

**IDENTIFICATION OF VOLATILE COMPONENTS OF FLOWERS OF
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Huangshan City, Anhui Province, 245041, P.R. China**Keywords: Zephyranthes candida, Volatiles, HS-SPME, GC-MS***Abstract**

The volatile components of *Zephyranthes candida* flowers were extracted using headspace-solid phase micro-extraction (HS-SPME) technique and then were analyzed by gas chromatography-mass spectrometry (GC-MS) for the first time. The major compounds of volatile extract were found to be 1-hexanol (15.4%), methyl heptanoate (12.8%), hexyl acetate (4.9%), 2-acetyl pyrrole (3.6%), methyl- α -D-rhamnopyranoside (3.0%), phenethyl alcohol (2.8%), heptanoic acid (2.7%), and 1-octanol (2.1%).

Amaryllidaceous plants recorded in the Bible as well-established treatments for cancer and used by the Greek physicians of the fourth century BC (Pettit *et al.* 1986) comprise about 85 genera and 1100 species that are distributed widely in tropical regions of the world (Luo *et al.* 2012). More than 500 Amaryllidaceae alkaloids exhibiting antitumor, analgesic, cytotoxic activities have been isolated and aroused great interest among the researchers in a wide range of biological fields. The genus *Zephyranthes* (Amaryllidaceae), native to the Western Hemisphere and mainly distributed in the warm-temperate regions of Western Hemisphere, is a large genus comprising about 70 species of which 2 species, *Z. candida* and *Z. carinata* have been recognized in China. Plants of the genus *Zephyranthes* reported to be rich sources of Amaryllidaceae alkaloids are well known in many countries for their pharmacological activities and are widely used in folk medicine (Wu *et al.* 2010). *Z. candida* native to South America is a bulbous herb. The plant with narrow and deep glossy green leaves bearing white flowers in fall is widely cultivated as an ornamental plant and is also used as medicinal plant to treat infantile convulsions, epilepsy, and tetanus in China. The leaves of *Z. candida* have been employed as a treatment for diabetes mellitus in Africa and the bulbs provided an extract that displayed antitumour and cytostatic activity (Pettit *et al.* 1990). Alkaloids are the principal active constituents of *Z. candida* and many kinds of Amaryllidaceae alkaloids such as haemanthamine, tazettine, 2-hydroxyalbomaculine, O-methylnerinine, 6 β -hydroxyhippeastidine were isolated from the leaves, bulbs or the whole parts of *Z. candida* (Nanase *et al.* 2014). Further more, previous phytochemical studies on *Z. candida* led to the isolation of flavonoid glycoside, ceramides, and sterol (Wu *et al.* 2008, Wu *et al.* 2009).

There is only one report on the essential oil of *Z. candida* (Bi *et al.* 2015). In a previous paper, the chemical constituents and free radical scavenging capacity of essential oil of *Z. candida* flowers from China obtained by hydrodistillation in a cleverger-type apparatus. The results showed that fatty acid, terpenes, and aldehydes were found to be dominant components in the essential oil exhibiting significant scavenging capacity against DPPH and ABTS free radical (Bi *et al.* 2015). In the present study, the volatile components from *Z. candida* flowers were extracted using headspace-solid phase micro-extraction (HS-SPME) technique for the first time and then were analyzed by gas chromatography-mass spectrometry (GC-MS).

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The white flowers of *Z. candida* were collected from Huangshan City, Anhui Province, P. R. China in October 2018. Identification was made by Associate Prof. Hui-chong Zhang, taxonomist at College of Life and Environment Science of Huangshan University, where a voucher specimen (HS1806) had been deposited. The flowers air-dried at 40 °C were ground to a fine powder and passed through a 20 mesh sieve.

Volatile materials were extracted using a manual SPME holder together with 5 ml head space vials sealed with polytetrafluoroethylene septum cap and 100 µm PDMS fibers (Supelco Inc.; Bellefonte, USA) aged for 30 min at 250°C. 1.5 g samples were put into 5 ml head space vial. The aged PDMS fiber was exposed in the upper space of the sealed vial at 70°C to adsorb the volatiles. After 40 min exposure, the fiber was withdrawn and immediately inserted into the GC injection port at 250°C for desorption of the volatile materials for 4 min.

Table 1. Volatiles of *Z. candida* flowers.

No.	Compounds	RI*	Percentage	Identification
1	1-Hexanol	867	15.4	MS, RI, Authentic compound
2	Heptaldehyde	903	1.6	MS, RI
3	Methyl hexanoate	924	1.1	MS, RI
4	Benzaldehyde	959	1.1	MS, RI
5	1-Heptanol	972	1.9	MS, RI
6	Hexyl acetate	1013	4.9	MS, RI, Authentic compound
7	Methyl heptanoate	1026	12.8	MS, RI, Authentic compound
8	Phenylacetaldehyde	1040	0.5	MS, RI
9	4-Hexanolide	1048	0.4	MS, RI
10	2-Acetyl pyrrole	1069	3.6	MS, RI
11	1-Octanol	1075	2.1	MS, RI
12	2,4-Dimethyl styrene	1091	1.4	MS, RI
13	Ethyl heptanoate	1099	1.8	MS, RI
14	Phenethyl alcohol	1112	2.8	MS, RI, Authentic compound
15	Heptanoic acid	1119	2.7	MS, RI
16	Methyl- α -D-rhamnopyranoside	1125	3.0	MS, RI
17	2-Camphanone	1146	0.3	MS, RI
18	Menthone	1155	0.2	MS, RI
19	<i>Trans,trans</i> -1,3,5-heptatriene	1171	0.8	MS, RI
20	1-Terpinen-4-ol	1179	0.3	MS, RI
21	Methyl-m-tolyl ketone	1182	0.2	MS, RI
22	1,3-Cycloheptadiene	1196	1.0	MS, RI
23	2,3-d\Hydro-2,2,6-trimethyl-benzalhyde	1198	0.7	MS, RI
24	Dodecane	1201	0.2	MS, RI
25	Methyl cyclohexane	1213	0.5	MS, RI
26	Methyl nonanoate	1225	0.4	MS, RI
27	Citronellol	1229	0.2	MS, RI
28	Hexyl-2-methyl-butanoate	1237	0.5	MS, RI
29	3-Methylbutyric acid hexyl ester	1244	0.3	MS, RI
30	<i>N</i> -Methylformanilide	1274	0.1	MS, RI
31	<i>L</i> -borneol acetate	1283	0.1	MS, RI
32	Safrole	1289	0.1	MS, RI

(Contd.)

No.	Compounds	RI*	Percentage	Identification
33	Methyl geraniate	1322	0.2	MS, RI
34	Methyl caprate	1326	1.8	MS, RI
35	3-Allylguaiacol	1350	0.1	MS, RI
36	Heptanoic acid, 3-methylbutyl ester	1352	0.1	MS, RI
37	Decanoic acid	1370	0.3	MS, RI
38	Copaene	1372	0.3	MS, RI
39	β -Elemen	1389	0.3	MS, RI
40	Ethyl caprate	1398	0.2	MS, RI
41	Methyl eugenol	1402	0.2	MS, RI
42	<i>A</i> -cedrene	1413	0.3	MS, RI
43	1-Caryophyllene	1416	0.5	MS, RI
44	<i>A</i> -ionone	1420	0.3	MS, RI
45	<i>Cis, trans</i> - α -farnesene	1432	0.1	MS, RI
46	1-(1,5-Dimethylhexyl)-4-methylbenzene	1447	0.2	MS, RI
47	<i>Z,Z,Z</i> -1,4,7,-cycloundecatriene, 1,5,9,9-tetramethyl	1455	0.2	MS, RI
48	β -ionone	1479	0.2	MS, RI
49	Curcumene	1481	1.3	MS, RI
50	Methyl isoeugenol	1497	0.2	MS, RI
51	1-Calamenene	1520	0.4	MS, RI
52	Methyl laurate	1528	0.1	MS, RI
53	2-Pentyl-2-nonenal	1565	0.2	MS, RI
54	Cadalin	1671	0.2	MS, RI
	Total identified		70.7	

* RI means retention indices relative to C₅–C₃₀ on the HP-5 MS column.

GC-MS analysis was carried out using a gas chromatograph (GC, model 7890N; Agilent Technologies, Palo Alto, CA, USA) equipped with a mass selective detector spectrometer (MS, Agilent 5975). Volatile substances were separated on a HP-5 MS column (5% phenyl methyl Silox, 30 m, 0.25 mm i.d.; 0.25 μ m film thickness). The temperature program was as follows: the initial column temperature 50°C, held for 3 min, and then programmed to 170°C at a rate of 2.5°C per min and held for 3 min; finally programmed to 250°C at a rate of 6°C per min, then held at 250°C for 10 min. The carrier gas was helium at 1 ml/min without split. The MS operating parameters were as follows: electron impact (EI) mode for molecular ionisation with a voltage of 70 eV; mass scan range of m/z 40-500 with full scan. Retention indices were calculated using the retention times of C₇–C₄₀ n-alkanes that were injected under the same chromatographic conditions. Identification of the volatile compounds were done by the interactive combination of MS data consisting of computer matching with NIST 98 libraries, IR, and MS analysis. The volatile compounds of *Z. candida* flowers and their percentages are presented in Table 1. Fifty-four compounds were identified, corresponding to 70.7% of the total composition. The major volatile matters of *Z. candida* flowers were esters (24.7%), alcohols (22.3%), and terpenes (5.3%) including five monoterpenes (1.7%) and nine sesquiterpenes (3.6%), while the major constituents of essential oil of *Z. candida* fresh flowers were fatty acids (22.05%), erpenes (13.53%), alkanes (13.19%), and aldehydes (6.93%) (Bi *et al.* 2015).

In the present investigation, the main components of the volatiles of *Z. candida* flowers were identified to be 1-hexanol (15.4%), methyl heptanoate (12.8%), hexyl acetate (4.9%), 2-acetyl pyrrole (3.6%), methyl- α -D-rhamnopyranoside (3.0%), phenethyl alcohol (2.8%), heptanoic acid (2.7%), and 1-octanol (2.1%), which together represented for 47.3% of the total composition. Many volatile compounds of *Z. candida* flowers such as benzaldehyde, phenylacetaldehyde, methyl hexanoate, 2-acetyl pyrrole, menthone, and β -ionone were widely used in flavor and food industry.

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