

## Comparative Analysis of Floral Scent Components Among Different *Osmanthus fragrans* Cultivars in Guilin Region (Postprint)

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### Abstract

Headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS) was employed to detect and analyze the petal volatile components of 12 osmanthus cultivars from the Guilin region, aiming to investigate the differences in floral scent composition among different osmanthus varieties in this area. The results showed that a total of 49 volatile components were detected, including 31 terpenoids, 10 fatty acids and their derivatives, 4 benzenoids, and 4 nitrogen-containing compounds, with terpenoids being the most abundant across all cultivar groups and even individual cultivars, accounting for 82.28%~94.83% of the total relative content. All osmanthus cultivars contained six floral scent components including trans- $\alpha$ -ocimene, but different varieties also possessed different components or different relative contents; for example, 'Xiangye Zhusha' lacked  $\alpha$ -ionone, while 'Longhuai Jingui' had the highest  $\alpha$ -ionone content (34.89%). Simultaneously, the main aroma components and their contents varied among cultivars; for instance, the primary aroma components of 'Longhuai Jingui' included five compounds such as  $\alpha$ -ionone, those of 'Yuetang Jingui' comprised eight compounds including  $\alpha$ -ionone, while 'Xiangye Zhusha' featured six compounds such as cis-linalool oxide. A total of 11 aroma-active compounds were identified, of which 10 belonged to terpenoids. 'Longhuai Jingui' exhibited the highest total content of aroma-active compounds (82.99%), along with the highest contents of ionone- and ocimene-type active substances; 'Xiangye Zhusha' and 'Tianxiang Taige' contained the highest levels of linalool-type active compounds (approximately 60%). In summary, terpenoid compounds constitute the main aroma components of osmanthus in the Guilin region; different osmanthus cultivars contain both common and distinct aroma components; 'Longhuai Jingui' is suitable for developing ocimene- and ionone-type products, while

'Xiangye Zhusha' and 'Tianxiang Taige' are appropriate for developing linalool-type products.

## Full Text

### Comparative Analysis of Aromatic Components from Different Varieties of *Osmanthus fragrans* in Guilin

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## Abstract

This study investigated the differences in aromatic components among twelve varieties of *Osmanthus fragrans* cultivated in the Guilin region using headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS). A total of 49 volatile compounds were identified across all varieties, including 31 terpenoids, 10 fatty acid-derived compounds, 4 benzenoids, and 4 nitrogen-containing compounds. Terpenoids dominated the volatile profile in every variety group, accounting for 82.28%-94.83% of total relative content. All twelve varieties shared six common aroma constituents: trans- $\alpha$ -ocimene, trans-linalool oxide, cis-linalool oxide, linalool,  $\alpha$ -ionone, and dihydro- $\alpha$ -ionone. However, distinct differences were observed among varieties in both composition and relative abundance. For instance,  $\alpha$ -ionone was absent in 'Xiangyehusha' but reached its highest concentration in 'Longhuai Jingui' (34.89%). The primary aroma components also varied by variety: 'Longhuai Jingui' featured five major compounds including  $\alpha$ -ionone, 'Yuetang Jingui' had eight major components including  $\alpha$ -ionone, while 'Xiangyehusha' was characterized by six compounds including cis-linalool oxide.

Eleven aroma-active compounds were identified, ten of which were terpenoids. 'Longhuai Jingui' exhibited the highest total content of aroma-active compounds (82.99%), along with the highest levels of ionone and ocimene compounds. 'Xiangyehusha' and 'Tianxiangtaige' showed the highest concentrations of linalool-type compounds (approximately 60%). These findings demonstrate that terpenoids constitute the primary aroma components of *O. fragrans* in

Guilin. While different varieties share common aroma constituents, each possesses unique compositional features. ‘Longhuai Jingui’ is particularly suitable for developing ocimene- and ionone-based products, whereas ‘Xiangyehusha’ and ‘Tianxiangtaige’ are optimal for linalool-based product development.

**Keywords:** *Osmanthus fragrans*, GC-MS, aroma constituents, aroma-active compounds

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## Introduction

*Osmanthus fragrans* Lour., belonging to the family Oleaceae and genus *Osmanthus*, is classified into four cultivar groups based on morphological characteristics, evolutionary patterns, and flowering habits: the Lutes Group (golden osmanthus), Albus Group (silver osmanthus), Aurantiacus Group (orange osmanthus), and Asiaticus Group (everblooming osmanthus) (Zang and Xiang, 2004). As an evergreen plant with delightful fragrance, *O. fragrans* is one of China’s traditional top ten flowers, integrating ornamental, aromatic, and ecological values (Wang et al., 2005). The petals are rich in nutrients and possess significant edible and medicinal properties (Zhao et al., 2017; Chen et al., 2013).

Previous studies have reported that *O. fragrans* contains abundant volatile aroma compounds such as ocimene, linalool and its oxides, and ionone derivatives, which are widely used in daily necessities, pharmaceuticals, and chemical industries (Aprotosoai et al., 2014; Arens et al., 2015) and hold substantial economic value (Lapczynski et al., 2008; Lalko et al., 2007). Consequently, numerous investigations have focused on the detection and application of floral aroma components (Wang et al., 2009; Wang et al., 2016) as well as the cloning and functional validation of aroma-related genes (Baldermann et al., 2010; Zeng et al., 2015; Chen et al., 2016). These studies indicate that the volatile composition of *O. fragrans* is complex, with variations arising from geographical location, cultivar differences, and analytical methodologies. Using HS-SPME-GC-MS, researchers have identified 25, 57, and 52 aroma compounds from different cultivars (Cao, 2009; Sun, 2012; Yang, 2015). Alternative extraction techniques including Soxhlet extraction, steam distillation, and supercritical CO<sub>2</sub> extraction have also been employed for essential oil analysis (He, 2010; Xia et al., 2017; Xia et al., 2015). These investigations consistently report the presence of linalool, ionone, ocimene, and pinene derivatives, along with other variable components that may reflect regional, cultivar, or methodological differences.

Guilin, Guangxi, represents one of China’s “five major *O. fragrans* production regions” (Zang, 2003) and is considered a central origin of the species (Huang and Huang, 2017). However, few reports have characterized the aroma components of *O. fragrans* from this region. To address this knowledge gap, we selected twelve cultivars from four cultivar groups widely grown in Guilin and analyzed their petal volatiles using HS-SPME-GC-MS. This study examines the primary aroma components and their relative abundance across varieties, iden-

tifies aroma-active compounds and their inter-varietal differences, and aims to provide a theoretical foundation for selecting superior cultivars and guiding the development of Guilin's *O. fragrans* industry.

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## Materials and Methods

### 1.1 Plant Materials

Twelve *O. fragrans* cultivars were collected from the Guilin region, with detailed information provided in . All trees were 9–12 years old with trunk diameters of 11–13 cm, exhibiting healthy growth without pest or disease issues.

### 1.2 Experimental Instruments

The analytical equipment included a manual solid-phase microextraction injector and 50/30  $\mu$ m PDMS/DVB fiber (Supelco, USA), an Agilent 6890N-5975B GC-MS system (USA), 40 mL headspace sampling vials, and a water bath (Shanghai Jingxue Scientific Instruments Co., Ltd.).

### 1.3 GC-MS Analysis of Floral Aroma Components

During the peak flowering period in mid-October, twenty fresh flowers per cultivar were collected between 9:00–10:00 AM after morning dew evaporation. The samples were placed in 25 mL headspace vials, and a 50/30  $\mu$ m PDMS/DVB fiber was inserted for headspace extraction at 40 °C for 30 minutes. After extraction, the fiber was withdrawn and inserted into the GC-MS inlet for 5 minutes of desorption before analysis.

**Chromatographic conditions:** HP-5MS quartz capillary column (30  $\mu$ m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m); carrier gas flow rate 0.8 mL  $\cdot$  min<sup>-1</sup>; high-purity helium (99.999%) as carrier gas; splitless mode; temperature program: initial 40 °C held for 3 min, ramped at 3 °C  $\cdot$  min<sup>-1</sup> to 73 °C held for 3 min, then ramped at 5 °C  $\cdot$  min<sup>-1</sup> to 220 °C held for 2 min.

**Mass spectrometry conditions:** Inlet temperature 230 °C; ion source temperature 150 °C; electron ionization (EI) at 70 eV; GC-MS transfer line temperature 250 °C; scan range 40–450 amu (Cai et al., 2014).

### 1.4 Identification and Quantification of Volatile Components

Based on the total ion chromatograms, individual peak mass spectra were interpreted using Xcalibur software (version 1.2) and matched against the NIST98 mass spectral library. Compounds were further confirmed by comparing Kovats Retention Indices (RI) calculated from n-alkane standards (C8–C40) under identical temperature programs with literature values from the NIST database. Relative content of each component was calculated using peak area normalization.

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## Results

### 2.1 Classification of Volatile Components in Different *O. fragrans* Cultivars from Guilin

A total of 49 volatile compounds were detected across the twelve cultivars, comprising 31 terpenoids, 10 fatty acid-derived compounds, 4 benzenoids, and 4 nitrogen-containing compounds (). The Lutes Group exhibited the greatest diversity with 36 compounds (21 terpenoids, 8 fatty acid derivatives, 3 benzenoids, and 4 nitrogen-containing compounds). The Albus Group contained 31 compounds (23 terpenoids, 3 fatty acid derivatives, 2 benzenoids, and 2 nitrogen-containing compounds). The Aurantiacus Group yielded 29 compounds (23 terpenoids, 5 fatty acid derivatives, 1 benzenoid, and no nitrogen-containing compounds). The Asiaticus Group similarly contained 28 compounds (19 terpenoids, 7 fatty acid derivatives, 2 benzenoids, and no nitrogen-containing compounds). Terpenoids dominated all cultivars, representing 82.28%-94.83% of total relative content, while other compound classes were present at much lower levels: fatty acid derivatives ranged from 1.20% to 12.69%, benzenoids from 0% to 8.89%, and nitrogen-containing compounds were detected in only a few cultivars at approximately 1.0%.

### 2.2 Analysis of Primary Floral Aroma Components

Based on relative abundance, six major aroma compounds were common to all twelve cultivars: trans-*-ocimene*, cis-linalool oxide, trans-linalool oxide, linalool, *-ionone*, and dihydro-*-ionone* ( and ). However, significant variations existed among cultivar groups and individual varieties in both component categories and relative content.

Trans-*-ocimene* showed relatively high levels in the Lutes Group (6.45%-15.64%), peaking in 'Longhuai Jingui' (15.64%). In the Albus Group, only 'Liuye Yingui' contained substantial amounts (8.81%), while the other two cultivars had approximately 2%. The three Aurantiacus cultivars ranged from 7.60% to 14.81%, with 'Guifeihong' showing the highest concentration. In the Asiaticus Group, 'Biansegui' (10.15%) exceeded 'Tianxiangtaige' (3.83%).

Cis-linalool oxide was universally abundant, though the Lutes Group (5.22%-10.67%) showed lower levels than other groups. Within the Lutes Group, 'Longhuai Jingui' contained the minimum (5.22%) while 'Jinqiugui' reached the maximum (10.67%). The Albus Group ranged from 15.43% to 18.76%, and the Aurantiacus Group from 10.71% to 23.18%, with 'Xiangyehusha' showing the highest value. The two Asiaticus cultivars contained 15.54% and 12.81%, respectively.

Trans-linalool oxide in the Lutes Group ranged from 3.75% to 10.17%. The Albus Group showed 8.51%-10.72%. The Aurantiacus Group exhibited greater

variation: ‘Guifeihong’ and ‘Xiangyehusha’ contained 16.57% and 17.12%, respectively, while ‘Taoye Dangu’ had the lowest concentration among all cultivars (2.79%). The two Asiaticus cultivars showed relatively high levels at 15.71% and 14.61%.

Linalool was abundant across all cultivars, with most exceeding 13.22%; only ‘Taoye Yingui’ and ‘Liuye Yingui’ contained less than 10%. ‘Tianxiangtaige’ showed the highest concentration (22.54%). -Ionone was generally low, with only ‘Guifeihong’ exceeding 5% (6.78%). Dihydro- -ionone varied considerably within groups: the Lutes Group ranged from 3.31% to 13.84%, with ‘Yanshan Jingui’ showing the highest level among all cultivars; the Albus Group contained 3.33%–10.88%; the Aurantiacus Group 3.25%–9.14%; and both Asiaticus cultivars contained less than 5.0%.

Beyond these six shared components, each cultivar possessed additional characteristic compounds ( and ). The four Lutes cultivars commonly contained four additional compounds: cis- -ocimene, -pinene, cis-linalool oxide (pyranoid), and notably high -ionone (22.05%–34.89%), with ‘Longhuai Jingui’ showing the maximum concentration across all cultivars. ‘Longhuai Jingui’ and ‘Yuetang Jingui’ also contained substantial cis-linalool oxide (pyranoid) at 6.58% and 6.66%, respectively. ‘Yanshan Jingui’ and ‘Yuetang Jingui’ additionally contained high levels of 2-aminobenzoate linalool (8.89% and 8.38%).

The Albus Group shared four other compounds, including -decalactone (3.63%–9.37%) and -ionone (20.38%–24.65%). The three Aurantiacus cultivars commonly contained n-octane and cis- -ocimene, though only ‘Xiangyehusha’ showed high n-octane content (8.60%). The Asiaticus Group shared 12 compounds, more than other groups, including six additional components such as ethyl crotonate, with ‘Tianxiangtaige’ containing notably high ethyl crotonate (6.69%). Several compounds were present at less than 1% in only one or two cultivars, such as hexyl butanoate, -terpinene, and neo-allo-ocimene, yet these may still contribute to the overall floral aroma.

### 2.3 Comparison of Aroma-Active Compounds Among *O. fragrans* Cultivars

Eleven aroma-active compounds were identified across the twelve cultivars, with ten belonging to the terpenoid class. Five compounds—trans- -ocimene, cis-linalool oxide, trans-linalool oxide, linalool, and -ionone—were present in all cultivars. The remaining six were detected only in specific varieties: -ionone was absent in ‘Xiangyehusha’ but abundant in others; cis- -ocimene was missing in ‘Liuye Yingui’ and Asiaticus cultivars; cis-linalool oxide (pyranoid) was absent in ‘Taoye Yingui’ and all Aurantiacus cultivars; hexyl butanoate occurred only in ‘Yanshan Jingui’ and ‘Yuetang Jingui’; -terpinene was found solely in ‘Yanshan Jingui’ and ‘Guifeihong’; and neo-allo-ocimene appeared in ‘Yuetang Jingui’, ‘Dutou Yingui’, ‘Taoye Yingui’, and ‘Taoye Dangu’.

The total relative content of aroma-active compounds was generally high across

all cultivars ([Figure 1: see original paper]). The four Lutes cultivars ranged from 69.10% to 82.99%, with ‘Longhuai Jingui’ showing the maximum value among all varieties. The three Albus cultivars contained 67.31%–75.23%, with ‘Liuye Yingui’ showing the lowest concentration overall. The three Aurantiacus cultivars exhibited relatively narrow variation (69.36%–73.81%). The two Asiaticus cultivars contained 77.57% and 79.59%.

Ocimenes, linalool and its oxides, and ionones represent three major classes of aroma-active compounds in *O. fragrans*. The eleven identified compounds were categorized accordingly, with hexyl butanoate and -terpinene classified as other actives. Linalool and its oxides were generally abundant but highly variable: ‘Tianxiangtaige’ and ‘Xiangyehusha’ reached 60.53% and 59.85%, respectively, while ‘Longhuai Jingui’ contained only 29.79%. Ionones showed substantial inter-varietal differences, peaking in ‘Longhuai Jingui’ (35.84%) and reaching a minimum in ‘Xiangyehusha’ (1.19%), with the remaining ten cultivars ranging from 11.32% to 22.96%. Ocimene content also varied considerably, with ‘Longhuai Jingui’ showing the highest level (17.36%) and ‘Dutou Yingui’ the lowest (5.41%). Trace amounts of hexyl butanoate or -terpinene were detected in ‘Yanshan Jingui’ , ‘Yuetang Jingui’ , and ‘Guifeihong’ .

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## Discussion

### 3.1 Terpenoids as the Primary Volatile Components of Guilin *O. fragrans*

Analysis of twelve *O. fragrans* cultivars from Guilin identified 49 volatile compounds, with terpenoids comprising 31 species. Terpenoids represented the most abundant compound class across all four cultivar groups and twelve individual varieties, accounting for 82.28%–94.83% of total volatile content. Monoterpenes and their oxides—including ocimene, linalool oxides, linalool, and ionones—constituted particularly large proportions. These results confirm that terpenoids are the principal volatile components of *O. fragrans* in Guilin, consistent with previous reports (Gang, 2005; Zou et al., 2017). Compared to the 17 aroma-active compounds identified by Cai et al. (2014), this study detected 11 aroma-active substances: trans- -ocimene, cis- -ocimene, neo-allo-ocimene, cis-linalool oxide (pyranoid), cis-linalool oxide, trans-linalool oxide, linalool, -ionone, -ionone, -terpinene, and hexyl butanoate. The first ten are terpenoid compounds, demonstrating that terpenoids play a crucial role in the fragrance emission of Guilin *O. fragrans*.

### 3.2 Inter-Varietal Variation in Shared and Unique Aroma Components

Floral aroma in *O. fragrans* originates from four major metabolic pathways—terpenoid, fatty acid derivative, benzenoid, and nitrogen-containing compound

biosynthesis—with each cultivar developing a unique fragrance profile through distinct combinations and proportions (Gang, 2005). All four cultivar groups in this study shared six terpenoid aroma components: trans- $\alpha$ -ocimene, cis-linalool oxide, trans-linalool oxide, linalool,  $\alpha$ -ionone, and dihydro- $\alpha$ -ionone, contributing to the characteristic fresh/grassy scent typical of *O. fragrans*. However, compositional differences in primary aroma components and their relative abundances created distinct fragrance characteristics for each cultivar. For example, ‘Longhuai Jingui’ was dominated by five compounds including  $\alpha$ -ionone (34.89%); ‘Yuetang Jingui’ featured eight major components including  $\alpha$ -ionone (22.74%); while ‘Xiangyehusha’ was characterized by six compounds including cis-linalool oxide (23.18%) but lacked the  $\alpha$ -ionone found in other cultivars. These differences in primary component composition and concentration underlie the unique fragrance features of each cultivar.

Previous research indicates that flavonoids and carotenoids constitute the main pigments in *O. fragrans* petals (Cai et al., 2010; Han et al., 2014; Zou et al., 2017). Studies have demonstrated linkages between pigment and aroma biosynthesis, suggesting that flower color may influence fragrance (Ben-Zvi et al., 2008). For example,  $\beta$ -carotene is cleaved by carotenoid cleavage dioxygenase (CCD) to produce important aroma compounds such as  $\alpha$ -ionone (Schwartz et al., 2001; Simkin et al., 2004; Zhang et al., 2016). Carotenoids are associated with red coloration. The two *Aurantiacus* cultivars (‘Guifeihong’ and ‘Xiangyehusha’) showed substantially lower  $\alpha$ -ionone content and deeper petal coloration than other varieties, possibly due to reduced CCD activity leading to  $\beta$ -carotene accumulation at the expense of ionone production, resulting in weaker fragrance intensity.

### 3.3 Selection of Superior Cultivars for Industrial Development in Guilin

*Osmanthus fragrans* is both a valuable spice plant and traditional medicinal herb with significant research and development potential. Fragrance primarily derives from abundant volatile components, though not all volatiles contribute equally—aroma-active compounds are the key determinants (Van Ruth, 2001). Therefore, the concentration of aroma-active substances critically influences fragrance intensity. This study identified 11 aroma-active compounds including trans- $\alpha$ -ocimene, with inter-varietal differences in relative abundance consistent with previous reports (Sun et al., 2012; Cai et al., 2014).

Among the twelve evaluated cultivars, ‘Longhuai Jingui’ showed the highest total aroma-active compound content (82.99%) and the maximum levels of both ionone and ocimene compounds, making it an excellent candidate for ocimene- and ionone-based product development. Several other cultivars—including ‘Yuetang Jingui’, ‘Jinqiugui’, ‘Biansegui’, ‘Tianxiangtaige’, and ‘Dutou Yingui’—also demonstrated total active compound contents exceeding 75%, representing promising varieties. Notably, ‘Xiangyehusha’ and ‘Tianxiangtaige’ contained exceptionally high linalool-type compounds (approximately 60%), making them

particularly suitable for linalool-based industrial applications.

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