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Article

In vivo and *in vitro* Volatile Constituents of the Flowers of *Xylopia aromatica* by HS-SPME/GC-MS

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Xylopia aromatica (Lam.) Mart. (Annonaceae) is a typical species from the Brazilian cerrado that presents medicinal properties. The plant is distinguished by its large white flowers which produce a pleasant fragrance. *X. aromatica* is characterized by a wide range of medicinal application. These characteristics have motivated us to investigate the flowers volatile organic compounds (VOCs) via *in vivo* and *in vitro* protocols by a headspace solid-phase microextraction (HS-SPME) technique combined with gas chromatography-mass spectrometry (HS-SPME/GC-MS). Four different fibers, extraction times and temperatures were the parameters changed to lead to the maximum profiling of the volatile constituents. Data were analyzed using principal component analysis (PCA). A total of 77 VOCs were extracted from the floral scent, with 52 and 68 extracted from *in vivo* and *in vitro* sampling, respectively, of which 48 were reported for the first time in the literature as volatile constituents from *X. aromatica* flowers. The extraction and identification of VOCs were successfully performed through HS-SPME/GC-MS. The PCA data allowed the identification of parameters that led to the maximum number of VOCs, which were polyacrylate (PA) and carboxen/polydimethylsiloxane (CAR/PDMS) fibers, 60 min extraction time and temperature of 29.0 °C. Among the volatile constituents identified, sesquiterpenes predominated, comprising about 61.04%.

Keywords: *Xylopia aromatica*, *in vivo* and *in vitro* sampling, HS-SPME/GC-MS, VOCs, multivariate analysis

Introduction

Annonaceae is a large family of aromatic trees, shrubs, or climbers broadly distributed in tropical and subtropical regions, and which comprises more than 130 genera and 2,300 species.[1,2] With considerable economic importance, this family has species that produce edible fruits, such as custard apple (*Annona squamosa* L.), soursop (*Annona muricata* L.) and numerous odorous species. These fragrances occur mainly due to the presence of essential oils,[3] which constitute one of the most important groups of raw materials for food and cosmetic industries.[1,2]

The genus *Xylopia* is one of the largest in this family, with approximately 160 species distributed in tropical and subtropical regions of America, Africa, Asia, and Oceania.[2] *X. aromatica* (Lam.) Mart., popularly known as "pimenta-de-macaco" ("monkey pepper" lit. translation into English)[3] is a small tree generally 4-5 m (12-15 feet) tall, commonly found in the coastal forest and cerrado of Brazil (Brazilian savanna). It is distinguished by its large white flowers that produce a pleasant fragrance.[4] The chemical composition of the flowers, leaves, fruits and stem bark contain the essential oil of *X. aromatica*,[3,5-7] and their pharmacological properties, which include cytotoxic,[8] trypanomicidal,[9] antimalarial,[10] anti-obesity,[11] anti-inflammatory[8] and antimicrobial activities[5] have all been reported.

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These remarkable characteristics, besides the wide medicinal application of the plant, prompted us to investigate the volatile organic compounds (VOCs) from its flowers by the solid-phase microextraction (SPME) technique. Nowadays, SPME combined with gas chromatography coupled to mass spectrometry (GC-MS) is a consolidated analytical approach widely used in the research of these compounds from plant materials. It is considered superior to other methods due to advantages related to the principles of green chemistry as being solvent-free, besides easy to operate and versatile.[12-14] In addition, the SPME technique enables *in vivo* and *in vitro* sample collection,[15] followed by the direct desorption of the analytes from the fiber inside a measuring system and detection of a large number of volatile compounds.[12-15]

In the previous study on the VOCs from *X. aromatica*, Andrade *et al*.,[16] using the SPME technique, performed an *in vitro* sampling of a specimen from Northern Brazil and identified limonene, α-pinene and β-pinene as the major components. However, no *in vivo* work of VOCs has been reported until this moment. Thus, this work investigated four different fibers, extraction times and temperatures for maximum extraction of volatile constituents from the flowers of *X. aromatica* via *in vivo* and *in vitro* techniques. In addition, the data obtained were analyzed using a multivariate analysis technique, principal component analysis (PCA).

Experimental

Plant material

The fully developed flowers of *X. aromatica* were randomly collected at the Federal University of Catalão farm in the city of Catalão (Goiás state), Brazil. Access to the genetic heritage was registered at the National System of Genetic Resource Management and Associated Traditional Knowledge (SisGen) under code No. A11AE20. This species was identified, and a voucher (specimen No. 6554) was deposited at the Centro-Norte-Matogrossense herbarium, Federal University of Mato Grosso, Campus Sinop. Three flowers were used for each fiber, resulting in twelve flowers *per* experiment.

Standard compounds and materials

Standard compounds *R*-limonene (analytical standard), β-elemene (analytical standard), *trans*-caryophyllene (≥ 98.5%), α-humulene (≥ 96%), tridecan-2-one (99%), pentadecan-2-one (≥ 98%) and C_8-C_{30} *n*-alkanes were all purchased from Sigma-Aldrich (Saint Louis, USA).

The extraction procedures for the VOCs were carried out using 20 mL headspace vials (Agilent, Santa Clara, USA) and 250 mL polypropylene vials (Plaszom, Orlenas, Brazil) with caps for *in vitro* and *in vivo* sampling, respectively. The SPME fibers coated with polydimethylsiloxane (PDMS, 100 μm), polyacrylate (PA, 85 μm), carboxen/polydimethylsiloxane (CAR/PDMS, 75 μm), divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 μm) and the manual SPME holder were acquired from Supelco® (Bellefonte, USA).

Headspace solid-phase microextraction (HS-SPME) optimization procedure

In vivo and *in vitro* sampling of VOCs from *X. aromatica* flowers were performed (Figure 1). For *in vivo* experiments, the compounds were obtained from the farm. After exposure, the fibers were transported to the laboratory in screw-capped glass tubes (20 × 150 mm, 30 mL, Flinn Scientific, Batavia, USA) inside a thermal box at 10 °C. For *in vitro* experiments, the flowers were collected, and the extraction procedures were carried out immediately on arrival in the laboratory, using a circulating air stove (Nova Ética, São Paulo, Brazil) (Figure 1). In both samplings, the fibers were placed in screw-capped glass tubes and store at 7 °C until their GC-MS analysis. The fibers were then retracted into the needle and inserted into the GC injector for 60 min in splitless mode. Extractions were carried out in triplicate for each fiber and one empty capped vial was used as the blank control.

Gas chromatography coupled to mass spectrometry (GC-MS)

GC-MS analyses were performed on an Agilent GC-7820A gas chromatogram system equipped with HP-5 MS fused silica capillary column (5% phenyl and 95% methylpolysiloxane) (30 m × 0.25 mm internal diameter (i.d.) and 0.25 μm film thickness) coupled to an Agilent MSD 5975 mass-selective detector (Agilent Technologies, Wilmington, USA). The system operating conditions were a programmed temperature at 60 °C for 2 min, followed by an increase of 4 °C *per* min until 250 °C, and then being kept at this temperature for 10.5 min, an injector temperature of 250 °C, an electron ionization (EI) mode at 70 eV. Helium was used as carrier gas at a constant flow rate of 1 mL min^{-1}; the scan range was set at *m/z* 45-450. The mass-selective detection (MSD) parameter source temperature was set at 230 °C, and the MS source temperature was 150 °C.

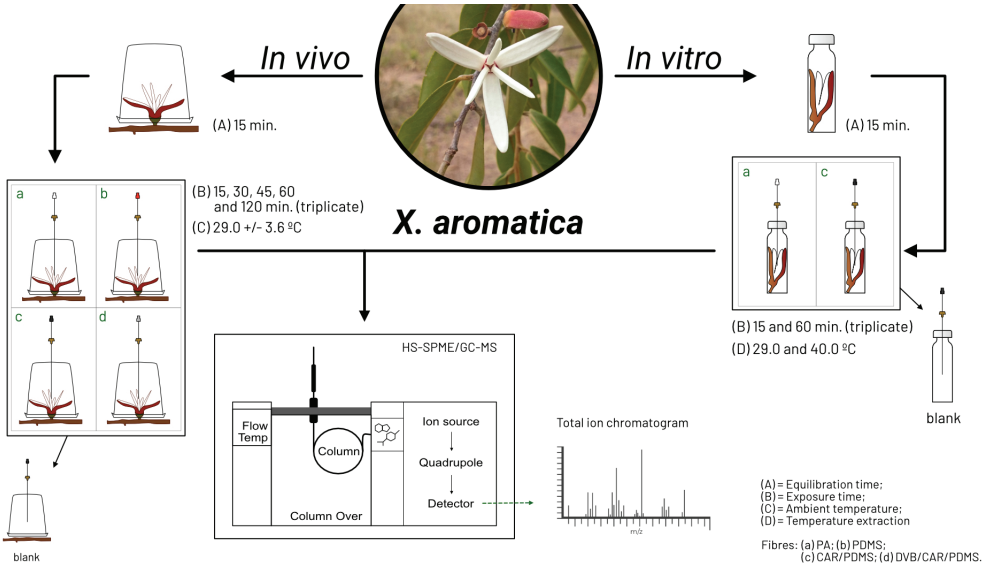

Figure 1. Steps of *in vivo* and *in vitro* experiments.

VOCs identification from *X. aromatica* flowers

The VOCs from *X. aromatica* flowers were identified based on the comparison of their mass spectra with those from the GC-MS data-base substances (NIST/EPA/NIH Mass Spectral Library, 2017).[17] Additionally, the retention indices (RI) of the compounds were compared with those reported in literature databases.[18-20] The RI values were determined by injecting a homologous series of *n*-alkanes (C_8-C_{30}) under the same operating conditions. Before injections in the GC-MS system, blank runs (with no sample) were carried out.

Multivariate analysis

The multivariate analyses of data sets obtained from *in vivo* and *in vitro* experiments were performed independently using Pirouette® software version 4.5.[21] Two distinct data matrices were created. These matrices were arbitrarily named **X** (n × m) for *in vivo* experiments and **Y** (n × m) for *in vitro* data, where n represents the number of chromatograms in each matrix and m is the 12.441 signals registered by the mass spectrometer during the 60 min of chromatograph run. Before the PCA, the raw data were normalized by the individual norm and mean-centered.

Results and Discussion

In vivo sampling of the flower scent of *X. aromatica*

In vivo sampling allows for obtaining a more representative floral scent metabolomic profile, since the specimen being investigated is in its habitat. In this way, this methodology eliminates the possibility of errors associated with the time taken to transport and store the sample, hence resulting in more precise, accurate and fast analytical data, besides its potential for allowing temporal and longitudinal studies.[22-24]

It is known that to guarantee the extraction of the largest possible number of VOCs, the variables need to be carefully investigated and optimized. Therefore, in this research, we decided to work with the fiber coating and extraction time variables simultaneously, while the extraction temperature was measured throughout all the experiments. Four different fibers (CAR/PDMS, DVB/CAR/PDMS, PA and PDMS) were evaluated during extraction times of 15, 30, 45, 60 and 120 min, with extraction temperatures of 29.0 ± 3.6 °C (Figure 2).

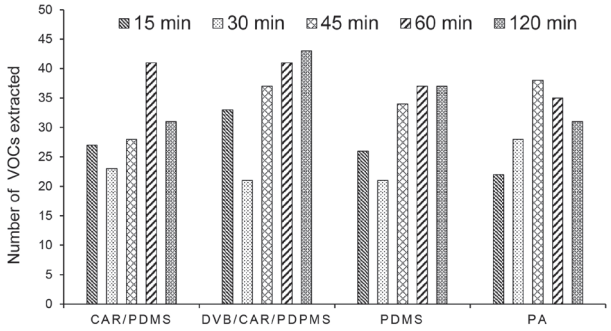

Figure 2. Effects of the extraction time and SPME fiber coating on the number of volatile compounds extracted from flowers of *X. aromatica* using *in vivo* experiments.

Our results showed that the times of 15, 30 and 45 min were not enough to extract the largest number of constituents of the volatile profile. However, when a longer extraction time (120 min) was used, the desorption of the

volatile constituents in most of the fibers resulted in a reduction in the number of VOCs. Therefore, the extraction time of 60 min was considered enough for the maximum extraction of volatile constituents from the flowers of *X. aromatica*.

As the number of compounds extracted was similar in the four different fibers, it was difficult to select a specific fiber. For this reason, to discriminate the VOCs investigated in the four fibers, the multivariate statistical method PCA was used.

PCA of the *in vivo* sampling

A PCA was performed to provide better visualization of VOCs data since this chemometric tool allows analyzing interrelationships between a large number of variables and explaining these variables in terms of their inherent dimensions (principal components, PCs).[25,26] The results of PCA for the data set obtained in the *in vivo* experiments are depicted in the score plot for the first and second principal components (Figure 3). Although PC1 and PC2 explained 56.8% out of the total variation of the data set, it was not possible to observe a clear separation in the chromatographic profiles for the four fibers. However, remarkable segregation of data in two groups was clearly perceived along the PC1 axis. The first group is composed of data from extractions performed using the PA and PDMS fibers, which were strongly correlated with variables (VOCs) that exhibit negative loadings for PC1. The second group is composed of data from extractions performed with CAR/PDMS and DVB/CAR/PDMS fibers, which were highly correlated with variables (VOCs) that exhibit positive loadings for PC1.

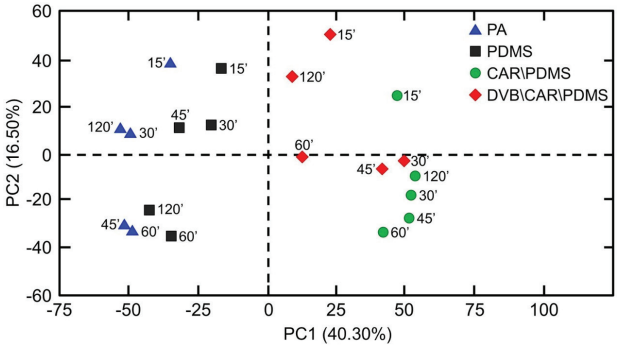

Figure 3. Scores plot of PCA for chromatograms obtained from *in vivo* experiments.

Beyond the molecular weight (MW) of the analytes extracted, the most probable reason to explain this separation of data can be the polarity of the VOCs associated with each fiber.

The extraction time of the samples also is believed to have interfered with the profile of the compounds. The influence of this experimental condition can be seen by the distance along the PC2 of the samples extracted by the same fiber at different exposure times. For PA, PDMS and CAR/PDMS fibers, the 15 and 60 min times were positioned at the horizontal ends, while the other times were included in this interval (Figure 3), proving that the 60 min time was the equilibrium for maximum extraction of volatile compounds. In other words, the temperature range for PC2 loadings was more influenced by exposure times between 15 and 60 min.

According to the PCA of the *in vivo* data, the PA and CAR/PDMS fibers were at the vertical ends, since both showed divergences in the extracted constituents. An example of this occurrence was observed with the CAR/PDMS fiber due to the extracted monoterpenes, unlike with the PA fiber.

To extract a significant number of different compounds, the PA and CAR/PDMS fibers were selected for the continuation of the experiments in the *in vitro* sampling, as together they provided a better evaluation of the floral scent. In addition, extraction times of 15 and 60 min were also selected in the extraction of VOCs for *in vitro* samples. Considering the multivariate approach of the data, the choice of fibers and the exposure times mentioned together leads to an experimental condition that allows for data collection along with all spaces of data variance provided by the four fibers measured in this study.

In vitro sampling of the flower scent of *X. aromatica*

In most of the scientific articles[13,16,27,28] in which the SPME technique is used, it is carried out in investigations with *in vitro* samples because of the facility in developing the entire analysis protocol in the laboratory. However, previous studies[29,30] have reported that the volatile composition of plants that have undergone some types of disturbances differ significantly from the live or undamaged specimen in response to biotic and/or abiotic stress. Hence, the impact of *X. aromatica* scent collection was assessed by comparing the volatile profiles obtained *in vivo* and *in vitro*.

The optimal extraction temperature in experiments of this nature is dependent on the matrixes used. Considering the temperature employed during the *in vivo* experiments (29.0 ± 3.6 °C) as well as the effect of the temperature increase, the *in vivo* extraction was evaluated at 29.0 and 40.0 °C and at the exposure times of 15 and 60 min in the PCA.

The amounts of compounds obtained at 29.0 and 40.0 °C of extraction times of 15 and 60 min are shown

(Figure 4) which indicated an increase in the extraction amounts over time leading to a greater number of VOCs being extracted at a temperature of 29.0 °C. The decrease in the number of VOCs at 40.0 °C may have occurred due to higher temperature coating headspace partition coefficients.[31] Consequently, there was less diffusion of the compounds in the coatings which directly influenced the retention capacity.

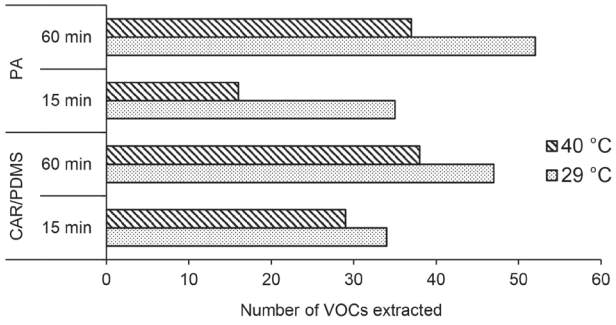

Figure 4. Effect of extraction temperature *in vitro* sampling of volatile compounds from *X. aromatica* flowers.

PCA of the *in vitro* sampling

To evaluate the temperature interference in the extraction of volatile compounds from the flowers of *X. aromatica*, a PCA was performed for *in vitro* samples of PA and CAR/PDMS fibers at selected extraction times of 15 and 60 min, which was extended to the PCA of the *in vivo* extraction, as previously mentioned. Thus, the scores plot obtained for PC1 and PC2 explained 62.10% of data variance (Figure 5). In the same way for the *in vivo* experiments, the segregation of samples in two groups is clearly obtained along the PC1.

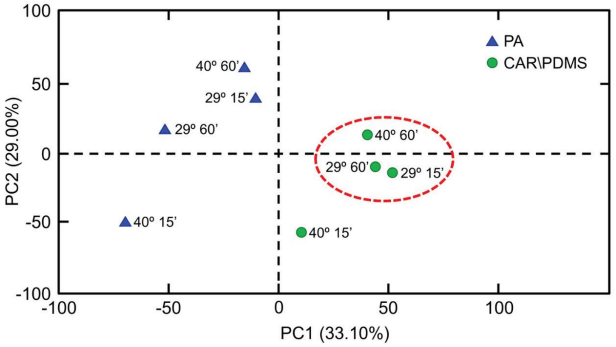

Figure 5. Scores plot of PCA for chromatograms obtained by evaluating the temperature from *in vitro* experiments.

As can easily be identified by the cluster highlighted (dashed line) in the scores plot shown in Figure 5 for the CAR/PDMS fiber, the variance of the data was small, except for the experiment performed at 15 min and 40 °C of exposure time and temperature, respectively. While for the PA fiber, the scores plot showed that the variance of the data was notably significant for the variables which also correlated with PC2.

Still, regarding the scores plot as with the case of PCA, the *in vitro* results obtained of significance in the sense that a simple and direct comparison against the data from Figure 4 may lead to erroneous conclusions. In Figure 4, the result is presented solely on quantitative data (number of detected and identified compounds), while on the other hand, the PCA analysis takes into account at the same time each signal and its respective intensities. This obviously denotes a quantitative influence on the analysis.

In vivo and *in vitro* composition of VOCs from *X. aromatica*

The HS-SPME optimized method (PA and CAR/PDMS fibers; 60 min and 29.0 °C extraction time and temperature, respectively) availed a total of 77 VOCs of which 48 were reported for the first time in the literature as constituents of the volatile profile of *X. aromatica* flowers. Table 1 summarizes the identification of the peaks of the *in vivo* and *in vitro* sampling, their relative retention times (RRt), RI values obtained by using an HP-5 MS column and comparisons to RI values from the literature.[18-20] Using *in vivo* and *in vitro* sampling, it was possible to extract 52 and 68 VOCs, respectively. Of these, nine (11.7%) and twenty-five (32.5%) were extracted exclusively by *in vivo* and *in vitro* sampling, respectively, while forty-three (55.8%) were extracted by both (Figure S1, Supplementary Information (SI) section).

Of the forty-three compounds common to both analyses, twenty-four ((*E*)-β-ocimene (**6**), terpinolene (**7**), indole (**15**), α-cubebene (**16**), α-copaene (**20**), β-cubebene (**22**), β-elemene (**23**), *trans*-caryophyllene (**24**), aromadendrene (**27**), α-humulene (**30**), 9-epi-(*E*)-caryophyllene (**31**), γ-muurolene (**35**), α-amorphene (**36**), valencene (**40**), α-muurolene (**41**), (*E*,*E*)-α-farnesene (**42**), δ-cadinene (**45**), zonarene (**46**), α-calacorene (**50**), spathulenol (**52**), 1-epi-cubenol (**60**), α-cadinol (**65**), pentadecan-2-one (**70**) and heptadecan-2-one (**76**)) were identified in previous studies in the leaves,[3,5,7,16,32-36] flowers,[5,16,37] fruits[16,27] and stems[32] of *X. aromatica*. It should be noted that spathulenol (**52**) has been reported in earlier research[3,5,7,16,27,32-37] in different parts of *X. aromatica* and can be considered a chemotaxonomic marker of this genera.[3,38]

The difference in the extraction efficiency of the volatile constituents may be related to the sizes of the vials used *in vivo* (250 mL) and *in vitro* (20 mL), as shown in Figure 1. Note that the equilibrium is more rapidly established in vials with reduced headspace volumes.[39] Thus, higher

Table 1. Composition *in vivo* and *in vitro* sampling of the VOCs from the floral scent of *X. aromatica* by HS-SPME

No.	RRt / min	RI[a]	RI[b]	Compound[c]	Fiber			
					PA		CAR/PDMS	
					In vivo	*In vitro*	*In vivo*	*In vitro*
1	7.46	987	990	β-pinene[17,18]	–	–	–	×
2	7.69	997	1002	myrcene[17,18]	–	×	–	–
3	8.62	1026	1029	*R*-limonene[d 17,18]	–	–	–	×
4	8.80	1032	1030	sylvestrene[17,18]	–	×	–	–
5	8.91	1035	1037	(*Z*)-β-ocimene[17,18]	–	–	–	×
6	9.36	1049	1050	(*E*)-β-ocimene[17,18]	–	×	×	×
7	10.81	1093	1088	terpinolene[17,18]	–	–	×	×
8	11.53	1115	1107	2-phenylethanol[17,18]	–	×	–	×
9	12.04	1130	1132	*allo*-ocimene[17,18]	–	–	×	×
10	12.46	1143	1144	neo-*allo*-ocimene[17,18]	–	–	×	×
11	15.44	1233	1229	nerol[17,18]	–	×	–	–
12	15.79	1244	1238	citronellol[17,18]	–	×	–	–
13	16.25	1258	1252	geraniol[17,18]	–	×	–	–
14	16.77	1274	1267	geranial[17,18]	–	×	–	–
15	17.65	1301	1291	indole[17,18]	×	×	×	×
16	19.37	1353	1348	α-cubebene[17,18]	×	×	×	×
17	19.83	1367	1361	neryl acetate[17,18]	–	×	–	–
18	19.89	1368	1371	cyclosativene[17,18]	–	–	–	×
19	20.05	1373	1375	α-ylangene[17,18]	–	×	×	×
20	20.21	1378	1376	α-copaene[17,18]	×	×	×	×
21	20.50	1387	1388	β-bourbonene[17,18]	×	–	×	–
22	20.67	1392	1388	β-cubebene[17,18]	–	–	×	×
23	20.72	1394	1390	β-elemene[d 17,18]	×	×	–	×
24	21.59	1422	1417	*trans*-caryophyllene[d 17,18]	×	×	×	×
25	21.89	1432	1432	β-copaene[17,18]	×	×	×	×
26	22.10	1438	1433	β-gurjunene[17,18]	–	×	–	–
27	22.38	1448	1441	aromadendrene[17,18]	×	×	–	–
28	22.43	1450	1450	*cis*-muurola-3,5-diene[17,18]	–	–	×	×
29	22.56	1454	1453	*trans*-muurola-3,5-diene[17,18]	×	×	×	×
30	22.66	1457	1454	α-humulene[d 17,18]	×	×	×	×
31	22.89	1465	1466	9-epi-(*E*)-caryophyllene[17,18]	×	×	×	×
32	22.96	1467	1466	*cis*-muurola-4(14),5-diene[17,18]	–	×	–	–
33	23.12	1473	1472	dauca-5,8-diene[17,18]	–	–	×	×
34	23.28	1478	1476	*trans*-cadina-1(6),4-diene[17,18]	×	×	×	×
35	23.39	1480	1479	γ-muurolene[17,18]	×	×	×	×
36	23.50	1484	1484	α-amorphene[17,18]	×	×	×	×
37	23.84	1496	1493	*trans*-muurola-4(14),5-diene[17,18]	×	×	×	×
38	23.91	1499	1496	tridecan-2-one[d 17,18]	–	×	–	–
39	23.93	1499	1495	*cis*-cadina-1,4-diene[17,18]	×	–	×	×
40	24.03	1502	1496	valencene[17,18]	–	×	×	–
41	24.08	1505	1500	α-muurolene[17,18]	×	×	×	×
42	24.30	1511	1505	(*E*,*E*)-α-farnesene[17,18]	–	×	×	×
43	24.50	1518	1512	δ-amorphene[17,18]	–	×	×	×
44	24.58	1520	1513	γ-cadinene[17,18]	–	×	–	×
45	24.62	1523	1523	δ-cadinene[17,18]	×	×	–	–
46	24.81	1529	1529	zonarene[17,18]	×	×	×	–
47	24.89	1531		n.i.[e]	×	×	×	×
48	25.06	1537	1534	*trans*-cadina-1,4-diene[17,18]	×	×	×	×
49	25.20	1542	1538	α-cadinene[17,18]	–	×	×	×
50	25.35	1546	1545	α-calacorene[17,18]	×	×	×	×
51	26.24	1576		n.i.[e]	×	–	–	–
52	26.32	1579	1578	spathulenol[17,18]	×	×	×	×
53	26.60	1588	1587	gleenol[17,18]	×	–	×	–

Table 1. Composition *in vivo* and *in vitro* sampling of the VOCs from the floral scent of *X. aromatica* by HS-SPME (cont.)

No.	RRt / min	RI[a]	RI[b]	Compound[c]	Fiber			
					PA		CAR/PDMS	
					In vivo	*In vitro*	*In vivo*	*In vitro*
54	26.64	1590		cadina-1(10),6,8-triene[f] [17,20]	–	–	×	×
55	26.88	1598	1597	tretadecan-2-one[17,19]	–	×	–	–
56	26.94	1600		α-patchoulene[g] [17,20]	–	–	–	×
57	27.29	1613	1607	β-oplopenone[17,18]	×	×	–	–
58	27.58	1623		n.i.[e]	–	–	–	×
59	27.66	1626	1623	α-corocalene[17,18]	–	–	×	–
60	27.82	1632	1628	1-epi-cubenol[17,18]	×	×	×	×
61	27.98	1638	1641	*allo*-aromadendrene epoxide[17,18]	×	–	–	–
62	27.99	1638		n.i.[e]	–	–	×	×
63	28.08	1642	1641	1,7-diepi-α-cedrenal[17,18]	×	–	–	–
64	28.19	1646	1646	α-muurolol[17,18]	×	×	×	×
65	28.57	1659	1654	α-cadinol[17,18]	–	×	×	×
66	28.69	1644		n.i.[e]	–	×	–	–
67	28.96	1674	1668	(6*Z*)-pentadecen-2-one[17,18]	–	×	–	–
68	29.13	1680	1676	cadalene[17,18]	–	–	×	×
69	29.46	1692	1686	germacra-4(15),5,10(14)-trien-1-α-ol[17,18]	×	–	–	–
70	29.72	1702	1697	pentadecan-2-one[d] [17,18]	×	×	×	×
71	30.86	1744	1740	oplopanone[17,18]	×	–	–	–
72	32.40	1800	1800	hexadecan-2-one[17,19]	–	×	–	–
73	32.63	1810	1803	14-hydroxy-δ-cadinene[17,18]	×	–	–	–
74	34.15	1871		(*Z*)-9,17-octadecadienal[h] [17,20]	–	×	–	–
75	34.30	1877		n.i.[e]	–	×	–	–
76	34.98	1904	1902	heptadecan-2-one[17,19]	×	×	–	–
77	39.64	2100	2100	*n*-heneicosane[17,18]	–	×	–	–
Total compounds identified *per* fiber					33	49	39	44
Classes of compounds from *in vivo* and *in vitro* sampling / %								
Monoterpenes					18.18			
Alcohols					1.30			
Aromatic heterocyclic					1.30			
Sesquiterpenes					61.04			
Hydrocarbons					1.30			
Ketones aliphatic					7.79			
Aldehyde aliphatic					1.30			
Unknown					7.79			

[a]Retention indices (RI) according to C_8-C_{30} *n*-alkanes on the HP-5 MS column; [b]obtained from the literature (Adams[18] or Rostad and Pereira[19]); [c]compound identification criteria, [d]identity confirmed by comparison of MS and retention time of commercial standard compounds, [e]not identified, NIST 17,[17] Adams,[18] Rostad and Pereira[19] and compound ID number (PubChem): [f]CID 518975; [g]CID 521710; [h]CID 5365667.[20] RRt: relative retention times; PA: polyacrylate; CAR/PDMS: carboxen/polydimethylsiloxane; ×: detected; –: not detected.

sensitivity was obtained in the *in vitro* experiments, as the space above the sample was kept smaller when compared with the *in vivo* experiments.

Apart from the processes of emission, other conditions are related and directly influence the number of these VOCs. For example, abiotic factors (light, radiation, solar, temperature, soil composition, water and others), plant or flowers age, different species and/or cultivars.[23] To maintain a homogeneous volatile profile, species with similar characteristics (aforementioned) should be sorted. Although in this study, different species of *X. aromatica* flowers were randomly sorted and used. Hence, this diversity of species could have influenced the VOCs reported here.

The composition of VOCs extracted *in vitro* was greater when compared with the *in vivo* procedure. This observation was attributed mainly to the nine new monoterpenes, (i.e., β-pinene (**1**), myrcene (**2**), *R*-limonene (**3**), sylvestrene (**4**), (*Z*)-β-ocimene (**5**), nerol (**11**), citronellol (**12**), geraniol (**13**) and geranial (**14**)) extracted. The same trend was observed for ketone hydrocarbons, where four new constituents (i.e., tridecan-2-one (**38**), tretadecan-2-one (**55**), (6*Z*)-pentadecen-2-one (**67**) and hexadecan-2-one (**72**)) were extracted. On the other hand, volatile constituents such as β-bourbonene (**21**), gleenol (**53**), α-corocalene (**59**), *allo*-aromadendrene epoxide (**61**), 1,7-diepi-α-cedrenal (**63**), germacra-

4(15),5,10(14)-trien-1-α-ol (**69**), oplopanone (**71**) and 14-hydroxy-δ-cadinene (**73**) were not observed in the *in vitro* sampling. This difference between the constituents extracted *in vivo* and *in vitro* may be connected with the fact that terpenoids easily undergo such reactions as oxidation, isomerization, cyclisation, or dehydrogenation reactions aided by the presence of enzymes or other chemicals. This is because VOCs are deprived of the protective compartmentalization provided in the plant matrix and are prone to oxidative damage or chemical transformations since these constituents under study are exposed to light, heat or air;[40] these conversion reactions may occur either when concentrated in the headspace or over time after the flower has been collected.[28]

The volatile compounds *cis*-muurola-4(14),5-diene (**32**), γ-cadinene (**44**) and *n*-heneicosane (**77**) were selective to DVB/CAR/PDMS and PDMS coatings in the *in vivo* procedure. Nevertheless, the combination of fiber optimization with the use of smaller vials made it possible to extract them even by using a different CAR/PDMS and PA fibers in the *in vitro* sampling, which demonstrates greater extraction efficiency.

In comparison with the previous study,[16] our analyses confirmed the importance of the extraction time and fiber selection for the VOCs extraction. Andrade *et al*.[16] identified 13 compounds in *X. aromatica* occurring in Northern Brazil using CAR/PDMS fiber and extraction time of 15 min. Similarly, this study identified 26 and 38 VOCs from extraction times of 15 and 60 min, respectively using the same fiber but from the West-Central region of the country.

In the current paper, sesquiterpenes represent 61.04% of the identified volatile constituents of *X. aromatica* flowers. Comparing the data reported of other extraction techniques, such as hydrodistillation[5] and dynamic headspace,[37] sesquiterpenes were also found to be the dominant volatile compounds in flowers. The above realization corroborates with the findings reported in this study.

Most studies[3,7,16,27] associated with the investigation of the volatile profile of the genus *Xylopia* have reported that its constituents are predominantly monoterpenes and sesquiterpenes. This same behavior is observed in our work, where both classes represent 79.22% of the total VOCs extracted.

The fact that the floral scent of *X. aromatica* flowers are mostly composed of terpenoids makes it the ideal species with great potential for exploration in the pharmaceutical, food and cosmetics industries, as these secondary metabolites are targets of several investigations in search of medicines, flavor enhancers and fragrances.[41]

Conclusions

In conclusion, this study provides the first and most comprehensive report of the volatile composition from the flowers of *X. aromatica* via *in vivo* and *in vitro* sampling. The use of HS-SPME and GC-MS techniques was efficient for the extraction and identification of the VOCs. A total of 77 VOCs was extracted from the floral scent, with 52 and 68 from *in vivo* and *in vitro* sampling, respectively, of which 48 were reported for the first time in the literature as volatile constituents from *X. aromatica* flowers. The PCA data was important to identify the most promising conditions of the experiments that led to the identification of the maximum number of VOCs. These conditions were the use of PA and CAR/PDMS fibers at as well as a 60 min extraction time and 29 °C temperature.

In addition, our study further revealed that there are volatile compositional scent differences between *in vivo* and *in vitro* sampling. Among the different classes of constituents identified, monoterpenes were the main ones responsible for such diversity.

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as a PDF file.

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Author Contributions

João G. M. Junqueira and Michelle N. G. do Nascimento were responsible for the conceptualization, methodology, validation, investigation, writing of original draft, review, editing and visualization; Lucas G. da Costa performed the formal analysis and visualization; Lincoln L. Romualdo was responsible for the conceptualization, methodology, resources, supervision, writing review and editing; Francisco W. B. de Aquino was responsible for the methodology, formal analysis, data curation, visualization, writing review and editing; Mustapha N. Abubakar carried out the writing review and editing; Ana P. Terezan and Gustavo

O. S. Cunha were responsible for the conceptualization, methodology, writing review and editing; Vanessa G. P. Severino performed the conceptualization, resources, supervision, project administration, funding acquisition and writing review and editing.

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