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Floral scent of brazilian *Passiflora*: five species analised by dynamic headspace

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ABSTRACT

This study describes for the first time the chemical composition and olfactive description of floral scent from Brazilian *Passiflora* (*Passiflora edulis* Sim, *Passiflora alata* Curtis, *Passiflora cincinnata* Mast., *Passiflora coccinea* Aubl. and *Passiflora quadrangularis* L.). Five species were grown in greenhouse at the Agronomic Institute (IAC), São Paulo, Brazil. Volatile compounds were collected using dynamic headspace. Analyses of scent composition were performed by gas chromatograph coupled to mass spectrometer. Identification of chemical constituents was conducted through of retention index followed by comparative analysis of mass spectra with specialized databases. The olfactive descriptions of floral scent from each species was evaluated for a professional perfumer. High interspecific diversity was found between chemical compositions of floral scent within *Passiflora* and different bouquets were observed amount the studied species. Mayor constituents were linalool (*P. alata),* geraniol (*P. quadrangularis*), 1,4-dimethoxybenzene (*P. edulis*), benzaldehyde (*P. cincinnata*) and 2-methyl-3-pentanone (*P. coccinea*).

Key words: chemical composition, fragrances, olfactive description, *Passiflora.*

INTRODUCTION

The genus *Passiflora* L. with more than 500 species described is the largest within the family Passifloraceae (MacDougal and Feuillet 2004). Vast majority of *Passiflora* species are distributed in Central America and South America, and much rarer in Asia, Australia, and tropical Africa (Dhawan et al. 2004, Ramaiya et al. 2014). Some species have commercial importance and are grown in many countries for fruit production, medicinal use or ornamental purposes (Ulmer and MacDougal 2004). The main centers of *Passiflora* diversity are found in Colombia and Brazil (Cervi 2006, Ocampo et al. 2007, Ramaiya et al. 2014). Relative abundant literature is available about phytochemistry of *Passiflora edulis* Sims and *P. incarnata* L., but only sporadic publications are focused on lest common species. Volatile compounds (VCs) of passion

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Figure 1 - Species of *Passiflora*: **(a)** *P. edulis*; **(b)** *P. alata*; **(c)** *P. cincinnata*; **(d)** *P. coccinea*; **(e)** *P. quadrangularis.* Photo: Daniel A.V. Montero, 2012.

fruit´s aroma have been collected and analyzed several times (Galvão et al. 2004, Janzantti et al. 2012, Werkhoff et al. 1998), although floral scent of passionflowers had received little attention. In Brazil, the composition and emission of floral scent in four species of *Passiflora* from the Atlantic Forest was investigated trough solvent extraction by Varassin et al. (2001); other authors have studied scent emissions by olfactory tests (Amela García 1999, MacDougal 1994, Amela García et al. 2007) and some other works have been focused on the role of floral scent in ecological dynamics like pollination syndromes among *Passiflora* species (Jazen 1968, Sazima and Sazima 1978, 1987, Koschnitzke and Sazima 1997).

The production of volatile compounds in *Passiflora* is highly variable and has major ecological importance (Varassin et al. 2001, Amela García et al. 2007). During the last twenty years, the collection and characterization of volatile compounds from live flowers by headspace/gas chromatograph coupled to mass spectrometer (HS/GC-MS) methods. HS/GC-MS methods have been utilized by many researchers to investigate the floral scent of several taxa (Dotterl et al. 2005, Kite and Salazar 2008, Knudsen et al. 2006, Kaiser 2006, Steenhuisen et al. 2012). However, the scent of passionflowers surprisingly, has received little attention in this matter and only the work of Lindberg et al. (2000) have addressed the chemical composition of passionflower´s scents by HS/GC-MS methods. For this reason, our research was carried out, with the aim of collect, identify and analyze for the first time, the floral fragrances of five Brazilian species of *Passiflora: Passiflora edulis* Sims, *Passiflora alata* Curtis, *Passiflora cincinnata* Mast., *Passiflora coccinea* Aubl. and *Passiflora quadrangularis* L. (Figure 1).

MATERIALS AND METHODS

Location and Plant Materials

The research was carried out during the years 2011-2012 at the Agronomic Institute (IAC), State of São

Paulo, Brazil. Five Brazilian passionflowers were used: *Passiflora edulis*, *P. alata*, *P. cincinnata*, *P. coccinea* and *P. quadrangularis*. Plant materials were obtained from the Active Bank of Germplasm of the IAC. Seeds were germinated in controlled conditions, following Meletti et al. (2010). After germination seedlings were planted in plastic bags and when reached 60 cm tall they were transplanted into 30 L pots. The substrate used for all pots was a mix of 100 kg of commercial organic substrate, 75 kg of coconut fibber, 1kg of dolomite calcareous, 1 kg of commercial phosphates and 0.5 kg of castor bean cake. Fifteen days after the transplant, the young plants were placed in a greenhouse (average temperature of 30°±5) adapted with wire lines at 1.70 m height and they were manually irrigated three times per week. When the plants reached the wire, the apical tissue was cut out to enhance formation of productive curtains, as the traditional passion fruit production system. During the first three months after transplant, foliar fertilization was made every fifteen days as follows: NPK 28-14-14 plus micro nutrients 0.1g Fe, 0.05 g Mn, 0.05 g Zn, 0.05 g Cu, 0.02 g B and 0.0005 g Mo.; after the third month, mensal fertilization was made using 100 g of granulose NPK 20-5-20 per plant. At the beginning of the flowering period 1kg of commercial KCl mixed with the substrate was provided into the pots and during the flowering 15 mL of liquid NPK 8-8-8 and 2.5 mL of Ca-Mg-B were provided every fifteen days.

Collection of Volatile Compounds

The floral scent from the flowers of each species was collected with the dynamic headspace technique adapted from Schilling et al. (2010). The traps used to capture the volatile compounds were made of glass tubings (external diameter: 6 mm, internal diameter: 3 mm, length: 1500 mm) inside which 100 mg of Porapak Q adsorbent (Supelco, 80-100 mesh) were packed and sealed at the ends with 1 cm of glass fiber (Supelco, pesticide degree). Before sampling, the traps were successively washed with 10 mL of methanol (Tedia Brazil, chromatography degree), 10 mL of dichloromethane (Merck, chromatography degree) and 10 mL of hexane (Tedia Brazil, chromatography degree) and kept in an oven at 170 °C for 8 hs, under Nitrogen flow (Praxair Inc., 99.99%) at 32 $mLmin^{-1}$ to ensure the removal of residual compounds.

One flower still attached to the plant was inserted into a glass funnel covered with a polyvinyl bag and the volatile compounds inside the system was captured for 1 hour through of the trap, using a portable vacuum pump with a flow of 100 mL min^{-1}. After sampling, the traps were eluted with 300 µL of hexane and ethyl acetate (1:1), chromatography degree (Tedia Brazil). The eluent was collected in a vial (1.5 mL, StepVial II, Sun-Sri) an ice bath and stored in the freezer at - 8 °C until the analyses of the volatile compounds. For each species were sampled five individuals (one flower per individual), plus a control with vegetative plant material.

Chromatographic Conditions

The chemical composition analysis of the floral scents was conducted on a gas chromatograph coupled to mass spectrometer (GC-MS, Shimadzu QP-5000, Kyoto, Japan). Gas chromatograph was equipped with a capillary silica column OV-5 (30 m x 0.25 mm x 0.25 µm) and used the following chromatographic conditions: helium as the carrier gas (1.0 mL min^{-1}), injector at 240 °C, splitless injection mode (Splitless time: 0.8 min), injected volume was 1 µL of solution of the volatile compounds. The OV-5 column temperature was programmed at 35 °C isotherm for 5 min; and then increased to 180 °C at a rate 3 °C min^{-1}, and then increased to 240 °C at a rate 8 °C min^{-1}. The mass spectrometer conditions were: ionization voltage 70 eV, interface at 230 °C, mass scan range: 35 - 450 mass units. The identification of the chemical constituents was carried out by comparative

analysis of mass spectra of the substances with the database system GC-MS (Nist 62.lib), retention index (Adams 1995, 2007) and complementary libraries when needed (NIST Chemistry WebBook, Givaudan database). The retention indices were obtained by injection a series of n-alkanes (Sigma-Aldrich, C_7-C_{24}) in same conditions indicated above for GC-MS analyses (Van den Dool and Kratz 1963).

Olfactory descriptions of the floral scents of each species were made with help of a professional perfumer.

Statistical Analysis

Based on the results of chromatographic analysis of fragrances, a graphic of similarity between species was generated by the distance of the correlation coefficient using the cluster analyses of the program BioDiversity Pro version 2. Chemical composition of the fragrances from the studied species were statistically analyzed by the method of principal component analysis also in the program BioDiversity Pro version 2, to describe the data variation between species.

RESULTS AND DISCUSSION

Each of the studied species showed its own and unique fragrance thanks to its chemical composition, proportion and relative abundance of volatile compounds. The fragrances of the studied species were very intense and pleasant, with the exception of *P. coccinea* which has a floral scent almost imperceptible by the human nose. Forty-six compounds were captured and identified (Table I).

According to similarity analyzes between floral scents of the studied species (Figure

TABLE I

Floral volatiles identified from *Passiflora* species.

SUBSTANCES	% (average)					IRexp[a]	Identification method[b]
	P. alata	*P. cincinnata*	*P. coccinea*	*P. edulis*	*P. quadrangularis*		
Alyphatic							
2-methyl-3-pentanone	2.2	14.5	16.3	4.7	2.8	-	a
heptane	0.7	2.5	8.1	1.6	2.7	702	a,b
methyl isobutyl ketone	-	-	-	0.3	-	716	a,b
2,5-dimethyl-2-hexene	1.9	9.6	8.8	4.2	-	745	a,b
isobutyl acetate	-	2.2	-	2.4	-	768	a,b
allyl acetate	0.9	-	-	3.0	2.2	785	a
2-hexanone	-	5.9	5.6	-	0	787	a,b
octane	-	2.6	-	-	0	792	a,b
2-hexanol	1.9	-	2.7	-	2.5	797	a,b
3-hexyl-hydroperoxide	-	2.7	2.1	0.8	1.2	801	a
3-penten-2-ol	-	3.6	-	1.3	-	806	a
butyl acetate	-	-	-	5.4	-	814	a,b
4-methyl -octane	-	1.9	-	-	-	863	a,b
decane	-	-	-	0.1	-	1000	a,b
2,4-dimethyl-decane	-	7.9	-	1.2	-	1060	a
undecane	-	5.5	3.5	0.5	-	1103	a,b
dodecane	-	-	15.9	2.7	-	1201	a,b

TABLE I (continuation)

SUBSTANCES	% (average)					IRexp[a]	Identification method[b]
	P. alata	*P. cincinnata*	*P. coccinea*	*P. edulis*	*P. quadrangularis*		
tridecane	-	2.4	-	0.6	-	1286	a,b
tetradecane	-	-	14.8	2.1	-	1403	a,b
hexadecane	-	-	4.3	2.0	-	1605	a,b
TOTAL	**7.6**	**61.3**	**82.1**	**32.9**	**11.4**		
Benzenoids and Phenylpropanoids							
benzaldehyde	-	14.7	-	-	-	956	a,b
benzyl alcohol	1.2	-	-	1.4	0.9	1032	a,b
phenylethylalcohol	-	2.4	-	-	2.7	1110	a,b
4-ethyl-benzaldehyde	trace	3.5	-	1.7	2.4	1161	a,b
1,4-dimethoxybenzene	6.2	-	-	44.7	-	1163	a,b
3,4-dimethylaceto phenone	0.5	2.7	-	1.1	1.6	1281	a,b
trans-methyl cinnamate	-	-	-	5.9	2.6	1382	a,b
1,3,5-trimethoxy-benzene	-	-	-	2.1	-	1409	a,b
prenyl benzoate	-	-	-	-	0.3	1487	a
benzyltiglate	-	-	-	-	10.9	1502	a,b
4-methyl-2,6-di-tert-butylphenol	-	-	3.0	-	-	1514	a,b
2-phenylethyltiglate	-	-	-	-	1.5	1589	a,b
TOTAL	**7.9**	**23.3**	**3.0**	**56.9**	**22.9**		
Monoterpenes							
mircene	0.4	-	-	-	2.0	992	a,b
limonene	0.2	-	-	0.7	1.6	1026	a,b
eucalyptol	-	-	-	0.5	0	1027	a,b
cis-ocimene	-	-	-	-	1.5	1040	a,b
trans-ocimene	1.0	-	2.7	-	3.7	1049	a,b
linalool	40.2	0.9	-	-	1.2	1102	a,b
citronellal	0.4	-	-	-	-	1151	a,b
nerol	0.3	-	-	-	1.4	1229	a,b
citronellol	22.4	-	-	-	-	1231	a,b
neral	4.5	-	-	-	-	1242	a,b
iso-geraniol	1.2	-	-	-	-	1250	a,b
geraniol	1.4	-	-	-	43.6	1254	a,b
geranial	7.8	-	-	-	1.6	1272	a,b
methyl geranate	2.0	-	-	-	0.9	1326	a,b
TOTAL	**81.8**	**0.9**	**2.7**	**1.2**	**57.5**		
TOTAL IDENTIFIED	**97.3**	**85.5**	**87.8**	**91.0**	**91.8**		

[a]IR (retention indices), comparison of RIs with reported in the literature (NIST Chemistry WebBook, Adams 2007, 1995).

[b]Identification method: a – mass spectra analyses**;** b – mass spectra and retention indices analyses.

2), two groups were associated. Close related species *P. alata* and *P. quadragularis* formed the first group with 77% of similarity between the compositions of their fragrances, both rich and dominated by monoterpenes. The other group was formed by the other three species, with higher similarity between *P. cincinnata* and *P. edulis*. This could be explained by the large proportion of benzenoids and phenylpropanoids present on the floral scents of these species. Moreover, *P. coccinea* was associated in this group but placed in the middle of the clad, since the fragrance of this species is mainly constituted by aliphatic compounds, also present in all the other studied species. The PC1 discriminated the species with monoterpenes as major constituents (*P. alata* and *P. quadrangularis*), and the PC2 discriminated the species with benzenoids and phenylpropanoids as major constituents (*P. edulis* and *P. cincinnata*). The principal component analyses (PCA) based on volatile compounds from different metabolic pathways explained 82% of variance between the studied species (Figure 3).

Floral Scent of *P. alata*

Based on the GC-MS analysis of the flower scent of *P. alata*, 21 compounds were identified. This fragrance consists primarily of terpenoids (81.8%), benzenoids and phenylpropanoids (7.9%) and aliphatic compounds (7.6%). Linalool (40.2%) and citronellol (22.4%) were the main constituents of fragrance. Olfactive description: rich and very interesting mix of white flower, fresh rose and very powerful citrus fruity notes and sub notes of honey with a creamy velvet undertone. This was a very elegant fragrance which cached our attention for further research.

Floral Scent of *P. cincinnata*

In the floral scent of *P. cincinnata*17 compounds were captured, identified and classified. The aliphatic compounds had the largest proportion (61.3%) with 12 identified compounds. Although the fragrance of *P. cincinnata* also presented a high proportion of benzenoids and phenylpropanoids (23.3%) of which five compounds were identified, including benzaldehyde (14.7%) as one of the main constituents. Only one terpenoid was identified (linalool) responsible for the 0.9% of the fragrance. Olfactive description: powerful white flower and a strong faint cresolic odor, intensely sweet, with an undertone of sweet crushed bitter almond, nut like, aspects of gardenia and mimosa flowers, sub notes of honey.

Floral Scent of *P. coccinea*

We noted that the flowers of *P. coccinea* emitted a fragrance so subtle that it was almost imperceptible for our noses, unless getting really close to the flower. However, a number of volatile compounds were collected in the headspace of these flowers. The flower scent composition of *P. coccinea* was primarily given by 10 aliphatic compounds (82.1%). In addition, two unidentified compounds constituted 5.2% of the fragrance. Major constituent of this fragrance was 2-methyl-3-pentanone (16.3%). Olfactive description: Weak mostly absent smell. Spots of vanillin and raspberry ketone.

Floral Scent of *P. edulis*

We captured, identified and classified 24 compounds belowing to the floral scent of this species. Most of the fragrance was made by six benzenoids and phenylpropanoids (56.9%).The compound 1,4-dimethoxybenzene (also known as dimethyl hydroquinone in the perfume industry) had the highest proportion in the fragrance (44.7%). The aliphatic compounds (32.9%) were the most diverse with 15 identified compounds. Interestingly, terpenoids only constituted 1.2% of the fragrance. Olfactive description:white flower, aspects of Ylang-Ylang, balsamic, anysic, carnation (eugenol,

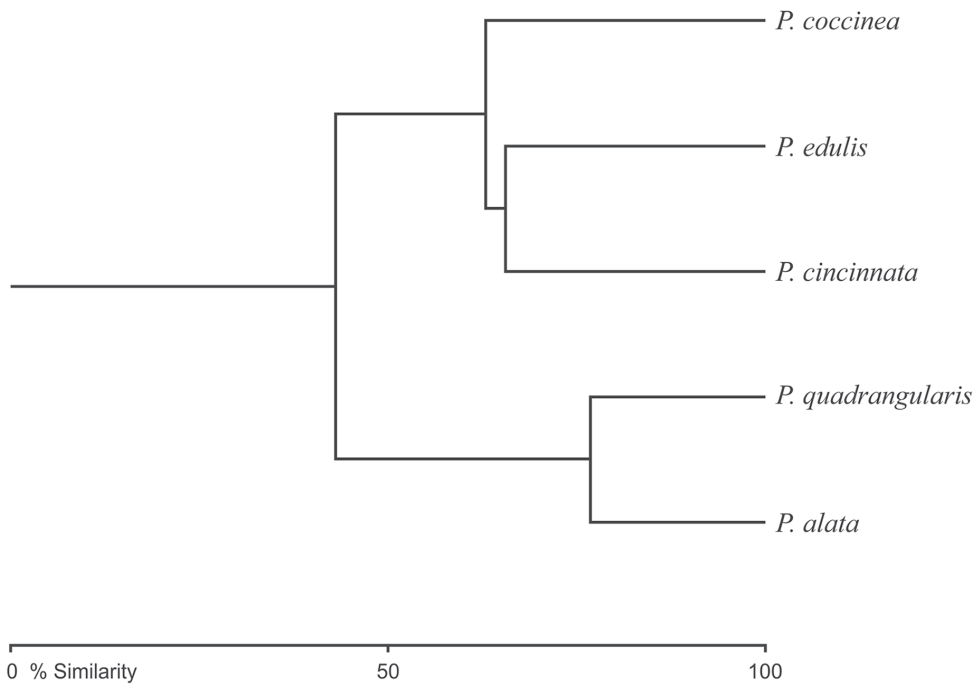

Figure 2 - Cluster analyze of similarity among floral scents of *Passiflora* species from Brazil.

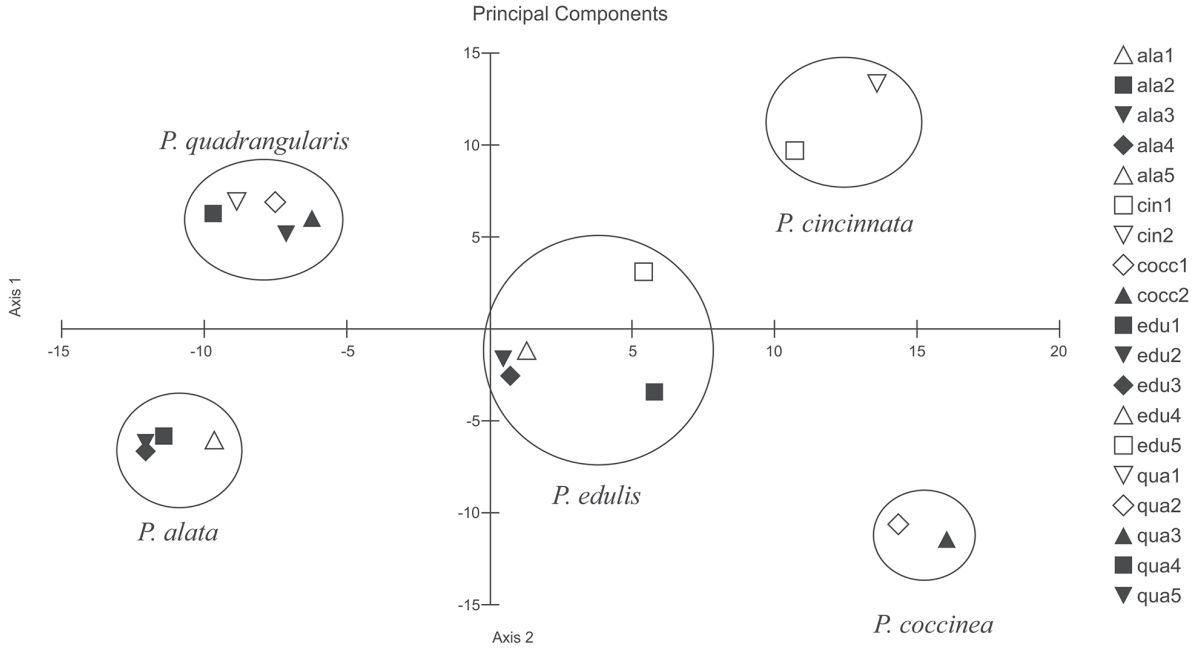

Figure 3 - Principal component analyses of floral scent of *Passiflora* based on volatile compounds.

heliotropine), animalic (creolic, methyl benzoate notes) with citric freshness of pamplemouse.

Floral Scent of *P. quadrangularis*

In the floral scent of *P. quadrangularis,* 22 compounds were identified. Floral scent was mainly constituted by nine terpenoids (57.5%) and nine benzenoids and phenylpropanoids (22.9%). Also, five aliphatic compounds (11.4%) were identified. The major compound of this fragrance was the geraniol (43.6%). Olfative description: velvet pinene odor. Ozone, sparkling / apple watermelon fruity notes. Has sulfur undertones with Rosy-floral-aldehydic-faint accord and rounded off by special fruitiness. Floral Fruity, Rose and Peach.

According to Amela García et al. (2007), the floral biology of approximately 23 species of *Passiflora* has been studied, although more than 525 species have been described (MacDougal and Feuillet 2004). At least 30 of these species produce floral fragrances perceivable by the human nose (see citation in Amela García et al. 2007), yet, until the year 2012, the volatile compounds of only a few species (c.a 12) of *Passiflora* had been identified (Lindberg et al. 2000, Varassin et al. 2001). Besides, olfactory tests with floral parts of nine species have been conducted, showing that the odors comprising the floral fragrances are emitted by different parts of the flower, particularly by the corona filaments (radii) in which the secretory tissue involves the entire perimeter of each filament (Amela García et al. 2007). Absence of fragrances also, has been reported in several species (Vanderplank 1996, Koschnitzke and Sazima 1997, Amela García 1999), including *P. edulis* (Lindberg et al. 2000), curiously. In our research *P. edulis* presented an intense fragrance very rich in benzenoids that may have a key function in pollination by carpenter bees, well documented for this specie (Meletti et al. 2010).

Like it was observed by Lindberg et al. (2000), the floral scent of the studied passionflowers had the volatile substances from the three main biosynthetic pathways of the secondary metabolism of plants: i.e. fatty acid derivatives of the polyketide; benzenoids from the shikimic acid; and isoprenoids of the mevalonic acid pathways. Most of these compounds are commonly found in floral scents of other species (Kaiser 1993, Knudsen et al. 2004) and its function as important signals for communication between plants and other organisms has been well documented (Raguso et al. 2003, Knudsen et al. 2006, Soler et al. 2011). The volatile compounds found by Lindberg et al. (2000) in *P. ligularis*, *P. riparia* and *P. maliformis* among other species of *Passiflora* which were also found in our research, included: benzaldehyde (*P. cincinnata*), benzylalcohol (*P. alata*, *P. edulis* and *P. quadrangularis*), 1,3,5-trimethoxybenzene (*P. edulis*), citronellol (*P. alata*), linalool (*P. alata*, *P. quadrangularis* and *P. cincinnata*), *trans*-ocimene (*P. alata* and *P. coccinea*) and myrcene, nerol, geraniol and geranial (*P. alata* and *P. quadrangularis*).

CONCLUSIONS

The chemical compositions of the floral fragrances from the studied species had large interspecific diversity, as well as interesting potential in the fragrance industry. Fragrances of *P. alata* and *P. quadrangularis* were very interesting mixes of floral and fruity notes with creamy sub notes and variable undertones. The main substances found were linalool for *P. alata*, benzaldehyde for *P. cincinnata*, 2-methyl-3-pentanone for *P. coccinea*, 1,4-dimethoxybenzene for *P. edulis* and geraniol for *P. quadrangularis*.

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