Analysis of Volatile Components in Flowers of Four Endemic *Begonia* Species in Guangxi

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Abstract

Begonia lanternaria, B. longgangensis, B. luzhaiensis, and B. pseudoleprosa, endemic plants of Guangxi, have important ecological value and ornamental potential. To clarify the composition, content, and interspecific differences of floral volatile components and provide a scientific basis for screening wild Begonia germplasm resources with regional characteristics, this study identified and analyzed the volatiles of fresh flowers of four Begonia species using headspace solid-phase microextraction combined with gas chromatography-mass spectrometry. The results showed that: (1) A total of 56 volatile compounds were detected, mainly including terpenes (17 types), hydrocarbons (excluding terpenes) (18 types), alcohols (8 types), phenols (1 type), esters (6 types), amides (1 type), and other compounds (5 types). Among them, the relative contents of terpenes in the flowers of B. longgangensis, B. luzhaiensis, and B. pseudoleprosa were extremely high (86.86%-98.01%), whereas alcohols had the highest relative content in the flowers of B. lanternaria. (2) The number of volatile components showed obvious interspecific differences, with richness in descending order as B. longgangensis (27 types), B. pseudoleprosa (22 types), B. luzhaiensis (10 types), and B. lanternaria (5 types), and no common volatile components were found among the four species. (3) The relative content of (Z)-β-ocimene was significantly enriched in the male flowers and female flowers of B. longgangensis and in the flowers of B. luzhaiensis, reaching 93.49%, 92.07%, and 81.72%, respectively, and it may be the main contributor to their characteristic aroma. In summary, the volatile substances in the fresh flowers of the four Begonia species differed in both component composition and relative content, thereby forming their unique aroma characteristics. The results of this study are expected to provide valuable references for the evaluation of Begonia germplasm resources, variety breeding, horticultural development, and wellness tourism.

Full Text

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Analysis of Volatile Components in Flowers of Four Endemic *Begonia* Species in Guangxi

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Abstract: The Guangxi endemic plants *Begonia lanternaria*, *B. longgangensis*, *B. luzhaiensis*, and *B. pseudoleprosa* have important ecological value and ornamental potential. To clarify the composition, contents, and interspecific differences of the volatile components in their flowers, and to provide a scientific basis for screening wild *Begonia* germplasm resources with regional characteristics, this study used headspace solid-phase microextraction combined with gas chromatography-mass spectrometry to identify and analyze the volatiles emitted from the fresh flowers of the four *Begonia* species. The results showed that: (1) A total of 56 volatile compounds were detected, mainly including terpenes (17 compounds), hydrocarbons (excluding terpenes) (18 compounds), alcohols (8 compounds), phenols (1 compound), esters (6 compounds), aldehydes and ketones (1 compound), and other compounds (5 compounds). Among them, terpenoids had extremely high relative contents in the flowers of *B. longgangensis*, *B. luzhaiensis*, and *B. pseudoleprosa* (86.86%-98.01%), whereas alcohols had the highest relative content in the flowers of *B. lanternaria*. (2) The number of volatile components differed markedly among species; in descending order of richness, they were *B. longgangensis* (27 compounds), *B. pseudoleprosa* (22 compounds), *B. luzhaiensis* (10 compounds), and *B. lanternaria* (5 compounds). No volatile component was shared among all four species. (3) The relative content of *(Z)-β-ocimene* was significantly enriched in the male and female flowers of *B. longgangensis* and in the flowers of *B. luzhaiensis*, reaching 93.49%, 92.07%, and 81.72%, respectively, suggesting that it may be the principal contributor to their characteristic fragrance. Overall, the fresh-flower volatile substances of the four *Begonia* species differ in both component composition and relative content, thereby forming distinct fragrance characteristics. The results of this study are expected to provide valuable references for the evaluation of germplasm resources, variety selection and breeding, horticultural development, and wellness tourism involving *Begonia* plants.

Keywords: *Begonia*; gas chromatography-mass spectrometry; fresh flowers; volatile components; karst plants

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Abstract: The Guangxi endemic plants *B. lanternaria*, *B. longgangensis*, *B.*

luzhaiensis, and *B. pseudoleprosa* have important ecological value and ornamental potential. This study aims to clarify the composition, content and interspecific differences of volatile components in their flowers, providing a scientific basis for screening wild *Begonia* germplasm resources with regional characteristics. In this study, headspace solid-phase microextraction combined with gas chromatography-mass spectrometry was used to identify and analyze the volatile compounds of four

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species of fresh *Begonia* flowers. The results were as follows: (1) A total of 56 volatile compounds were detected, including terpenes (17), hydrocarbons (excluding terpenes) (18), alcohols (8), phenols (1), esters (6), amides (1), and other compounds (5). The relative content of terpenes in the flowers of *B. longgangensis*, *B. luzhaiensis*, and *B. pseudoleprosa* was extremely high (86.86%–98.01%), whereas alcohols had the highest relative content in the flowers of *B. lanternaria*. (2) The number of volatile components differed significantly among species. The richness of volatile components ranked from highest to lowest as follows: *B. longgangensis* (27 types), *B. pseudoleprosa* (22 types), *B. luzhaiensis* (10 types), and *B. lanternaria* (5 types). No common volatile components were found among the four species. (3) The relative contents of *(Z)*-β-ocimene in the male and female flowers of *B. longgangensis* and in the flowers of *B. luzhaiensis* were significantly enriched, reaching as high as 93.49%, 92.07%, and 81.72%, respectively, which may be the main contributor to their characteristic aroma. In summary, the volatile components of the flowers of the four *Begonia* species differed in composition and relative content, resulting in unique aroma profiles. These findings provide valuable references for the evaluation of germplasm resources, variety breeding, horticultural development, and health tourism related to *Begonia* plants.

Keywords: *Begonia*, GC-MS, fresh flower, volatile components, karst plants

Begonia L. is one of the most diverse ornamental plant groups, with more than 2,000 recorded taxa, of which approximately 290 species are distributed in China (Hu et al., 2025); new taxa and newly recorded species continue to be added. In recent years, extensive and in-depth studies on *Begonia* have been carried out in multiple fields, including plant taxonomy (Shui Yumin et al., 2019), new cultivar breeding (Deng Tao et al., 2023), resource surveys and evaluations of landscape application (Li Jingxiu et al., 2021; Gao Yangyang et al., 2023), molecular systematics (Du Wenwen et al., 2021; Xue Zhenzhen et al., 2023; Chao et al., 2023), genomics (Lu et al., 2023; Chen et al., 2024), and introduction, domestication, and adaptive mechanisms (Yang Xuan, 2023; Zhao et al., 2024).

Volatile organic compounds (VOCs) released by plants are important chemical signaling substances in ecosystems (Li Jun, 2016). They play important roles in processes such as attracting pollinators, mediating chemical communication among plants, participating in plant resistance to environmental stress, biological defense, signal transduction, and reproductive strategies (Hu Shujing and Zhang Rumin, 2022). The chemical composition of floral scent, its emission rhythm, and its differences among species not only carry evolutionary information on species-specific pollination strategies, but also have multiple potential values, including the development of new aromatic resources, the elucidation of ecological adaptation mechanisms, guidance for artificial breeding, and expansion of health and wellness applications. However, although the ornamental value of *Begonia* plants has been widely recognized, the development and utilization of their floral fragrance traits remain at an initial stage. At present, studies on floral volatile substances in this genus are still relatively few. Owing to factors such as the difficulty of obtaining wild-resource samples, existing work has mostly focused on a small number of cultivated species or species with pronounced fragrance. For example, Ding Youfang et al. (2021) and Liang Xiaoli et al. (2022, 2025) analyzed the composition and content of volatiles in fragrant-flowered *Begonia* and two of its varieties (female flowers of prostrate *Begonia* and male flowers of red-haired fragrant-flowered *Begonia*); Du Wenwen et al. (2022) compared differences in aroma components among *B. pseudo-dryadis*, *B. sinensis*, *B. wilsonii*, and *B. rex*. These studies preliminarily revealed interspecific diversity in floral scent in *Begonia*, but research on the floral volatile components of wild species in special karst habitats remains lacking. Karst regions are characterized by strong habitat heterogeneity (He Jie et al., 2021). Under such environments, whether plant secondary metabolites—including floral VOCs—adapt to special pollination environments or respond to abiotic stress by releasing unique chemical information is a question of important scientific exploratory value. Guangxi is one of the most typical and complex karst-landform regions in China. Its unique karst ecosystem harbors rich biodiversity and is one of the centers of modern distribution and diversification of *Begonia* in China. The *Begonia* plants in this region not only have a large number of species and high endemism, but, in the course of long-term adaptation to karst habitats, many species have evolved extremely high ornamental value, including highly variable and unusual leaf shapes, brilliant variegated foliage, rich and diverse flower colors, and long flowering periods. Most also have multiple values in medicine, food, and culture, making them excellent materials for basic scientific research and development and utilization (Tian Daike, 2020). However, most species have not yet been fully developed or applied. The present study selected *B. lanternaria*, *B. longgangensis*, *B. luzhaiensis*, and *B. pseudoleprosa*

All are endemic species of Guangxi, distributed mainly in karst mountains in Daxin, Jingxi, Longzhou, Luzhai, Rongshui, Xincheng, and other areas. In the most recently issued *China Biodiversity Red List*, *Begonia luzhaiensis* and *B.*

longgangensis are listed as threatened species (Ministry of Ecology and Environment and Chinese Academy of Sciences, 2023). *Begonia dengguoensis* and *B. pseudodryadis* are also narrowly distributed species: the former has so far been found only in Longzhou and Jingxi, and the latter only in Daxin. Their strong adaptation to karst habitats makes them excellent materials for studying plant–environment interactions. In landscape applications, these four begonias all show high ornamental value in their leaves, flowers, and plant form, making them promising materials for landscape construction and the development of new potted flowers. Since their introduction to the Guilin Botanical Garden for long-term observation, they have also shown strong adaptability to acidic lateritic red soil, providing a scientific basis for their wider application in broader ecological environments. However, systematic development and utilization studies have not yet been carried out, and the chemical composition of their floral volatile compounds has not yet been reported. To better conserve and develop these plants, it is necessary to identify and analyze their floral volatile compounds.

There are diverse techniques for extracting floral volatile constituents from plants, including traditional solvent extraction, distillation, and modern headspace solid-phase microextraction (HS-SPME). Analytical identification methods include gas chromatography, gas chromatography–mass spectrometry (GC–MS), and gas chromatography–infrared spectroscopy, among others. In recent years, combined multi-technology strategies have become mainstream in studies of plant volatile organic compounds. Among them, headspace solid-phase microextraction combined with gas chromatography–mass spectrometry (HS-SPME–GC–MS), owing to its advantages of convenient operation, speed and efficiency, high sensitivity, no solvent requirement, and minimal damage to living samples (Pang Jiwei et al., 2023), has become a commonly used method for analyzing plant volatiles, and has been successfully applied in the detection of floral volatile components in many plant groups, such as minority species of *Begonia* (Ding Youfang et al., 2021; Du Wenwen et al., 2022; Liang Xiaoli et al., 2022, 2025), *Primula* (You Shuo et al., 2019; Xia Ke et al., 2021), *Rhododendron* (Wang Shuai et al., 2024), *Clematis* (Teng Xinlei et al., 2024), and *Arthropodium* (Huang Bei et al., 2023). In view of this, the present study adopted headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry to conduct, for the first time, a systematic analysis of the volatile organic compounds of fresh flowers of four Guangxi-endemic begonias: *B. dengguoensis*, *B. longgangensis*, *B. luzhaiensis*, and *B. pseudodryadis*. The aim was to clarify their floral fragrance chemical composition and interspecific differences, fill gaps in research on the fragrance of regionally endemic species, enrich data on floral fragrance chemical diversity in *Begonia*, and provide baseline data for germplasm innovation, targeted breeding of fragrant cultivars, commercial development, and the characteristic flower industry in karst regions.

1 Materials and Methods

1.1 Experimental Materials

The test materials were four Guangxi-endemic begonias potted in the *Begonia* germplasm resource nursery of the Guangxi Institute of Botany (110°18$ ‘04’ ‘$ E, 25°04$’ 28’ ’ $ N). All four plants are perennial herbs that are monoecious with separate male and female flowers on the same plant. Among them, *B. dengguoensis* and *B. longgangensis* were introduced from Longzhou, *B. luzhaiensis* from Xincheng, and *B. pseudodryadis* from Daxin.

1.2 Experimental Instruments and Equipment

The main instruments and equipment used in this experiment were as follows: a manual solid-phase microextraction sampling device produced by SUPELCO; an SPME extraction fiber with a 50/30 μm DVB/CAR/PDMS composite coating; an HP-5MS capillary chromatographic column from Agilent (30 m × 0.25 mm × 0.25 μm); a 6890N–5975B gas chromatography–mass spectrometry system; matching 40 mL brown headspace sampling vials; and a DHP-9602 constant-temperature drying oven with digital display manufactured by Shanghai Yiheng Co., Ltd.

1.3 Experimental Methods

1.3.1 Flower Collection and Extraction of Volatile Components

During the full-bloom period of the four begonias in late August, flowers were collected on sunny mornings from 9:00 to 11:30. Whole flowers from healthy, vigorous plants were selected. In samples of *B. longgangensis*, where individual plants showed an obvious predominance of either male or female flowers, female and male flowers were collected and measured separately. After the base of the pedicel was cut with medical scissors, the flowers were quickly placed into 40 mL brown headspace sampling vials and promptly transported to the laboratory. For each sample, 10 whole flowers were collected, with three replicates.

This study used headspace solid-phase microextraction (HS-SPME) to enrich volatile organic compounds. The experimental method referred to the volatile-compound extraction methods of Zhu Xiaozhen et al. (2020) and Xia Ke et al. (2018), with appropriate modifications. The specific operating procedure

as follows: first, the extraction head was aged at the GC-MS injection port at 250 °C for 30 min, and then inserted into the sampling vial containing the begonia flowers. Headspace adsorption was continued for 30 min in a constant-temperature environment at 37 °C. The enriched extraction head was transferred to the GC-MS injection port, desorbed for 5 min, and then gas chromatography-mass spectrometry analysis was initiated.

1.3.2 GC-MS Analysis

The GC-MS analysis was performed with reference to the method of Zhu Xiaozhen et al. (2020).

Gas chromatography (GC): (1) chromatographic column selection: an HP-5MS quartz capillary column was used (30 m × 0.25 mm × 0.25 μm); (2) temperature-program setting: the initial column temperature was set to 50 °C, then increased at a rate of 5 °C per minute to 200 °C and held at that temperature for 3 min; (3) injection conditions: the injection-port temperature was 230 °C, a split ratio of 1:10 was used, and the injection volume was 2 μL each time; (4) carrier-gas parameters: ultra-high-purity helium was used (purity $ $99.999%), and the flow rate was controlled at 1.0 mL per minute under constant-flow mode.

Mass spectrometry (MS): (1) ionization and ion transfer: electron-impact ionization technology (EI source) was used, with the electron-beam energy set to 70 eV; (2) system temperature parameters: the ion source was heated to 230 °C, the transfer-line temperature was set to 280 °C, and the quadrupole temperature was maintained at 150 °C; (3) mass analysis range: full-scan acquisition mode was used, and the detection mass range was set to m/z 35–450.

1.3.3 Statistical Analysis of Data

For the total ion chromatograms obtained by GC-MS, the mass spectra corresponding to each chromatographic peak were analyzed (Xia Ke et al., 2021). The NIST05a.L standard mass spectral database was used for bidirectional matching with the acquired mass spectral data, and the detected components were identified with reference to the literature. The relative content of volatile components was calculated by the peak-area normalization method. WPS software was used to statistically analyze the number and relative contents of volatile components, and OriginPro 2024b software was used to draw stacked bar charts, PCA plots, and Venn diagrams.

2 Results and Analysis

2.1 Types and Principal Component Analysis of Volatile Components in the Flowers of Four Begonia Species

2.1.1 Types of Volatile Components A total of 56 volatile substances were detected in the fresh flower samples of the four Begonia species (Table 1). The main types included terpenoids, hydrocarbons other than terpenoids, alcohols, ketones, esters, amides, and other compounds such as aromatic compounds and carboxylic acid compounds. The volatile-component types of the flowers differed among species. Based on Table 1 and Figure 1, the following differences can be observed.

(1) Terpenoids. A total of 17 terpenoids were detected in the flowers of the four Begonia species. The total relative contents of this type of sub-

stance in each species ranked as follows: male flowers of Nonggang begonia (98.01%), female flowers of Nonggang begonia (96.10%), Luzhai begonia (96.38%), Jialaiye begonia (86.86%), and Dengguo begonia (0%). The results indicate that the volatile substances in the flowers of Nonggang begonia, Luzhai begonia, and Jialaiye begonia are dominated by terpenoids, whereas no terpenoid compounds were detected in the flower samples of Dengguo begonia.

(2) Hydrocarbons. Eighteen hydrocarbon substances other than terpenoids were present in the fresh flowers of the four Begonia species. However, except for Dengguo begonia, in which the relative content was relatively high (23.23%), the total relative contents of this class of substances in the other species were all below 3%.

(3) Alcohols. Eight alcohol substances were detected in the flowers of the tested samples. Among them, the content of this class of substances was highest in Dengguo begonia flowers (45.9%), followed by Jialaiye begonia (4.09%) and Nonggang begonia (male flowers 0.04%, female flowers 0.19%); no alcohol substances were detected in the fresh flowers of Luzhai begonia.

(4) Ketones. This class of substances was detected only in the female flowers of Nonggang begonia, and its relative content was low (0.15%).

(5) Esters. A total of 6 compounds of this class were identified. They were present only in the flowers of Nonggang begonia and Jialaiye begonia. The total relative content in the former (male flowers 0.44%, female flowers 0.72%) was significantly lower than that in the latter (6.01%), whereas no ester components were detected in the flowers of Dengguo begonia or Luzhai begonia.

(6) Amides. This class of volatile substances was present only in the flowers of Dengguo begonia, accounting for 26.20%; it was not detected in the fresh flowers of the other three Begonia species.

(7) Other compounds. This class of substances included aromatic compounds, N-containing compounds, carboxylic acid compounds, and pyridine derivatives, among others. Among the other compounds, Luzhai begonia had the highest proportion (6.31%), followed by Dengguo begonia (4.67%) and Nonggang

The proportions in male flowers of begonia and in false scabrous-leaved begonia were low, and no other compounds were detected in female flowers of Nonggang begonia.

Table 1. Analysis of volatile components in flowers of four *Begonia* species

No.	Retention time (min)	Compound	A	B	C	D	E
Terpenes	**Terpenes**	**Terpenes**					
1	7.144	1R-α-Pinene; R-α-Pinene	–	–	–	–	4.40
2	7.525	Camphene	–	–	–	–	2.05
3	8.214	β-Pinene	–	–	–	0.35	5.59
4	8.54	β-Myrcene	–	0.74	1.06	–	22.43
5	8.941	α-Phellandrene	–	–	–	–	0.75
6	9.288	Cyclohexene, 3-methyl-6-(1-methylethylidene)-	–	–	–	–	0.38
7	9.639	(S)-(-)-Limonene; Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)-	–	–	–	–	42.34
8	9.80	(E)-β-Ocimene; 1,3,6-Octatriene, 3,7-dimethyl-, (E)-	–	1.72	2.19	1.76	–

No.	Retention time (min)	Compound	A	B	C	D	E
9	10.172	(Z)-β-Ocimene; 1,3,6-Octatriene, 3,7-dimethyl-, (Z)-	–	93.49	92.07	81.72	0.47
10	10.392	Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	–	–	–	–	0.37
11	11.233	Ocimene	–	0.04	–	–	–
12	11.233	(+)-4-Carene	–	–	–	–	8.08
13	12.717	Allo-ocimene; 2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)-	–	1.23	–	–	–
14	14.316	1-Methylene-4-(1-methylethenyl)cyclohexane	–	0.05	–	–	–
15	21.077	(E)-β-Farnesene; 1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (Z)-	–	–	0.78	–	–

No.	Retention time (min)	Compound	A	B	C	D	E
16	21.086	trans-β-Farnesene; 1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (E)-	–	0.74	–	–	–
17	22.388	α-Farnesene	–	–	–	8.55	–
		Total	0	98.01	96.10	92.38	86.86
Hydrocarbons (excluding terpenes)	**Hydrocarbons (excluding terpenes)**	**Hydrocarbons (excluding terpenes)**					
18	9.588	trans-1,3-Pentadiene; 1,3-Pentadiene, (E)-	–	–	0.05	–	–
19	6.328	Benzocyclobutene; Bicyclo[4.2.0]octa-1,3,5-triene	–	–	–	–	2.48
20	8.996	5-Methyl-1,3,6-heptatriene; 1,3,6-Heptatriene, 5-methyl-	–	0.20	–	–	–
21	9.571	1,2-Cyclononadiene	–	0.03	–	–	–

No.	Retention time (min)	Compound	A	B	C	D	E
22	9.576	1,8-Nonadiyne	15.76	–	–	–	–
23	10.40	Cyclopropane, 1,1-dimethyl-2-(3-methyl-1,3-butadienyl)-	–	0.33	0.72	–	–

Category	No.	Retention time	Compound	[[unclear: column header not visible]]	[[unclear: column header not visible]]	[[unclear: column header not visible]]	[[unclear: column header not visible]]	[[unclear: column header not visible]]
	24	11.018	7-Ethenyl-bicyclo[4.2.0]oct-1-eneBicyclo[4.2.0]oct-1-ene, 7-exo-ethenyl-	–	0.07	–	–	–
	25	11.246	2,5-Dimethyl-3-ethenyl-1,4-hexadieneSantolina triene	–	–	0.21	–	–

Category	No.	Retention time	Compound	[[unclear: column header not visible]]	[[unclear: column header not visible]]	[[unclear: column header not visible]]	[[unclear: column header not visible]]	[[unclear: column header not visible]]
	26	11.436	1,3,3-Trimethyl-2-ethenylcyclohexeneCyclohexene, 2-ethenyl-1,3,3-trimethyl-	–	0.08	–	–	–
	27	12.180	3-Methyl-2-methylene-bicyclo[3.2.1]oct-3-eneBicyclo[3.2.1]oct-2-ene, 3-methyl-4-methylene-	–	0.06	0.37	–	–
	28	12.358	1,3,5,5-Tetramethyl-1,3-cyclohexadiene1,3-Cyclohexadiene, 1,3,5,5-tetramethyl-	–	–	1.28	0.43	–

Category	No.	Retention time	Compound	[[unclear: column header not visible]]	[[unclear: column header not visible]]	[[unclear: column header not visible]]	[[unclear: column header not visible]]	[[unclear: column header not visible]]
	29	12.438	(3E,5E)-2,6-Dimethyl-1,3,5,7-octatetraene(3E,5E)-2,6-Dimethyl-1,3,5,7-octatetraene	—	0.45	—	—	—
	30	12.447	1,2,3,4,5,8-Hexahydronaphthalene1,2,3,4,5,8-Hexahydronaphthalene	—	—	—	0.24	—
	31	13.09	1,4-Pentadiene1,4-Pentadiene	7.47	0.06	0.21	—	—
	32	15.301	3,3,6-Trimethyl-1,4-heptadiene3,3,6-Trimethyl-1,4-heptadiene	—	0.03	—	—	—
	33	18.405	4-Methyl-1,4-heptadiene4-Methyl-1,4-heptadiene	—	—	—	—	0.11
	34	20.291	3-PentylcyclohexeneCyclohexene, 3-pentyl-	—	—	—	0.52	—

Category	No.	Retention time	Compound	[[unclear: column header not visible]]	[[unclear: column header not visible]]	[[unclear: column header not visible]]	[[unclear: column header not visible]]	[[unclear: column header not visible]]
	35	22.025	1,1-DicyclopropyletheneCyclopropane, 1,1′-ethenylidenebis-	—	—	—	0.12	—
			Total	23.23	1.31	2.79	1.31	2.59
Alcohols								
	36	10.84	2-Isopropylidene-α-methyl-cyclopropanemethanolCyclopropanemethanol, 2-isopropylidene-.alpha.-methyl-	—	—	0.19	—	—
	37	11.584	Linalool1,6-Octadien-3-ol, 3,7-dimethyl-	—	—	—	—	1.34
	38	13.842	(-)-4-Terpineol3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	—	—	—	—	0.15
	39	14.269	α-Terpineolp-menth-1-en-8-ol	—	—	—	—	1.99

Category	No.	Retention time	Compound	[[unclear: column header not visible]]	[[unclear: column header not visible]]	[[unclear: column header not visible]]	[[unclear: column header not visible]]	[[unclear: column header not visible]]
	40	15.314	2,5-Dimethyl-2-ethenyl-hex-4-en-1-ol4-Hexen-1-ol, 2-ethenyl-2,5-dimethyl-	–	0.04	–	–	–
	41	15.999	2,7-Dimethyl-2,6-octadien-1-ol2,6-Octadien-1-ol, 2,7-dimethyl-	–	–	–	–	0.54
	42	17.276	6-Methyl-1-heptanol1-Heptanol, 6-methyl-	–	–	–	–	0.07

Category	No.	Retention time	Compound	[[unclear: column header not visible]]	[[unclear: column header not visible]]	[[unclear: column header not visible]]	[[unclear: column header not visible]]	[[unclear: column header not visible]]
	43	17.487	3,6-Dimethoxy-9-(2-phenylethynyl)-9-fluorenolFluoren-9-ol, 3,6-dimethoxy-9-(2-phenylethynyl)-	45.90	–	–	–	–
			Total	45.90	0.04	0.19	0	4.09
Phenols	44	11.432	4-tert-Butylphenol4-tert-Butylphenol	–	–	0.15	–	–
			Total	0	0	0.15	0	0

Compound class	No.	Retention time	Compound	A	B	C	D	E
Esters	45	11.593	1,5-Dimethyl-1-vinyl-4-hexenyl butyrate	–	–	0.72	–	–

Compound class	No.	Retention time	Compound	A	B	C	D	E
Esters	46	11.601	Linalyl propionate; 1,6-Octadien-3-ol, 3,7-dimethyl-, propanoate	—	0.44	—	—	—
Esters	47	16.701	Bornyl acetate	—	—	—	—	1.68
Esters	48	18.709	(Z)-3,7-Dimethyl-2,6-octadien-1-ol acetate; 2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	—	—	—	—	0.34
Esters	49	19.225	Geranyl acetate; 2,6-Octadien-1-ol, 3,7-dimethyl-, acetate	—	—	—	—	3.89

Compound class	No.	Retention time	Compound	A	B	C	D	E
Esters	50	19.526	2-Propenoic acid, 3-phenyl-, 4-methyl-2-oxo-2H-1-benzopyran-7-yl ester	—	—	—	—	0.10
Esters			Total	0	0.44	0.72	0	6.01
Amides	51	8.518	3-Ethenyl-2-(3-pentenylidene)-*N*-phenylcyclopentanecarboxamide, [1α,2Z(E),3α] configuration; Cyclopentanecarboxamide, 3-ethenyl-2-(3-pentenylidene)-*N*-phenyl-, [1α,2Z(E),3α]- (9CI)	26.20	—	—	—	—

Compound class	No.	Retention time	Compound	A	B	C	D	E
Amides			Total	26.20	0	0	0	0
Other compounds	52	12.722	(1α,2β,4β,5α)-3,3,6,6-Tetramethyltricyclo[3.1.0.02,4]hexane	–	–	–	0.23	–
Other compounds	53	12.916	Benzyl nitrile	–	–	–	6.08	–
Other compounds	54	14.434	1,3,5,7-Cyclooctatetraene, 1-(methoxymethyl)-	4.67	–	–	–	–
Other compounds	55	17.483	4-(4-Chlorophenyl)-2,6-diphenylpyridine	–	0.17	–	–	0.45
Other compounds	56	17.711	4-Methyl-2-pentenoic acid; 2-Pentenoic acid, 4-methyl-	–	0.03	–	–	–
Other compounds			Total	4.67	0.20	0	6.31	0.45

Note: **A.** *Begonia lanternaria*; **B.** *B. longgangensis* male flower; **C.** *B. longgangensis* female flower; **D.** *B. luzhaiensis* flower; **E.** *B. pseudoleprosa*. –indicates not detected.

Figure 1 (plot labels). Y-axis: Relative content (%). Legend categories: Terpenes; Amides; Phenols; Alcohols; Esters; Other compounds; Hydrocarbons (excluding terpenes). Percent labels visible by bar, from bottom to top: A, 23.23%, 45.90%, 26.20%, 4.67%; B, 98.01%, 1.31%, 0.44%, 0.20%, 0.04%; C, 96.10%, 2.84%, 0.19%, 0.15%, 0.72%; D, 92.38%, 1.31%, 6.31%; E, 86.86%, 2.59%, 4.09%, 6.01%, 0.45%.

A. *B. lanternaria*; B. *B. longgangensis* male flower; C. *B. longgangensis* female flower; D. *B. luzhaiensis* flower; E. *B. pseudoleprosa*. The same below.

Figure 1. Types and proportions of volatile components in flowers of four *Begonia* species

2.1.2 Principal component analysis

Seventeen components with relative contents greater than 2% among the floral volatile components of the four *Begonia* species were selected for principal component analysis. Figure 2 shows that the contribution rate of PC1 was 52.7%, and that of PC2 was 33.0%; together they contributed 85.7%, indicating that these two principal components can largely reflect the major characteristic information of the original samples. Among them, 1R-α-pinene (1), camphene (2), β-pinene (3), and β-myrcene (4) had positive effects on PC1; compounds such as (*E*)-β-ocimene (8) had a marked negative effect on PC1; compounds such as 1,8-nonadiyne (22) had a positive effect on PC2; and (*Z*)-β-ocimene (9) and α-farnesene (17) had negative effects on PC2.

According to the positions of the sample points in Figure 2, the volatile components of the five samples from four *Begonia* species were distributed across three quadrants. *B. luzhaiensis*, the female flowers of *B. longgangensis*, and the male flowers of *B. longgangensis* were all located in the third quadrant (the region of the negative PC1 axis and negative PC2 axis), indicating that their volatiles had relatively similar chemical compositions; the principal substances in this region included (*Z*)-β-ocimene (9). *B. lanternaria* and *B. pseudoleprosa* were distributed in different quadrants from the other samples, indicating that the volatile components of these two flowers differed markedly. The main substances in *B. lanternaria* included 1,8-nonadiyne (22) and four others; the main substances in *B. pseudoleprosa* included 1R-α-pinene (1), camphene (2), and β-pinene (3).

(Figure labels: PC1 (52.7%); PC2 (33.0%); legend: A, B, C, D, E.)

The numbers in the figure correspond to the serial numbers in Table 1.
The number in the figure is the same as the serial number in Table 1.

Figure 2 Principal-Component (PCA) Analysis
Fig. 2 Principal component analysis

2.2 Quantitative Analysis of Floral Volatile Components in Four *Begonia* Species

The quantitative analysis mainly involved two aspects: the types of floral volatile substances and their relative contents in the four species. In terms of component richness, the order from high to low was: Nonggang begonia (27 types) > false rough-leaved begonia (22 types) > Luzhai begonia (10 types) > Dengguo begonia (5 types). As shown by the Venn diagram in Figure 3, the four *Begonia* species had no component shared by all of them. The floral volatile substances of false rough-leaved begonia, Nonggang begonia, and Luzhai begonia shared the common component (Z)-β-ocimene, but its relative content differed among the species.

(1) **Dengguo begonia:** This species had only five volatile components in its flowers, namely (methoxymethyl)-1,3,5,7-cyclooctatetraene (8.64%), 1,4-pentadiene (13.80%), 3-vinyl-2-(3-pentenylidene)-N-phenylcyclopentanecarboxamide, $[1\alpha, 2Z(E), 3\alpha]$ configuration (48.43%), and 1,8-nonadiyne (29.13%). Among these, the latter two components were unique to Dengguo begonia and were its major volatile substances.

(2) **Nonggang begonia:** A total of 27 volatile compounds were detected in the fresh flowers of this species; 13 and 20 types were detected in female and male flowers, respectively. Both female and male flowers contained six compounds, including (Z)-β-ocimene, β-myrcene, (E)-β-ocimene, and 1,4-pentadiene. The relative contents of (Z)-β-ocimene in female and male flowers were as high as 92.07% and 93.49%, respectively. The male flowers had 13 unique components (3.52%), but their contents were all relatively low; even the most abundant, allo-ocimene, accounted for only 1.23%. The female flowers had fewer volatile components than the male flowers, only 13 types, including six unique components (3.38%). This indicates that the floral volatile components of female and male flowers of this species differed to some extent in type, but the differences were not significant; both were dominated by terpenes. Nonggang begonia shared one common component each with Dengguo begonia and Luzhai begonia, respectively [$1,4$-pentadiene, (E)-β-ocimene].

(3) **Luzhai begonia:** A total of 10 volatile substances were analyzed from the flowers of this species. The major components included (Z)-β-ocimene (81.48%), α-farnesene (8.52%), phenylacetonitrile (6.07%), and (E)-β-ocimene (1.76%). It had eight unique components, among which the relatively abundant ones were α-farnesene (8.52%) and phenylacetonitrile (6.07%).

(4) **False rough-leaved begonia:** Twenty-two volatile components were detected in the flowers of this species. The major components included (S)-(-)-limonene (42.34%), β-myrcene (22.43%), and 4-terpinene (8.08%). It had as many as 18 unique components (accounting for 71.06%), among which (S)-(-)-limonene had the highest content. It had, respectively, 1 common component with Luzhai begonia and with the female and male flowers of Nonggang begonia.

one component (β-pinene), two components [β-myrcene and (Z)-β-ocimene], and three components [β-myrcene, (Z)-β-ocimene, and 4-(4-chlorophenyl)-2,6-diphenylpyridine], respectively; no common components were shared with *B. lanternaria*.

[Figure: Venn diagram with four groups labeled B. lanternaria*,* B. luzhaiensis*,* B. pseudoleprosa*, and* B. longgangensis*.]*

Figure 3. Venn diagram analysis of volatile components in four *Begonia* species

Fig. 3 Venn diagram analysis of volatile components in four *Begonia* species

3 Discussion and Conclusions

3.1 Discussion

3.1.1 Interspecific differences in volatile components of *Begonia* Existing studies on floral volatile substances in *Begonia* have all used HS-SPME-GC-MS technology, but because of differences in provenance, growth environment, and material-processing methods, the volatile components and their numbers differ among species (Liang Xiaoli et al., 2022). For example, Ding Youfang et al. (2021) identified 21 volatile substances from the male flowers of fragrant-flowered begonia produced in Xiamen, Fujian, dominated by aldehydes (57.12%), among which furfural accounted for 43.53%. In contrast, the volatile substances of its varieties, prostrate begonia (female flowers) and red-haired fragrant-flowered begonia (male flowers), numbered 32 and 44, respectively; both varieties were dominated by alcohols (50.10% and 38.22%), with cis-linalool oxide accounting for the highest proportions (21.90% and 20.00%). Liang Xiaoli et al. (2022), in their study of fresh flowers and frozen flowers of fragrant-flowered begonia introduced and cultivated in Guangzhou, likewise found alcohols to be dominant (65.10% and 62.40%), with 1-octen-3-ol accounting for the highest proportions (33.48% and 20.78%). The fresh flowers contained as many as 63 volatile components, markedly higher in number than the components reported by Ding Youfang et al. (2021) for male flowers of the same plant species (21), which may be attributable to differences in ecological factors and in the flower parts sampled. Meanwhile, the study by Liang Xiaoli et al. (2022) also found that, after fresh flowers were subjected to freezing treatment, the proportions of alcohols, ketones, and heterocyclic compounds in frozen-flower samples decreased, whereas the proportions of terpenes and aldehydes increased. It can thus be seen that differences in species provenance, floral organ type (male/female flowers), and physiological state (fresh/frozen flowers) have important effects on the distribution and contents of volatile components.

In addition, Du Wenwen et al. (2022) found that false thick-leaved begonia and Chinese begonia were dominated by terpenes (75.44% and 78.61%), while willow-leaved begonia and Li' s begonia were dominated by benzenoids/phenylpropanoids (both accounting for 49.63%). In terms of volatile-component richness, the order from high to low was willow-leaved begonia (28 compounds), false thick-leaved begonia (23 compounds), Chinese begonia (21 compounds), and Li' s begonia (15 compounds). Subsequently, Liang Xiaoli et al. (2025) detected 30 floral volatile components in beautifully striped begonia, dominated by heterocyclic compounds (60.14%); whereas black-leaved begonia (56 compounds) and Basaros begonia (44 compounds) were both dominated by alcohols (46.90% and 56.09%). Comparison of the present study with the above literature shows that volatile components in *Begonia* exhibit both the shared feature that terpenoid substances are enriched among the floral volatile components of certain species and pronounced species specificity. *B. longgangensis*, *B. luzhaiensis*, and *B. pseudoleprosa* were all dominated by terpenoid substances, consistent with the high terpene proportions (75.44%-78.61%) reported by Du Wenwen et al. (2022) for false thick-leaved begonia and Chinese begonia. The differences are reflected in the differentiation between monoterpenes and sesquiterpenes: the volatile substances in the flowers of *B. longgangensis* and *B. luzhaiensis* were dominated by

monoterpene [(Z)-β-ocimene] predominated; false rough-leaved begonia was dominated by monoterpenes [(S)-(-)-limonene and β-myrcene], whereas false thick-leaved begonia and Chinese begonia were dominated by sesquiterpenes such as β-caryophyllene and α-farnesene. In addition, the number of compound types detected in this study was smaller than in other studies, and the relative content of (Z)-β-ocimene was abnormally high; this is the first discovery in *Begonia* of a phenomenon in which a single component is highly enriched. Although no previous report has documented an ultra-high proportion of a single volatile constituent in *Begonia*, cross-taxon studies support the biological plausibility of such a phenomenon. Qin Yongzhi et al. (2025) found that the relative content of (E)-β-ocimene in the fresh flowers of four cultivars of Bauhinia reached 78.26$\pm$6.50%, and that monoterpenes and sesquiterpenes together accounted for more than 95%, although the specific component ratios differed among cultivars. Hu Guiting et al. (2025) also detected β-phellandrene (77.49%-80.65%) as the dominant floral scent component of willow-leaved wintersweet. In addition, Lu Yonggui et al. (2025) confirmed that limonene contents in the peels of seven citrus species all exceeded 82%. The factors affecting the production of terpenoid compounds in plants are mainly genetic control and ecological environmental conditions (Peng Shaolin et al., 2002). Nonggang begonia, Luzhai begonia, and false rough-leaved begonia were introduced from karst areas of Guangxi. In their native habitats, soils are shallow, poor, and mostly weakly alkaline. Such environmental conditions drive plants to develop distinctive physiological and biochemical characteristics to adapt to extreme soil and water conditions; for example, the release of special volatile organic compounds can help plants resist harsh environments and may also participate in plant defense (Hu Shujing and Zhang Rumin, 2022). β-Ocimene is a signaling molecule closely associated with the initiation of plant defense (Liu Wu et al., 2012). It can induce defensive responses in plants and is beneficial for species in resisting external biological stress. As an isomer of β-ocimene, the extremely high proportion of (Z)-β-ocimene in the flowers of Nonggang begonia and Luzhai begonia may be an adaptive response of the species to pollinator attraction or insect-resistance defense, and may be closely related to an insect-mediated pollination strategy. The flowers of lantern-fruited begonia contained few volatile substances and lacked terpenoid compounds; the release of special alcohols may also be related to its distinctive ecological niche or reproductive strategy, or may have been caused by cultivation conditions and environment (potted in a greenhouse), extraction methods, analytical instrument parameter settings, and other factors. Compared with previous studies on floral volatiles of *Begonia*, this study detected, for the first time in *Begonia* plants, components such as 3-methyl-6-(1-methylethylidene)cyclohexene, trans-1,3-pentadiene, and 4-terpinene. This adds new data and perspectives to research on volatile constituents in *Begonia*, while also providing new directions for chemical classification, mechanisms of ecological adaptation, and resource development and utilization in this genus.

3.1.2 Multidimensional application value of volatile constituents in *Begonia* plants

Plant volatile organic compounds (VOCs), as important products of plant secondary metabolism, play multiple roles in nature and in human life. These low-molecular-weight, readily volatile compounds not only participate in information exchange between plants and the environment (Li Jun, 2016), but also show broad application prospects in fields such as food, medicine and health, agricultural production, horticultural therapy and wellness tourism, and mental-health regulation.

Terpenoid compounds exhibit important value in multiple fields because of their diverse biological activities and natural attributes. Ocimene—especially its cis isomer, (Z)-β-ocimene—has a fresh citrus note and also possesses antibacterial and antioxidant activities, giving it significant application potential in organic foods and natural cosmetics. Studies have shown (Liu Wu et al., 2012; Liu Rong et al., 2021) that β-ocimene, as a plant communication signaling molecule, can induce systemic defense responses in plants and has major application prospects in green pesticides and insect-resistance inducers. Limonene, owing to its pleasant lemon aroma and biological activities such as antibacterial, antioxidant, anti-inflammatory, and antitumor effects, has been widely applied in food preservation, pharmaceutical preparations, and natural flavor additives. At the same time, its insecticidal, bactericidal, and herbicidal activities allow it to serve as a pesticide adjuvant to improve the control efficacy against diseases, insect pests, and weeds, showing important potential in green prevention and control of agricultural diseases, pests, and weeds (Li Yeqing et al., 2023). Myrcene has analgesic, anti-inflammatory, and anticatabolic effects (Wang Yuancheng et al., 2022), and its balsamic aroma makes it an intermediate for fragrance synthesis. In addition, terpenes such as linalool and α-pinene have also been confirmed to be associated with anti-inflammatory activity (Dong Lihua et al., 2017). Guan Kaiyun et al. (2005) also confirmed through research that the volatile substances released by 13 *Begonia* species, including thick-walled begonia and false thick-leaved begonia, have inhibitory effects on various pathogenic microorganisms. Given the high relative proportion of (Z)-β-ocimene in the volatile constituents of Nonggang begonia and Luzhai begonia flowers, the authors believe that the flowers of these two plants can be used as extraction materials for natural plant-derived ocimene and as new raw materials for plant essential oils. In the flowers of false rough-leaved begonia, the content of (S)-(-)-limonene reached as high as 42.34%; this component can not only be used as a natural flavoring agent and antioxidant, but also as a plant-derived pesticide for biological control.

At present, the health-regulating functions of terpenoids are promoting their application in horticultural therapy and wellness. α-Pinene, β-pinene, limonene, myrcene, and others can reduce stress and relieve anxiety (Donelli et al., 2023); ocimene can lower the risk of cardiovascular disease; and myrcene, limonene, phellandrene, and others can promote sedation and calming, clear the mind and open the orifices, and increase sleep duration (Hossain et al., 2004; Yamada et al., 2005). In addition, these terpenoids also possess antifungal, insect-repellent, and broad-spectrum antibacterial functions. Among the 56 floral volatile substances detected in this study, 29 volatile components with obvious aroma could be found through the literature (Hao Ruijie, 2014;

(Du Wenwen et al., 2022; Zhang Peng et al., 2025). Among them, 14 aroma components were detected in fresh flowers of Jialaiye begonia, including relatively high contents of β-pinene (5.57%), β-myrcene (22.35%), and *(S)*-(-)-limonene (up to 42.20%); β-myrcene presents a light resinous aroma, while *(S)*-(-)-limonene has a fresh lemon-like scent. In Nonggang begonia and Luzhai begonia, 7 and 6 aroma components were detected, respectively, mainly the citrus-aroma compound *(Z)*-β-ocimene. In addition, compounds with relative contents exceeding 5% in fresh flowers of Luzhai begonia included phenylacetonitrile (6.07%) and α-farnesene (8.52%). For the volatile substances in fresh flowers of Dengguo begonia, no direct literature reports currently describe the odors of the compounds in its flowers, but a faint aromatic fragrance can be perceived at close range. These findings further support the application of these four begonias in horticultural therapy and wellness-oriented tourism landscape development. Among the 29 identified aromatic volatile substances, *(S)*-(-)-limonene, which is unique to Jialaiye begonia, and *(Z)*-β-ocimene, which occurs at high contents in the flowers of Nonggang begonia and Luzhai begonia, can both serve as key aroma markers for scent-based breeding. However, caution is also needed in application: limonene and ocimene may irritate the skin, and direct contact with the flowers may cause allergic reactions such as redness, itching, and burning sensations. Therefore, high-risk groups such as pregnant women, children, and individuals with allergic constitutions should avoid exposure during use.

3.2 Conclusion

In summary, plants of the genus *Begonia* exhibit extremely high interspecific diversity in volatile components. This diversity is reflected not only in the types and contents of volatile compounds among different species, but also in the significant differences in their aroma characteristics. Using HS-SPME-GC-MS technology, this study identified 56 volatile organic compounds from the fresh flowers of four *Begonia* species characteristic of Guangxi. The richness of these volatile components, in descending order, was Nonggang begonia (27 compounds), Jialaiye begonia (22 compounds), Luzhai begonia (10 compounds), and

Dengguo begonia (5 compounds). No common component was shared among the floral volatiles of these four begonias. Jialaiye begonia had the greatest number of species-specific volatile components, with 18; Nonggang begonia possessed 13 male-flower-specific and 6 female-flower-specific volatiles; Luzhai begonia contained 8; and Dengguo begonia had only 4 unique components. Nonggang begonia, Luzhai begonia, and Jialaiye begonia belong to the monoterpene-dominant type, whereas Dengguo begonia is dominated by special alcohol components. The *(Z)*-β-ocimene-dominant type reported for the first time in this study adds a new chemotype to research on volatile components in begonias, while the special alcohol components of Dengguo begonia suggest that this species may possess previously undiscovered secondary metabolic pathways. These findings not only expand our understanding of the chemical diversity of begonias, but also provide new clues for subsequent studies on the pollination ecology and evolutionary adaptation of this genus. At the same time, the marked differences in these volatile components confer unique aromatic characteristics on different begonia species, providing valuable reference information for germplasm resource evaluation, cultivar improvement, horticultural application development, and wellness tourism. Future studies may further explore the effects of different environmental conditions on volatile components, as well as the distribution patterns of volatile components in different plant tissues, in order to provide scientific support for fragrance improvement, new cultivar selection, development and utilization of regionally distinctive flowers, and sustainable use and ecological conservation of plants in the genus *Begonia*.

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