i.e., wasps and small bees) is predominant. For other species, such as *P. trinervia* and *P. penduliflora* that are pollinated by hummingbirds and bats, different and extreme mutualistic specializations between flower and pollinator are reported.<sup>10</sup> Regarding the floral structure, other mechanisms (e.g., floral scent chemistry) play an important role both in controlling flower visiting and in the foraging behavior of insects. This process is considered as the major specialization that provides the mutualistic relationship plant-pollinator.

The significant increase in organic acids (31.9%) and mono unsaturated hydrocarbons (20.0%) detected in flower of *P. trifasciata* could attest a different insect attraction mechanism, in comparison with *P. sexocellata* that highlighted lower percentages - 17.1% and 13.7% respectively - for the examined compounds. Simultaneously, the opposite trend observed by



comparing the leaf extracts of the two plant species (higher amount of both organic acids (18.3%) and mono unsaturated hydrocarbons (9.4%) in leaves of *P. sexocellata*) suggested an involvement of these compounds in the reproductive mechanism commonly due to the flower traits.

Despite the absence of any physiological evidences concerning the nectar dynamics and reproductive biology of *P. trifasciata*, our findings support the hypothesis of a divergence in the scent production related to a different pollinator syndrome.

Regarding Passiflora family and its nectar production dynamic, high rates of nectar like the storage of starch in *P. actinia* (subgenus *Passiflora*) have been shown to be associated with structural characteristics of nectaries and pollinator behavior and breeding strategies.<sup>11</sup> For *P. biflora* (supersection *Decaloba*) starch storage was also described by Durkee et al. (1981).<sup>12</sup> The high content in total acid and alcohols recorded in *P. trifasciata*, could be considered as an additive mechanism playing a role in the selective pressures exerted on the pollinator species, towards larger and long-distance flight capable bees. On the other hand, *P. sexocellata* belonging to a different taxonomical supersection (*Cieca*) co-evolved a close association with a limited number of herbivores - only one or two known - and limited its pollination to small or medium guild bees.<sup>3</sup> Concerning our findings, a conspicuous content in terpenes (52.6%) was recorded in the flower extracts of *P. sexocellata*, associated to a reduced amount of total acids and alcohols (17.1% and 8.2%, respectively), comparing to *P. trifasciata* and to a complete absence of detectable ketones and esters. On this regard, Porter-Utley (2014) reports the absence of flavonoid compounds as a distinctive feature of most species of supersection *Cieca*, analyzing dried leaf samples.<sup>3</sup> Accordingly, the absence of ketones and esters in the volatiles of the flower fraction should be further investigated as a distinctive trait of *P. sexocellata*, including additional species within the same supersection. Terpenes are a large class of compounds usually detected in the volatile constituents of essential oils of different plant species.<sup>13</sup> Several studies investigating the complex pollination mechanisms of orchids have largely highlighted the involvement of terpenes in the pollination by sexual deception.<sup>14</sup> This latter is a highly specialized pollination strategy that mainly mimic the sex pheromone of females of their pollinator species to attract male insects that then pollinate the flowers.<sup>15</sup> Although considerable progress, many gaps remain in the understanding of the terpene's biosynthesis, but evidence was provided on the attraction exercised by short-chain terpenes on pollinators, that seem to be attracted from distance to their mates.<sup>14</sup> Different kinds of chemicals, other than terpenes are involved in this mechanism (cyanogenic glucosides, alkaloids, flavonoids, saponins and tannins). On the contrary, chemical mimicry is widely known in different *Passiflora* species as a coevolutionary process occurring between passion flower and heliconiines, in terms of a protection system evolved by the plant against its predators.<sup>16</sup> In particular, the high terpenes content detected in the volatile fraction of flower extracts of *P. sexocellata* was ascribable to a high content in  $\alpha$ -humulene. On this

respect, high level of (*E*)-caryophyllene and  $\alpha$ -humulene, were reported by Da Silva et al. (2015) in *Commiphora leptophloeos* leaf oil, and the oviposition deterrent activity of the two terpenes were investigated, suggesting an insecticides role for those chemicals.<sup>17</sup> However, interactions with environment and insects in plants, promoted by terpenes and terpenoids, involve a myriad of mechanisms, underlining their important role as chemical mediators.<sup>18</sup> In the contest of the present paper, the high amount of terpenes detected in *P. sexocellata* suggests a mechanism aimed to control the flower visitors, both repelling the presence of undesired predators and as a selective approach promoting specific coevolved pollinators.

## Conclusions

The present paper provides the first qualitative and quantitative characterization of the essential oil volatile fraction isolated from fresh leaves and flowers of *P. trifasciata* and *P. sexocellata*. Terpenes, organic acids and unsaturated hydrocarbons represent the major constituents of flowers by acting as semiochemicals to attract insect pollinators. These compounds are deemed to be crucial for *P. sexocellata* and *P. trifasciata* reproduction. Further investigations of the involvement of these compounds in pollinators' interaction mechanism might be of great interest.

## Experimental

### Plant Material

*Passiflora trifasciata* Lem. and *Passiflora sexocellata* Schtdl. are part of a greenhouse-grown plant collection including wild species, varieties and hybrids, belonging to the subgenera *Passiflora* and *Decaloba*, was established in Sanremo (NW-Italy) UTM WGS84, Zone 32T North: Long 400142.51 Lat 4852298.79. The plants were grown in 30 cm pots supplied with plastic structures to allow climbing of the tendrils and they were heated during winter and shadowed during summer time, with temperatures ranging from 10 °C (min) to 32 °C (max). At the flowering stage, flowers and young leaves produced in the current year of the same phenological stage and without any defect were collected at about 9.30 am, put into Pyrex 250 ml sealed bottles and stored at -80 °C until analyzed. A voucher specimen was deposited at CREA-OF (Sanremo, Italy) with code PASS017 and PASS027, respectively. The specimen was fully characterized by morphological characters and by the ISSR markers.<sup>19</sup>

### Isolation of Essential Oil

samples of flowers and leaves of *P. sexocellata* (7.12 g and 18.67, respectively) and *P. trifasciata* (8.55 g and 20.52, respectively) to which octyl octanoate was added as internal standard, were steam distilled for 3 hours. Steam distillation was performed in the steam distillation system. Briefly, a body for the distillation was made of heat-resistant glass, including a plate of stainless steel, and the steam passing through the plants was water-cooled, thereby producing the essential oil and plant water

simultaneously. The distillate was extracted three times with 100 ml of methylene chloride, dried over anhydrous  $\text{Na}_2\text{SO}_4$  concentrated with a rotary evaporator and finally using a gentle stream of  $\text{N}_2$ , stored at  $-20^\circ\text{C}$  until analyzed.

### Fractionation and Alkylthiolation of Alkenes

after analyzes of the whole essential oil, a portion from each sample was subjected to selective purification process<sup>8</sup> and alkylthiolation reaction.<sup>20</sup> The dimethyl disulfide adducts were identified, and the positions of the methyl sulfide substituents were deduced from the fragmentation pattern.

### GC-FID Analysis

The analyzes were carried out using a Hewlett Packard model 5980 GC, equipped with Elite-5MS (5% phenyl methyl polysiloxane) capillary column of (30 m  $\times$  0.32 mm i.d.) and film 0.32  $\mu\text{m}$  thick. The carrier gas was He at a flow of 1 ml/min. An aliquot (1  $\mu\text{L}$ ) of essential oil were manually injected in “split” mode (30:1). The oven temperature program included an initial isotherm of  $40^\circ\text{C}$  for 5 minutes, followed by a temperature ramp to  $260^\circ\text{C}$  at  $4^\circ\text{C}/\text{min}$ , and a final isotherm at this temperature for 10 minutes. Injector and detector temperatures were set at  $250^\circ\text{C}$  and  $280^\circ\text{C}$ , respectively. The relative amount of each component was calculated based on the corresponding FID peak area without response factor correction.

### GC-MS Analysis

The analyzes were carried out using a GC Model 6890 n, coupled to a bench top MS Agilent 5973 Network, equipped with the same capillary column and following the same chromatographic conditions used for the GC/FID analyzes. The carrier gas was He at constant flow of 1.0 mL/min. The essential oils were diluted prior to analysis and 1.0  $\mu\text{L}$  was manually injected into the GC system with a split ratio of 30:1. The ion source temperature was set at  $200^\circ\text{C}$ , while the transfer line was at  $300^\circ\text{C}$ . The acquisition range was 40-500 amu in electron-impact (EI) positive ionization mode using an ionization voltage of 70 eV.

### Identification of the Components of the Volatile Fractions

The components of the essential oils were identified by comparing their mass spectra with NIST 98 and Wiley 5 MS libraries, as well as by comparing their Kovats retention indices, relative to a C8-C23 homologous series of n-alkanes and calculated according to Van Den Dool and Kratz,<sup>21</sup> with literature values.<sup>7</sup>

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