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ORIGINAL PAPER

Isolation and characterization of the volatile aroma compounds from the concrete headspace and the absolute of *Jasminum sambac* (L.) Ait. (Oleaceae) flowers grown in Egypt

Amr E. Edris · Remigus Chizzola · Chlodwig Franz

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Abstract The composition of the volatile fraction of Egyptian *Jasminum sambac* (L.) Ait. (Family: Oleaceae) flowers picked in July (the climax of the flowering season), was studied using GC/MS. The flowers' concrete headspace (HS) volatiles had been analyzed by SPME (solid phase microextraction) and compared with those from the flowers' absolute. The main volatile constituents of the concrete HS and the absolute, respectively, were: benzyl acetate (23.7 and 14.2%), indole (13.1 and 13.4%), E-E-α-farnesene (15.9 and 13.1%), Z-3-hexenyl benzoate (4.9 and 9.4%), benzyl alcohol (7.7 and 8.4%), linalool (10.6 and 6.3%), and methyl anthranilate (5.0 and 4.7%). The major volatile constituents of Egyptian *J. sambac* absolute are almost qualitatively similar but quantitatively different from those grown in other geographical regions. The proportion of some major volatile constituents at this investigation are also different from those reported in a previous investigation for Egyptian *J. sambac*. The study also revealed that, headspace-solid phase microextraction (HS-SPME), equipped with a polydimethylsiloxane (PDMS) fiber, can give an indication about the composition of some of the major volatile constituents of *J. sambac,* e.g., indole, methyl anthranilate and E-E-α-farnesene, directly from the concrete HS.

Keywords *Jasminum sambac* (L.) Ait. (Oleaceae) · Absolute · Concrete headspace · Volatile composition · HS-SPME · Geographical origin

Introduction

Jasminum sambac (L.) Ait. (Family: Oleaceae) is a shrub with white flowers which have characteristic volatile compounds differing from that of *Jasminum grandiflorum* L. The flower petals are thicker and waxier, and the stem of the shrub is thicker than that of *J. grandiflorum* L. [1]. In Egypt, the flowering period of *J. sambac* begins in April; the flowering reaches its climax in July and then declines in September. The buds are odorless but the flowers develop their characteristic odor during bloom at night. The fragrance of the flower is formed from glycosidic precursors by endogenous enzymatic hydrolysis just before the opening of the flowers [2]. Different glycosidic volatile precursors of *J. sambac* were previously investigated [3–5].

The flowers of *J. sambac* are used as flavor for tea leaves to provide a characteristic jasmine impact [6, 7]. *J. sambac* absolute and essential oil are extensively used in perfumery industry due to their fine, sweet and elegant fragrance impact. The fully bloomed flowers are extracted using organic solvents to get the concrete followed by ethanol extraction to obtain the absolute [8]. On the other hand, the essential oil is prepared using hydrodistillation [9], simultaneous steam distillation-solvent extraction [10] and super critical carbon dioxide extraction [11].

The volatile composition of *J. sambac* absolute varies depending on the environmental conditions and the agricultural practices [12–14]. The major and the minor volatile constituents of *J. sambac* absolute and essential oil were previously studied [11, 15–17]. The volatile composition of

A. E. Edris (✉)
Aroma and Flavor Chemistry Department,
National Research Center, Dokki, 12622 Cairo, Egypt
e-mail: amr_edris@hotmail.com

R. Chizzola · C. Franz
Institute of Applied Botany and Pharmacognosy,
Department of Veterinary Public Health,
University of Veterinary Medicine,
Veterinärplatz 1, 1210 Vienna, Austria

the headspace collected directly from the flowers [18, 19] as well as from the concrete [15] was also reported.

Since the early study of Chaput et al. [20], no investigators had reported the volatile composition of *J. sambac* absolute from flowers grown in Egypt. Moreover, unlike the investigation of Chaput et al., who started with a commercial concrete sample imported from Egypt, the current study re-investigates the composition of the absolute as well as the concrete HS of *J. sambac* flowers picked at a specific period of the flowering season (July). This period is considered to be the climax of the flowering season, which starts at April and ends late at September. Correlating volatiles' composition to a specific period of the flowering season will give a more realistic volatile profile to the absolute of *J. sambac*. From our point of view, this profile may not be the same from April to September due to the change in the environmental conditions along that period (e.g., temperature and day light intervals). So we started this study on July crop as a beginning, aiming for further evaluations all around the flowering season.

Also in this investigation, the headspace-solid phase microextraction (HS-SPME) technique using a polydimethylsiloxane (PDMS) fiber, was applied for the first time on the concrete, to examine the possibility of determining some of the major volatile components of *J. sambac* directly from the concrete headspace. Previous investigation [15] did not use this technique in their evaluation of concrete HS.

Materials and methods

Plant materials

Buds of *J. sambac* (L.) Ait, were picked early in the morning of the first week of July 2005, (the climax of the flowering season). The buds were collected from different shrubs, which are genetically homogenous and equally distributed over an area of about one and half Feddan (approx. 6,100 m^2) in a farm 20 km to the South of Cairo, Egypt. A plant sample including flowers was identified by a taxonomist affiliated to the herbarium of National Research Center, Cairo, Egypt, in which a voucher specimen had been deposited.

Chemicals

Five liters of *n*-hexane (98% pure) were re-purified by fractional distillation using a 30 cm long, 2 cm internal diameter, fractionating column filled with raching rings. The distilled solvent was kept under anhydrous sodium sulfate for 24 h, then filtered and used for the preparation of the concrete.

Concrete extraction

The closed buds of *J. sambac* (700 g), were spread in a thin layer over linen sheets on the ground and kept in shade until they fully bloomed at night. Then the flowers were soaked in five liters of distilled n-hexane with gentle stirring for 60 min, which were found appropriate period from a previous preliminary experiment. Afterwards, the hexane extract was decanted, dried over anhydrous sodium sulfate, filtered and the solvent was evaporated at 40 °C under reduced pressure (350 mbar). A yellowish-brown viscose concrete (2.2 g) was obtained which solidified on refrigeration.

Absolute extraction

The absolute was extracted from the concrete according to the method of Tamogami et al. [21]. The concrete was extracted with 5 ml of 95% (w/v) ethanol by stirring at room temperature for 5 min. The mixture was cooled to −20 °C and filtered. The extraction process was repeated twice with the concrete residue. Finally, the combined filtrates were concentrated under a gentle nitrogen stream to get the *J. sambac* absolute (0.94 g).

Collection of volatiles from the concrete HS using HS-SPME

Jasminum sambac concrete was spread in 100 ml bottle which was hermetically sealed with a parafilm sheet. The bottle was transferred into a thermostated water bath at 30 ± 2 °C for 60 min to equilibrate. This time span was found to be appropriate for the sample headspace to equilibrate, as confirmed by three successive GC–MS analyses. A manual SPME holder with a 1-cm fiber assembly (Supelco, Bellefonte, PA, USA) coated with a 100-μm layer of polydimethylsiloxane (PDMS) was used for the volatile collection from the headspace of the bottle. This type of fiber is reported to adsorb very effectively a wide range of polar and nonpolar analytes [22], so that no other fiber coatings were tried in this investigation.

Prior to volatile collection, the fiber was conditioned for 1 h in the injector port of the gas chromatograph at 250 °C. Then, the fiber was exposed to the equilibrated headspace of the sample bottle by puncturing through the parafilm sheet barrier at a constant depth for 15 min. This time interval was found to be enough for impregnating the fiber without overloading it.

HS-SPME volatile desorption and GC–MS analysis

The SPME fiber was immediately placed and left for 10 min in the injector port (250 °C) of an HP 6,890 gas chromatograph coupled with the HP 5972 MS quadrupole mass selective detector. The injector port was equipped with a 0.75 mm i.d. splitless glass liner, the injection took place in the splitless mode. The desorbed volatiles were separated on Restek (Bellefonte, PA, USA) capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness) coated with RTX-5 MS phase. The condition of analysis was: initial temperature 60 °C for 2 min, final temperature 200 °C reached at a rate of 2 °C/min, final temperature hold 10 min, the injector temperature was 250 °C. Helium was used as a carrier gas at a rate of 1.2 ml/min with an average velocity of 40 cm/s, operated in the constant flow mode. The interface to the mass selective detector was set at 280 °C. The scanning of the mass fragments was conducted from 40 to 350 mass units.

GC–MS analysis of the absolute

One microliter of the absolute was diluted with 300 µl dichloromethane and 1 µl of this mixture was injected into GC–MS with a splite ration 1:10. The conditions for the separation were the same as for the headspace analysis. The mean area percentages of the individual peaks were calculated based on the total ion current from three analyses of both the concrete HS and the absolute. The volatile compounds were identified according to their mass spectra (NIST 1994 and Mc Lafferty 1989) and their retention indices [23]. In addition, the available authentic samples (Aldrich Chemical Co.) were used to confirm the identity of some components.

Statistical analysis

For the statistical analysis of the data MSTAT software ver. 1.41 (Knowledge Dynamics Corp., Texas, USA) was used, to test whether there are significant differences between the volatile composition in the concrete HS and the absolute.

Results and discussion

Table 1 shows the volatile composition of *J. sambac* concrete HS and the absolute for flowers picked at July. The volatiles profile of July flowers is characterized by the presence of seven major components in both the concrete HS and the absolute, respectively, including: benzyl acetate (23.7 and 14.2%), indole (13.1 and 13.4%), E-E-α-farnesene (15.9 and 13.1%), Z-3-hexenyl benzoate (4.9 and 9.4%), benzyl alcohol (7.7 and 8.4%), linalool (10.6 and 6.3%), and methyl anthranilate (5.0 and 4.7%). It is not surprising that the percentage of most components in the concrete HS is significantly different from that of the absolute (Table 1). This is due to the high proportion of the more volatile compounds in the concrete HS and the differential adsorption of components on the SPME fiber coating [24].

The major constituents, indole, methyl anthranilate and E-E-α-farnesene are an exception, in which there was no significant difference between their proportion in the concrete HS (at equilibrium) and the absolute. That was previously reported for Chinese *J. sambac* in which indole and methyl anthranilate were also found in comparable proportions in the concrete HS and in the absolute [17].

Esters are the main volatile constituents of *J. sambac* concrete HS and absolute (about 37%), taking in consideration that the PDMS fiber-coating material used in this investigation was reported to give the best recovery for esters [25]. Benzyl acetate, with a strong jasmine character, along with Z-3-hexenyl benzoate, are the major volatile esters of *J. sambac* absolute and concrete HS. Methyl anthranilate with orange blossoms character is the only nitrogen-containing ester detected. Unlike the other publications, we did not detect dimethyl anthranilate.

Alcohols represent about 35% of the concrete HS and the absolute, with a high contribution of indole followed by linalool. Indole is formed as a natural decomposition product of nitrogenous compounds (specially amino acids), which takes place in the cut-off flowers [26]. Thus, the time elapsed between the collection of the buds and the extraction of the concrete may contribute to the proportion of indole in the concrete and consequently in the absolute.

It is worth mentioning that, *R*-(−)-linalool is the predominant and the only enantiomer in *J. sambac* flowers [6],which differentiates it from *J. grandiflorum* flowers in which the *S*-(+)-linalool is the predominant enantiomer, while the *R*-(−)-linalool is minor[21].

(−)-Germacra-1,6-dien-4-ol (1.1 and 2.3%, for concrete HS and absolute, respectively), is also a key component differentiating *J. sambac* volatiles from *J. grandiflorum,* where it is completely absent [15]. In the present study, the content of this alcohol in the absolute is noticeably low (2.3%) compared with the Indonesian origin flowers (20.0%) [15]. That should affect the whole olfactoric perception of both flowers.

Hydrocarbons comprised about 18.0% of the concrete HS and the absolute composition. E,E-α-farnesene is the major sesquiterpene hydrocarbon. This component can be oxidized at atmospheric conditions into conjugated trienols, which can cause injury and death to cell membrane. This phenomena is known as “scald disorder” which can happen to farnesene-rich fruits [27]. Thus, oxidation of α-farnesene may also be responsible for the high perishability and appearance of scalding symptoms of *J. sambac* flowers if left without extraction overnight. However, no investigations were conducted so far to detect the presence of the conjugated trienols in *J. sambac* scald flowers’ absolute.

Table 1 Volatile constituents of *Jasminum sambac* concrete HS and the absolute, for flower picked in July

Volatile component	RI	Mean peak area % (from GC–MS data)[a]		*T* value at 0.5%
		HS-SPME[b]	Absolute	
Z-3-hexenol[c]	841	0.2 ± 0.03	ND due to solvent delay	–
Benzaldehyde[c]	960	0.1 ± 0.008	ND due to solvent delay	–
Z-3-hexenyl acetate[c]	1,006	1.3 ± 0.15	0.7 ± 0.03	**
Benzyl alcohol[c]	1,043	7.7 ± 0.17	8.4 ± 0.22	*
Z-linalool oxide[f]	1,073	0.5 ± 0.04	0.2 ± 0.005	*
Methyl benzoate[c]	1,095	1.3 ± 0.09	0.9 ± 0.03	*
Linalool[c]	1,107	10.6 ± 0.20	6.3 ± 0.15	**
Phenyl ethyl alcohol[c]	1,117	1.9 ± 0.05	1.7 ± 0.03	NS
Benzyl nitrile[f]	1,145	0.6 ± 0.04	0.3 ± 0.02	**
Benzyl acetate[c]	1,174	23.7 ± 0.19	14.2 ± 0.23	**
Epoxylinalool[f]	1,179	0.1 ± 0.007	0.1 ± 0.01	NS
Methyl salicylate[c]	1,192	0.4 ± 0.02	0.2 ± 0.008	*
syn-phenylacetaldoxime[d]	1,253	1.6 ± 0.08	1.8 ± 0.10	*
2-Phenylethyl acetate[c]	1,257	1.0 ± 0.12	0.9 ± 0.15	NS
anti-phenylacetaldoxime[e]	1,278	1.4 ± 0.1	2.6 ± 0.23	**
Indole[c]	1,297	13.1 ± 0.17	13.4 ± 0.14	NS
Cinammyl alcohol[c]	1,306	0.1 ± 0.01	0.3 ± 0.04	**
Methyl anthranilate[c]	1,343	5.0 ± 0.15	4.7 ± 0.10	NS
8-Hydroxylinalool[f]	1,366	ND	0.1 ± 0.007	**
β-Elemene[f]	1,385	0.2 ± 0.02	0.1 ± 0.03	**
β-E-caryphyllene[f]	1,411	0.1 ± 0.01	0.1 ± 0.02	NS
α-Humulene[f]	1,448	0.1 ± 0.01	0.1 ± 0.007	**
Germacrene-D[f]	1,474	0.7 ± 0.03	0.5 ± 0.01	*
Bicyclogermacrene[f]	1,487	0.3 ± 0.01	0.2 ± 0.01	*
Z-E-α-farnesene[f]	1,490	0.3 ± 0.04	0.1 ± 0.01	**
E-E-α-farnesene[f]	1,509	15.9 ± 0.20	13.1 ± 0.13	NS
δ-cadenine[f]	1,515	0.3 ± 0.04	0.2 ± 0.01	*
E-nerolidole[f]	1,562	0.2 ± 0.007	0.5 ± 0.03	**
Z-3-hexenyl benzoate	1,571	4.9 ± 0.21	9.4 ± 0.12	**
Germacra-diene-4-ol[f]	1,572	1.1 ± 0.08	2.3 ± 0.14	*
E-3-hexenyl benzoate[f]	1,585	0.04 ± 0.004	0.1 ± 0.01	NS
α-cadinol[f]	1,648	0.03 ± 0.002	0.1 ± 0.07	**
Farnesol[f,g]	1,713	ND	0.2 ± 0.02	**
Geranyl linalool[f]	1,747	0.2 ± 0.03	1.7 ± 0.05	**
Benzyl benzoate[c]	1,760	0.1 ± 0.01	0.6 ± 0.04	**
Farnesyl acetate[f,g]	1,831	ND	0.2 ± 0.01	**
B-phenylethyl benzoate[c]	1,844	0.02 ± 0.002	0.3 ± 0.02	**
Benzyl salicylate[f]	1,857	ND	0.1 ± 0.09	**
Methyl palmitate[c]	1,926	0.02 ± 0.002	0.6 ± 0.04	**
Methyl linoleate[c]	2,089	0.04 ± 0.003	0.4 ± 0.07	**
Methyl linolenate[c]	2,098	0.1 ± 0.01	3.9 ± 0.15	**
Methyl stearate[c]	2,126	ND	0.3 ± 0.03	**
Z-11-tricosene[f]	2,161	0.1 ± 0.02	3.5 ± 0.19	**
Total esters		37.9 ± 0.96	37.3 ± 1.13	
Total alcohols		35.7 ± 0.76	35.3 ± 0.90	
Total hydrocarbons		18.0 ± 0.38	17.8 ± 0.42	
Other components		3.7 ± 0.27	4.8 ± 0.35	

NS non-significant, *ND* not detected

* Significant, ** highly significant

[a] The data reported are mean values of three determinations ($n = 3$) ± SD

[b] HS-SPME was conducted for the concrete HS of *J. sambac*

[c] Authentic sample was injected for more confirmation of component identity

[d] Newly and tentatively identified by MS: *m*/*z*: 91(100%), 117(69%), 90(43.4%), 118(27.3%), 135(26.7%), 65(25.2%), 89(20.7%), 51(14.1%), 63(12.2%), 92(10.5%)

[e] Newly and tentatively identified by MS: *m*/*z*: 91(100%), 117(78.6%), 90(60.1%), 65(29.0%), 89(27.0%), 135(24.4%), 118(19.3%), 51(15.1%), 63(13.9%), 92(10.7%). Both d and e are tentatively identified by MS only because their reference Kovats index and their authentic samples are not available to us

[f] Tentatively identified by MS, however, these compounds were previously reported by other investigators among the volatiles of *J. sambac*

[g] Correct isomer is not specified

Beside the previous components, this investigation also revealed the presence of some other nitrogen-containing volatiles. These include (*syn*- and *anti*-)-phenylacetaldoxime (3.0 and 4.4%) and benzyl nitrile (0.6 and 0.3%), for the concrete HS and the absolute, respectively. These two components are bioconversion products of the amino acid L-phenylalanine [28, 29]. To our knowledge, previous literature did not mention phenylacetaldoxime as a constituent in *J. sambac* absolute. Thus in this investigation, we reported the tentative identification of the *syn*- and *anti*-isomers of this component by mass spectroscopy, taking into consideration their possible interconversion during the GC analysis [30]. Phenylacetaldoxime's odor is reminiscent of phenylacetaldehyde with orange blossom notes over 10 ppm [31].

Benzyl nitrile was previously identified in Egyptian *J. sambac* absolute [20]. It can also be formed as an artifact due to the dehydration of phenylacetaldoxime at the injector port of the GC due to the high temperature.

Table 2 shows the absolute composition of the seven major volatile constituents of *J. sambac* from different geographical regions. In the current investigation, benzyl alcohol and indole contents, were higher than those from other regions, while linalool content was the lowest. The high indole content (13.4%) indicates that *J. sambac* absolute in this investigation belongs to the "indole-rich" type compared with Egyptian-2, Chinese, Indonesian and Philippine, which belong to "indole-poor" type [15]. The other major components reported in the current investigation were in the same average range as in the absolutes from other geographical regions.

In spite of belonging to the same geographical region, a noticeable variation was detected in the proportion of benzyle acetate, indole, and benzyl alcohol between July flowers' absolute in the current investigation and those from the previous Egyptian-2 investigation [20]. This variation could be, in part, attributed to the difference in the time period at which the flowers were picked. In Egyptian-2 investigation, Chaput et al. [20] used flowers' concrete of unknown harvesting date. The variation of temperature and daylight intervals throughout the flowering season (April–September) can participate to a great extent to rationalize this variation in the absolute composition, keeping in consideration the possible contribution of other factors like agronomic treatments, age of the plants and genetic diversity.

Conclusion

The study of the volatile composition of the absolute isolated from Egyptian *J. sambac* flowers picked in July indicated that it almost has a common qualitative pattern of components as those from other regions. However, its quantitative composition differs considerably depending, partly, on the geographical environment and/or the season of harvesting the flowers. The study also revealed that, HS-SPME, can give a preliminary indication, (at equilibrium), about the composition of some major volatile components of *J. sambac* directly from concrete HS, e.g., indole, methyl anthranilate and E-E-α-farnesene. With this technique one can also judge whether a concrete sample is derived from indole-rich or indole-poor flowers, which will be of interest to quality control labs.

Finally, future studies are of interest to us including the continuous investigation of the absolute volatile composition all around the flowering season (April–September) to determine the quantitative and/or qualitative change of volatile composition along that period. That will help to determine the optimum harvesting time for flowers that possess a maximum composition of the major volatile components. In addition, investigating the role of oxidation of E-E-α-farnesene in the development of the scald symptoms to *J. sambac* flowers is also of our future interest.

Table 2 Composition of the major volatile components of *Jasminum sambac* absolute from different geographical regions

	Average area %							Reference
	Benzyl acetate[a]	Indole[b]	E-E-α-farnesene[f]	Z-3-hexenyl benzoate[f]	Benzyl alcohol[c]	Linalool[d]	Methyl anthranilate[e]	
Current investigation	14.2	13.4	13.1	9.6	8.4	6.3	4.73	–
Egyptian-2	21.2	Trace	12.4	2.5	1.1	7.4	4.90	[20]
Chinese	8.0	1.5	10.0	10.0	5.0	20.0	6.0	[15]
Indonesian	3.0	0.1	10.0–15.0	5.0	3.0	20.0–30.0	3.0	[15]
Philippine	2.0	1.0–2.0	15.0–20.0	2.0	0.1	15.0–20.0	5.0	[15]

For current investigation the area % was reported from GC–MS quantification and for others the area % was reported from GC–FID quantification. To compensate for the different responses between FID and MS, a standard mixture composed of components a, b, c, d, and e was injected into GC–MS and GC–FID simultaneously. The corrected area % of these components which is equivalent to FID response was found to be (a) 13.8%, (b) 13.5%, (c) 11.0 %, (d) 4.6% and (e) 4.41%

[f] Tentatively identified by MS, however, these compounds were previously reported by other investigators among the volatiles of *J. sambac*

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