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Characterization of Volatile Organic Compounds and Aroma of Eight Bamboo Species Leaves

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Abstract: Bamboo forest healthcare tourism is a practical and sustainable management model that utilizes the medicinal functions of bamboo. However, the mechanism and potential functions of bamboo's healthcare functions are still unclear. In this study, the volatile organic compounds (VOCs) that are the core factor of bamboo forest healthcare were analyzed. The foliar VOCs of eight bamboo species, including *Pleioblastus amarus* (Keng) P. C. Keng, *Pleioblastus maculatus* (McClure) C. D. Chu et C. S. Chao, *Pleioblastus juxianensis* T. H. Wen, C. Y. Yao et S. Y. Chen, *Acidosasa chienouensis* (T. H. Wen) C. S. Chao et T. H. Wen, *Pseudosasa amabilis* (McClure) P. C. Keng ex S. L. Chen et al., *Pseudosasa amabilis* (McClure) Keng f., *Phyllostachys rubromarginata* McClure, and *Phyllostachys hirtivagina* G. H. Lai were qualitatively and semi-quantitatively analyzed by headspace solid-phase microextraction (HS-SPME)–gas chromatography–mass spectrometry (GC-MS). Screening compounds by aroma vitality value (OAV) determined the key aromas. The results showed that a total of 40 VOCs were identified from the leaves of the eight bamboo species. The compounds with relatively high content were (*Z*)-3-Hexen-1-ol, (*E*)-2-Hexen-1-ol, 1-Hexanol, (*E*, *E*)-2,4-Hexadienal, Limonene, and so on. The commonality of different bamboo species was that the dominant groups consisted of alcohols and aldehydes. The significant differences in leaf VOCs among species presented classification. *Pleioblastus amarus*, *Acidosasa chienouensis*, *Pseudosasa amabilis*, and *Phyllostachys rubromarginata* were noticeably clustered together. The aroma of bamboo leaves is a combination of grassy, fruity, and piney notes by 24 VOCs. The key aroma from *Pleioblastus amarus* is leaf alcohol, which contributes to the grassy scent, while the piney aroma is dominant in *Pseudosasa amabilis* and *Phyllostachys rubromarginata*. The study provides a reference value for enriching the chemical information of subtropical bamboo and developing the functional potential of bamboo forest healthcare tourism.

Keywords: bamboo; volatile organic compounds (VOCs); odor activity value (OAV); healthcare function

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1. Introduction

Bamboo is naturally distributed in about 80 countries and regions around the world, with a total bamboo forest area of more than 50 million hm^2 in 39 countries known globally [1]. China is one of the richest countries in the world in terms of bamboo resources, with bamboo forests covering 7.02 million hectares [2]. Bamboo forests play an important role in local ecosystems and social economies due to their carbon sequestration, soil and water conservation, building materials, and biomass energy [3], and are widely welcomed in tropical and subtropical regions [4]. It is noteworthy that the global area of bamboo forests is increasing at a rate of 3% per annum at a time when the world's forest area is declining sharply, thus showing that bamboo is fulfilling great potential [5]. However, in recent years, the above traditional use has led to long-term intensive management and over-harvesting of bamboo monoculture commercial forests, severely degrading soil

fertility and productivity [6], and damaging bamboo forest ecosystems. Meanwhile, in the context of rising raw material production and labor costs, farmers' enthusiasm for managing bamboo forests has been affected [7]. Therefore, it is urgent to explore effective sustainable management models for bamboo forests.

The healing functions of utilizing bamboo have been a tradition since ancient times [8]. In recent years, bamboo forest healthcare tourism has received increasing attention and popularity in Asia as a mode of sustainable bamboo forest management. For example, Shunan Bamboo Sea in Sichuan, China, the micro-scale bamboo forest space provides a comfortable thermal environment, clean air in all seasons, and strong bactericidal ability [9,10]. Research in Korea has predicted that microclimatic factors within moso bamboo forests can influence the release of natural volatiles, which in turn affects the effectiveness of bamboo forest recreation [11]. In terms of psychological regulation, comparing volunteers in an urban environment, bamboo forest therapy was able to significantly increase positive emotions and decrease negative emotions [12]. These practical pieces of evidence demonstrate the availability of bamboo forest healthcare as an important forest resource model. Studies have confirmed the nutritional edible and medicinal capacity of bamboo plants, which contain steroids, terpenoids, tannins, flavonoids, polyphenols, alkaloids, glycosides, and phytosterols [13,14] that can be antioxidant, anti-inflammatory, antibacterial, and anticancer [8,15,16]. However, existing studies focus on extracting ingredients and natural flavors in the pharmaceutical and cosmetics industries. For example, in vitro and/or in vivo studies have shown that bamboo leaf extracts have significant antioxidant activity and treat respiratory illness [17,18]. These empirical studies have proved the recreational potential of bamboo leaves. According to reports [19,20], the natural volatile organic compounds (VOCs) released by most plants have strong physiological activity and aromatic odor and have healthcare effects on human physiological and psychological diseases, which is the core factor of forest health functions. However, there are few relevant studies on VOCs in bamboo species.

Considering that bamboo has a wide geographical distribution, and the diversity is a repository of untapped sources of aromatic plants. China is the country with the highest diversity of bamboo species in the world [21], but its expressive richness is highly underdeveloped. Plants rich in bioactive compounds are receiving increasing attention and interest due to increased health awareness and support from various scientific claims [22]. Therefore, it is a very meaningful work to increase the study of VOCs from a wide range of bamboo. Headspace solid-phase microextraction–gas chromatography–mass spectrometry (HS-SPME-GC-MS) is the most versatile strategy for capturing volatility from the sample headspace in static mode. Since solid-phase microextraction (SPME) uses fibers coated with an adsorbent phase to combine extraction and preconcentration of compounds, it exhibits better extraction efficiency. Gas chromatography–mass spectrometry (GC-MS) has high sensitivity and is able to collect and analyze as many metabolites as possible [23]. Given the advantages of this method, it is by far the most commonly used technique in all areas of plant volatile analysis. For example, studies have been conducted to characterize key odor-active compounds (OAV) in moso bamboo leaves by SPME-GCMS [24]. The concept of odor landscape (smellscape) was first proposed by Porteous and was gradually focused on by landscape research [25]. Liu et al. [26] concluded that negative odor landscapes can directly affect the experience of visiting forest parks. To verify the odor perception of the components in different bamboo species, this study combined the aroma vitality value to characterize the key aroma components of bamboo leaves based on the extraction of VOCs, providing a reference for the further development and utilization of the odor landscape in bamboo forest healthcare tourism.

This study focused on the VOCs' health efficacy of different bamboo leaves and conducted qualitative and semi-quantitative research on the components of eight bamboo compounds that have never been reported before. To provide scientific references for the sustainable management mode of bamboo forests, VOCs of the eight bamboo leaves were characterized by HS-SPME-GC-MS, and their key contributing aroma components were analyzed by OAV. The aims were to determine the following: (a) the compositional and content characteristics of VOCs and the differences among eight bamboo species; and (b) the key aroma components of different bamboo species.

2. Materials and Methods

2.1. Study Sites and Experiment Design

Anhui Province, located in East China, is characterized by its diverse bamboo species and a climate conducive to the growth of multiple bamboo varieties. The sampling site is located in the Hengshan National Forest Park in Jingde City, Anhui Province (30°12′26″ N–30°16′24″ N, 118°24′09″ E–118°27′34″ E). The park belongs to the subtropical humid monsoon climate zone, with an annual average temperature of 15 °C, annual average precipitation of 1643 mm, and annual average sunshine of 1972 h. The zonal forest vegetation is a subtropical evergreen broad-leaved forest. The park has a bamboo resource garden with over 200 bamboo species, including common landscape bamboo species distributed over a large area in Anhui Province. Due to overlogging and timber production, there is less native vegetation in the forest park, and most of the existing vegetation is plantations and natural secondary forests.

Through literature review, it was found that the plants release VOCs with the most significant effects during summer [27]. Additionally, summer months are more popular for people's recreational experiences and escape the heat in the study area. Therefore, the investigation and sampling were scheduled in August 2020. Based on the principles of similar altitude and even distribution of sample points, bamboo forests with similar canopy density and other conditions were manually selected. The eight bamboo species included *Pleioblastus amarus* (Keng) P. C. Keng, *Pleioblastus maculatus* (McClure) C. D. Chu et C. S. Chao, *Pleioblastus juxianensis* T. H. Wen, C. Y. Yao et S. Y. Chen, *Acidosasa chienouensis* (T. H. Wen) C. S. Chao et T. H. Wen, *Pseudosasa amabilis* (McClure) P. C. Keng ex S. L. Chen et al., *Pseudosasa amabilis* (McClure) Keng f., *Phyllostachys rubromarginata* McClure, and *Phyllostachys hirtivagina* G. H. Lai. Within each stand, six trees of the same age, with good growth and free from diseases and pests, were chosen. Leaf samples were collected in equal proportions from the southeast, northwest, northeast, and southwest directions at the same height position on each tree. The collected bamboo leaves were promptly placed in liquid nitrogen and stored in a −80 °C freezer for extraction and HS-SPME-GC-MS analysis.

2.2. Sample Extraction and Analysis

2.2.1. SPME Extraction and GC-MS Analysis

After conducting preliminary experiments and comparisons to account for variations caused by different conditions, the following experimental parameters were standardized. After grinding the plant leaf sample, weigh 0.2 g and place it in a 20 mL injection bottle, add 0.5 μL of internal standard (2-Octanol), and heat in a water bath at 40 °C. For headspace extraction, an SPME fiber assembly, specifically an aromatic-type divinylbenzene/carboxen/polydimethylsiloxane fiber (50 μm/30 μm DVB/CAR/PDMS) was used [28]. Before each extraction, the gas chromatography inlet was aged and desorbed at 250 °C for 5 min, then inserted into a sampling bottle with a polytetrafluoroethylene silicone stopper, and the fiber head was pushed out and placed in the sample headspace for adsorption for 45 min. After the extraction, the fiber head was inserted into the gas chromatograph inlet for a 5 min analysis immediately, followed by GC-MS analysis.

Analysis conditions were as follows: the chromatographic column used was a TG-5MS (30 m × 250 μm × 0.25 μm); the injection port temperature was set at 250 °C; high-purity helium (He) was used as the carrier gas at a column flow rate of 1 mL min^{-1}. The

temperature program for the oven to heat up from 40 °C to 210 °C, with a heating rate of 4 °C min^{-1} and hold for 5 min. The transfer line temperature was maintained at 260 °C, the ion source temperature at 230 °C, and the quadrupole temperature at 150 °C. The electron ionization voltage was set at −70 eV, with a splitless mode of injection. The detection mode employed was full scan, covering a mass range (m/z) from 30 to 500.

The data obtained through GC/MS analysis were subjected to format conversion and imported into the MSDIAL software (version 4.70) for total ion chromatogram peak detection, matching, and alignment. Based on NIST, METLIN, MASSBANK, and other databases and combined with n-alkanes, the retention index is calculated to assist in analyzing the compound components. Following internal standard correction of compound peak areas, any values with over 80% missing data were considered invalid and removed. For the remaining missing values, a multiple imputation method was employed. Subsequently, data were subjected to interquartile range (IQR) filtering and normalization, resulting in the quantification of compound components. Since only 2-Octanol was used for the internal standard, the content was determined semi-quantitatively, which is calculated using the following formula [29]:

$$Q_i = A_i \times M_s / A_s \times M \quad (1)$$

In the equation, Q_i represents the content of the compound to be measured, g/g FW, while M_s is the mass/g of internal standard s added, A_i and A_s are the peak areas of component i and internal standard s, respectively; M is the mass/g of the sample to be measured.

2.2.2. OAV Analysis

In general, the determination of whether an individual component qualifies as a key contributor to the overall aroma profile relies on the assessment of its OAV, which is calculated using the following formula [30]:

$$\mathrm{OAV} = C_i / T_i \quad (2)$$

In the equation, C_i represents the absolute content of the volatile aroma component, μg/g, while T_i represents the perception threshold of the aroma component in water, μg/g.

Due to variations in human olfactory sensitivity to different substances, the lowest detectable concentration of a specific substance, often referred to as the perception threshold, is typically measured in mg/mL. In most cases, the unit of mg/kg is used for threshold values for conversion purposes. The OAV is calculated by comparing the content of volatile organic components in bamboo leaves to their respective thresholds. The threshold, representing the lowest concentration at which aromatic compounds can be perceived, is a key indicator of the aroma contribution of a compound. OAV > 1 indicates that the compound has a direct impact on aroma. It is generally believed that volatile organic compounds with higher OAV values contribute more to plant aroma [30]. In this study, 24 compounds were selected with OAV values exceeding 1, which are considered to have a significant impact on aroma contribution.

2.3. Statistical Analysis

The pre-processing of GC-MS raw tags was conducted in Trace Finder software (version 5.1). Data cleaning processes were calculated on the R package "MetaboAnalyst". One-way ANOVA combined with the Fisher's least significant difference (LSD) test to compare VOCs and OAV contents across eight bamboo species leaves. Since our database includes more than 5 groups, the UpSet version can provide a more intuitive representation of the intersections of VOCs [31]. Bubble plot was used to display the content values of each VOC. Principal component analysis (PCA) was used to analyze VOCs of eight bamboo species composition structures. Hierarchical clustering by ward. D was used to analyze the OAV of eight bamboo species.

3. Results

3.1. Volatile Organic Compounds in Leaves of Different Bamboo Species

The VOCs in the leaves of eight bamboo species were analyzed and determined by GC-MS. The ion flow peak diagrams were obtained (Figure 1), and 57 peaks were identified. A total of 40 compounds were identified after excluding impurities such as siloxanes, which are likely bleeding of the column (Table 1). Six species were dominant in all the samples, the mean contents followed by alcohols (55.49%), aldehydes (37.78%), esters (1.58%), terpenoids (4.51%), alkanes (0.45%), and ketones (0.19%) (Figure 2). UpSet datasets showed nineteen compounds to eight bamboo species for shared (Figure 3): (*E*)-2-Hexen-1-ol, 1-Hexanol, 2-Ethyl-1-Hexanol, (*Z*)-3-Hexenal, Hexanal, (*E*)-2-Hexenal, 2-Hexenal, (*E*, *E*)-2,4-Hexadienal, Benzaldehyde, 2-Phenylethanal, Ethyl hexanoic (*Z*)-3-Hexen acetate, acetic acid, hexyl ester, alpha-Pinene, 3-Carene, Limonene, Terpinolene, Germacrene D, beta-Cadinene, 4-Decyne. *P. amabilis* and *Ph. rubromarginata* have the most diverse variety of compounds by 39, while *P. juxianensis* has the fewest by 26. The content of VOCs is mainly concentrated in alcohols and aldehydes in all samples. 2-Hexenal was found the highest content in *P. amarus*, *P. maculatus*, *P. amabilis*, and *Ph. hirtivagina*. (*Z*)-3-Hexen-1-ol was the highest content in *P. juxianensis*, *A. chienouensis*, and *P. amabilis*. The highest content in *Ph. rubromarginata* was 1-Hexenal (Figure 4a). Terpenes are the most diverse volatile organic compounds, with 14 kinds. Among these, Limonene has the highest content of all bamboo species. In addition to 19 common VOCs of all samples, there are unique compounds in some bamboo species. For example, Caryophyllene, beta-Copaene, gamma-Muurolene, and beta-Cyclogermacrane only exists in *P. amarus*, *A. chienouensis*, *P. amabilis* and *Ph. rubromarginata*. Additionally, the total contents of terpenes in *Ph. rubromarginata* and *P. amabilis* have significant advantages.

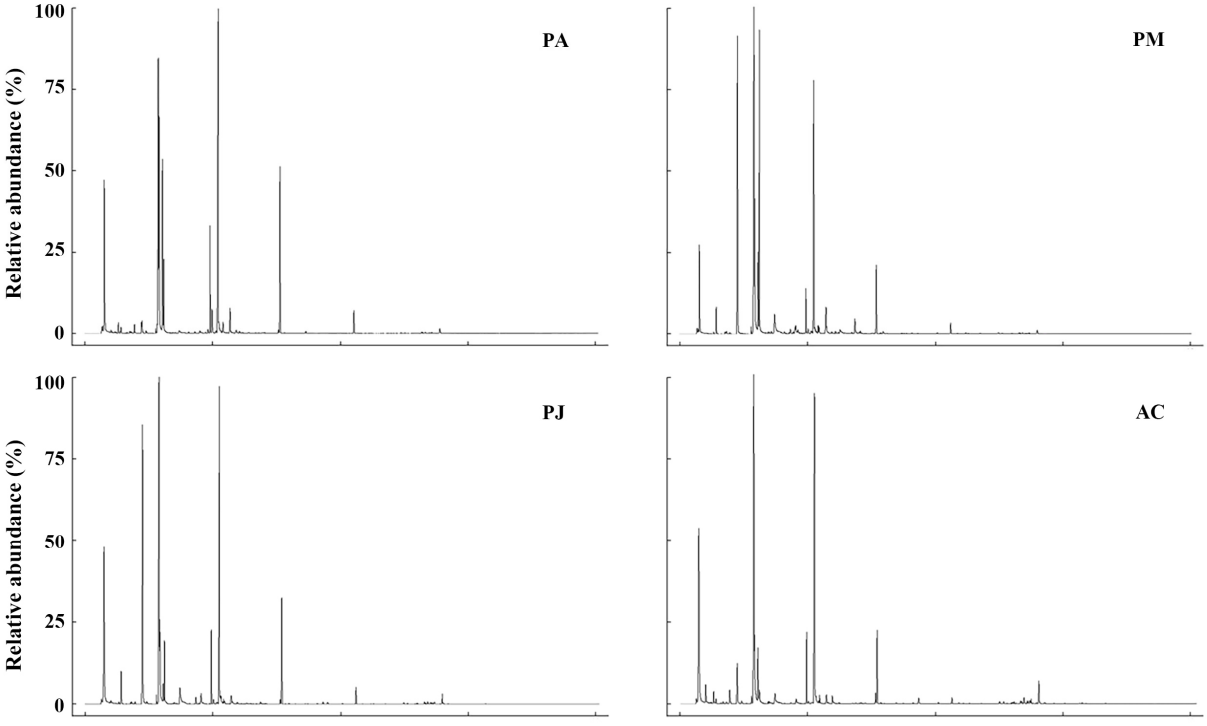

Figure 1. *Cont.*

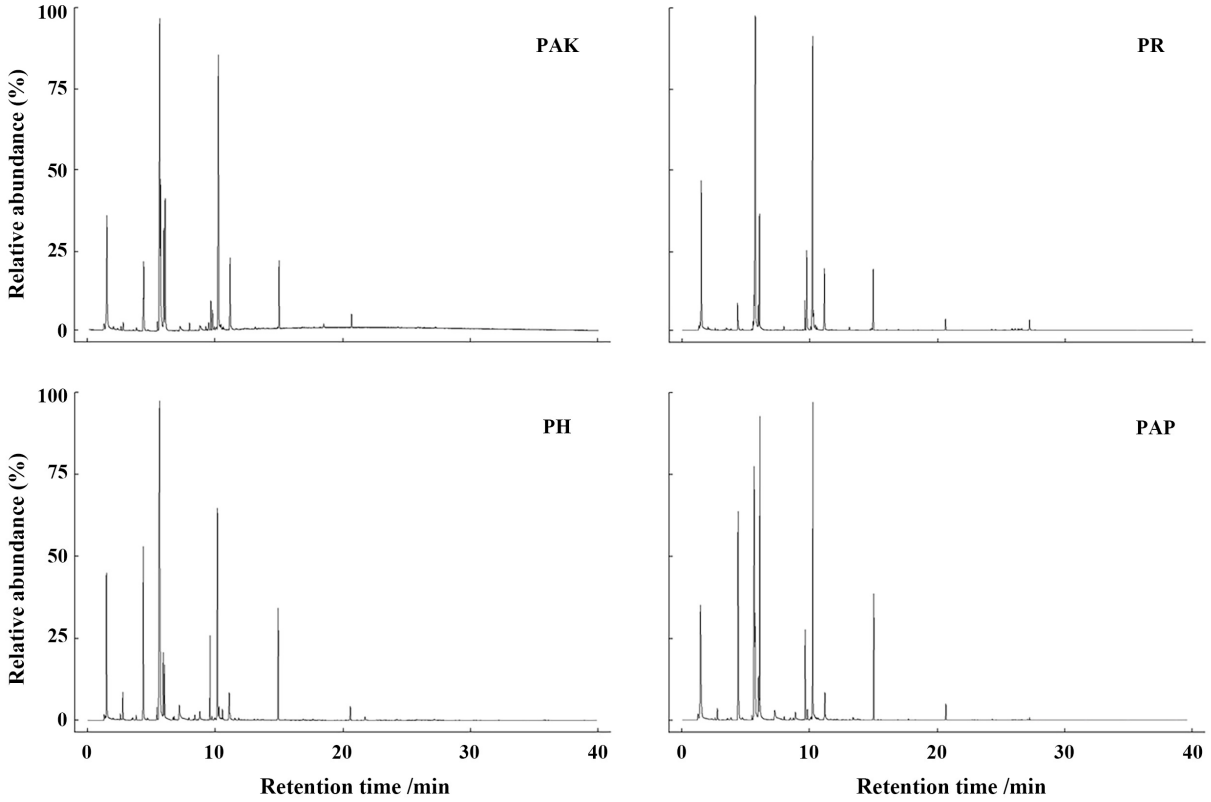

Figure 1. Total ionic chromatogram of eight bamboo species leaves. PA: *Pleioblastus amarus* (Keng) P. C. Keng, PM: *Pleioblastus maculatus* (McClure) C. D. Chu et C. S. Chao, PJ: *Pleioblastus juxianensis* T. H. Wen, C. Y. Yao et S. Y. Chen, AC: *Acidosasa chienouensis* (T. H. Wen) C. S. Chao et T. H. Wen, PAP: *Pseudosasa amabilis* (McClure) P. C. Keng ex S. L. Chen et al., PAK: *Pseudosasa amabilis* (McClure) Keng f., PR: *Phyllostachys rubromarginata* McClure, and PH: *Phyllostachys hirtivagina* G. H. Lai. The same as below.

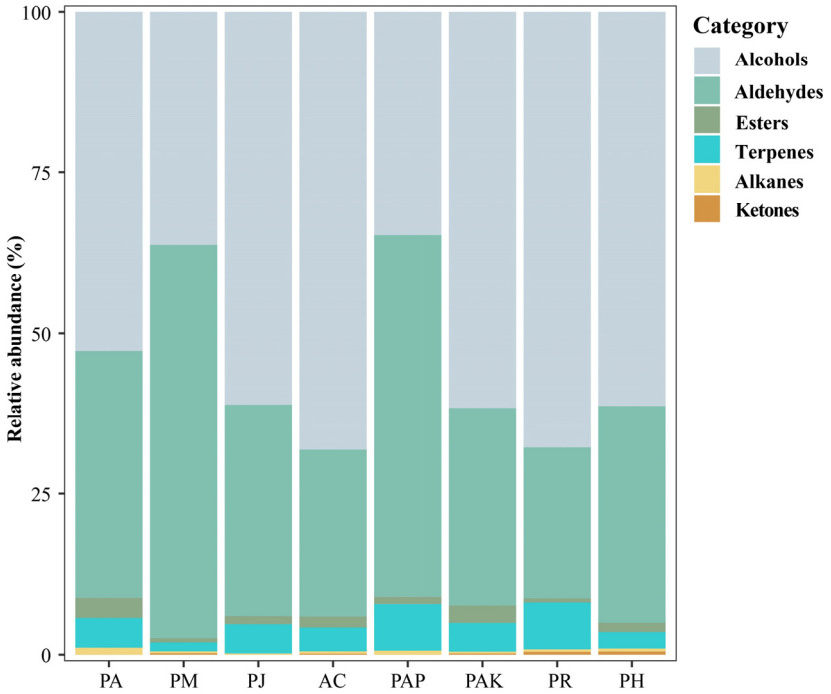

Figure 2. Relative abundance of VOCs among eight bamboo species leaves.

Table 1. Volatile organic components (VOCs) and contents of eight bamboo species leaves.

Category	Compound	CAS#	Content/(μg·g^{-1})							
			PA	PM	PJ	AC	PAK	PR	PH	PAP
Alcohols	(Z)-3-Hexen-1-ol	928-96-1	150.91 ± 6.40 bc	117.06 ± 12.00 c	156.35 ± 29.92 bc	223.22 ± 37.32 b	322.35 ± 48.73 a	143.16 ± 18.07 bc	71.74 ± 12.00 c	-
	(*E*)-2-Hexen-1-ol	928-95-0	131.76 ± 13.45 a	45.46 ± 4.25 cd	95.90 ± 13.24 b	87.02 ± 11.49 b	23.50 ± 4.90 d	45.92 ± 3.43 cd	53.51 ± 7.16 c	51.81 ± 2.80 c
	1-Hexanol	111-27-3	35.97 ± 3.73 e	45.92 ± 6.07 de	90.48 ± 25.10 bc	54.73 ± 3.73 cde	79.40 ± 14.77 bd	177.09 ± 11.83 a	169.95 ± 15.84 a	107.47 ± 8.00 b
	2-Furanmethanol, tetrahydro-	97-99-4	1.77 ± 0.19 a	1.02 ± 0.07 b	0.99 ± 0.10 b	-	-	0.58 ± 0.05 c	0.90 ± 0.11 bc	-
	1-Octen-3-ol	3391-86-4	-	4.52 ± 0.48 a	1.97 ± 0.32 b	4.32 ± 0.29 a	0.91 ± 0.06 c	0.71 ± 0.05 c	0.61 ± 0.03 c	-
	3-Methyl-3-Heptanol	5582-82-1	4.94 ± 0.60 ab	6.08 ± 0.46 a	4.15 ± 0.44 ab	3.27 ± 1.85 bc	2.72 ± 0.53 bc	1.01 ± 0.08 c	0.88 ± 0.09 c	-
	2-Ethyl-1-Hexanol	104-76-7	2.96 ± 0.24 c	8.31 ± 0.84 ab	7.59 ± 0.89 ab	3.63 ± 0.72 c	9.76 ± 1.98 a	7.54 ± 1.12 ab	5.78 ± 1.01 bc	10.19 ± 0.65 a
Aldehydes	(Z)-3-Hexenal	6789-80-6	4.59 ± 0.74 d	21.06 ± 3.43 b	6.22 ± 0.94 cd	31.84 ± 4.57 a	12.49 ± 2.17 c	8.71 ± 1.28 cd	5.89 ± 0.73 cd	5.25 ± 0.49 d
	4-Oxohex-2-enal	20697-55-6	2.63 ± 0.54 b	19.79 ± 1.38 a	5.41 ± 1.17 b	4.42 ± 1.07 b	5.07 ± 0.74 b	3.41 ± 0.54 b	2.90 ± 0.33 b	-
	Hexanal	66-25-1	17.29 ± 1.26 de	63.91 ± 7.58 b	25.03 ± 6.23 d	5.46 ± 0.76 e	42.26 ± 2.24 c	25.75 ± 4.43 d	42.18 ± 2.74 c	92.53 ± 7.63 a
	(*E*)-2-Hexenal	6728-26-3	3.04 ± 0.51 b	6.00 ± 0.16 a	2.85 ± 0.45 bc	1.53 ± 0.18 de	2.46 ± 0.17 bd	1.96 ± 0.33 cde	1.22 ± 0.05 e	2.36 ± 0.35 bd
	2-Hexenal	505-57-7	162.16 ± 11.01 b	217.74 ± 12.80 a	140.43 ± 13.46 b	90.39 ± 9.23 c	129.34 ± 17.88 b	63.84 ± 6.22 c	82.23 ± 2.73 c	132.23 ± 11.40 b
	(*E*, *E*)-2,4-Hexadienal	142-83-6	32.09 ± 4.46 b	48.16 ± 2.22 a	8.73 ± 1.67 d	5.65 ± 1.21 d	19.35 ± 0.67 c	19.14 ± 1.61 c	23.51 ± 3.41 c	33.72 ± 2.30 b
	Benzaldehyde	100-52-7	3.58 ± 0.19 a	1.79 ± 0.11 b	2.02 ± 0.12 b	1.05 ± 0.17 b	1.94 ± 0.21 b	4.07 ± 0.47 a	4.67 ± 0.84 a	1.52 ± 0.31 b
	(*E*, *E*)-2,4-Heptadienal	4313-3-35	2.03 ± 0.19 bc	3.93 ± 0.22 a	-	1.30 ± 0.18 d	1.88 ± 0.10 bd	1.61 ± 0.11 cd	1.84 ± 0.32 cd	2.52 ± 0.26 b
	2-Phenylethanal	122-78-1	9.91 ± 1.00 a	1.86 ± 0.15 bc	1.22 ± 0.13 c	1.25 ± 0.10 c	2.77 ± 0.18 b	1.20 ± 0.09 c	1.38 ± 0.12 c	2.80 ± 0.24 b
	Nonanal	124-19-6	0.40 ± 0.05 bc	0.34 ± 0.03 cd	-	0.18 ± 0.01 d	0.61 ± 0.08 a	0.28 ± 0.01 cd	0.31 ± 0.02 cd	0.53 ± 0.10 ab
	beta-Cyclocitral	432-25-7	0.25 ± 0.01 ab	0.29 ± 0.02 ab	-	-	0.29 ± 0.03 ab	0.24 ± 0.04 b	0.24 ± 0.03 b	0.35 ± 0.05 a
Esters	Ethyl hexanoic	123-66-0	0.46 ± 0.09 c	0.40 ± 0.05 c	2.35 ± 0.31 a	0.90 ± 0.19 bc	1.49 ± 0.30 b	1.15 ± 0.21 b	1.29 ± 0.14 b	0.46 ± 0.07 c
	(*Z*)-3-Hexen acetate	3681-71-8	12.06 ± 1.84 a	2.82 ± 0.36 bc	2.03 ± 0.25 c	5.78 ± 0.53 b	10.80 ± 2.14 a	1.23 ± 0.26 c	4.44 ± 0.86 bc	2.65 ± 0.44 bc
	Acetic acid, hexyl ester	142-92-7	2.44 ± 0.36 ab	0.86 ± 0.19 b	1.44 ± 0.19 b	1.04 ± 0.04 b	4.04 ± 1.07 a	1.05 ± 0.13 b	1.35 ± 0.15 b	2.27 ± 0.85 b
	2-Octyl acetate	2051-50-5	4.22 ± 0.61 a	-	1.43 ± 0.32 c	1.62 ± 0.33 bc	2.65 ± 0.39 b	-	0.56 ± 0.04 c	-
Terpenes	alpha-Pinene	80-56-8	0.86 ± 0.22 b	0.67 ± 0.18 b	2.33 ± 0.40 a	0.88 ± 0.17 b	1.88 ± 0.28 a	2.08 ± 0.40 a	0.74 ± 0.14 b	2.31 ± 0.54 a
	beta-Pinene	127-91-3	-	-	1.60 ± 0.19 a	0.85 ± 0.14 cd	1.11 ± 0.09 bc	1.55 ± 0.20 ab	0.54 ± 0.10 d	-
	3-Carene	13466-78-9	1.02 ± 0.18 b	0.55 ± 0.07 b	1.83 ± 0.27 a	0.85 ± 0.16 b	1.13 ± 0.22 ab	1.86 ± 0.21 a	0.58 ± 0.11 b	1.88 ± 0.49 a
	Limonene	138-86-3	16.56 ± 1.67 c	5.47 ± 0.99 d	19.85 ± 2.87 bc	9.92 ± 1.13 d	18.62 ± 2.65 c	26.31 ± 1.82 ab	7.95 ± 1.28 d	28.86 ± 3.08 a
	Terpinolene	586-62-9	0.65 ± 0.07 ab	0.29 ± 0.05 b	0.64 ± 0.09 ab	0.37 ± 0.05 b	0.60 ± 0.09 b	1.13 ± 0.17 a	0.52 ± 0.10 b	0.80 ± 0.14 ab
	Caryophyllene	87-44-5	0.35 ± 0.04	-	-	0.40 ± 0.09	0.32 ± 0.04	0.35 ± 0.05	-	-
	beta-copaene	18252-44-3	0.46 ± 0.06	-	-	0.45 ± 0.09	0.39 ± 0.02	0.39 ± 0.07	-	-
	gamma-Muurolene	30021-74-0	0.46 ± 0.09	-	-	0.45 ± 0.08	0.41 ± 0.03	0.37 ± 0.07	-	-
	Germacrene D	23986-74-5	0.85 ± 0.24 a	0.19 ± 0.04 c	-	0.70 ± 0.15 ab	0.76 ± 0.07 ab	0.46 ± 0.08 bc	0.22 ± 0.03 c	0.24 ± 0.02 c
	Bicyclosesquiphellandrene	54324-03-7	0.65 ± 0.05 a	-	-	0.57 ± 0.10 a	0.60 ± 0.05 a	0.47 ± 0.11 a	-	0.20 ± 0.02 b
	beta-Cyclogermacrane	24703-35-3	0.44 ± 0.09	-	-	0.28 ± 0.04	0.30 ± 0.03	0.27 ± 0.07	-	-
	alpha-Muurolene	10208-80-7	1.00 ± 0.21 a	0.24 ± 0.05 b	-	0.79 ± 0.10 a	0.98 ± 0.11 a	0.83 ± 0.18 a	0.29 ± 0.06 b	0.33 ± 0.03 b
	beta-Cadinene	523-47-7	3.77 ± 0.58 a	0.57 ± 0.03 c	0.69 ± 0.17 c	2.39 ± 0.24 b	3.19 ± 0.36 ab	2.83 ± 0.60 ab	0.73 ± 0.17 c	0.82 ± 0.14 c
	trans-Calamenene	73209-42-4	2.08 ± 0.38 a	0.65 ± 0.04 b	-	1.90 ± 0.24 a	1.91 ± 0.19 a	1.69 ± 0.29 a	0.90 ± 0.07 b	-
Alkanes	4-Decyne	2384-86-3	5.20 ± 0.27 a	1.49 ± 0.26 bc	1.10 ± 0.22 c	1.40 ± 0.18 c	1.22 ± 0.30 c	0.99 ± 0.12 c	1.04 ± 0.14 c	2.15 ± 0.24 b
	Dodecane, 2,6,11-trimethyl-	31295-56-4	0.47 ± 0.06 a	0.34 ± 0.06 b	-	0.28 ± 0.02 b	0.28 ± 0.03 b	0.30 ± 0.03 b	0.34 ± 0.03 ab	0.39 ± 0.05 ab
	Pentadecane	629-62-9	0.92 ± 0.07 a	-	-	0.31 ± 0.03 b	0.52 ± 0.11 b	0.84 ± 0.12 a	0.83 ± 0.06 a	0.46 ± 0.13 b
Ketones	3-Octanone, 2-methyl-	923-28-4	-	1.40 ± 0.09 b	-	0.86 ± 0.07 c	1.30 ± 0.14 b	2.44 ± 0.13 a	2.45 ± 0.08 a	-

Content values represent mean ± standard deviation (S.D) (n = 6). Significant differences among the species are indicated by different letters ($p < 0.05$).

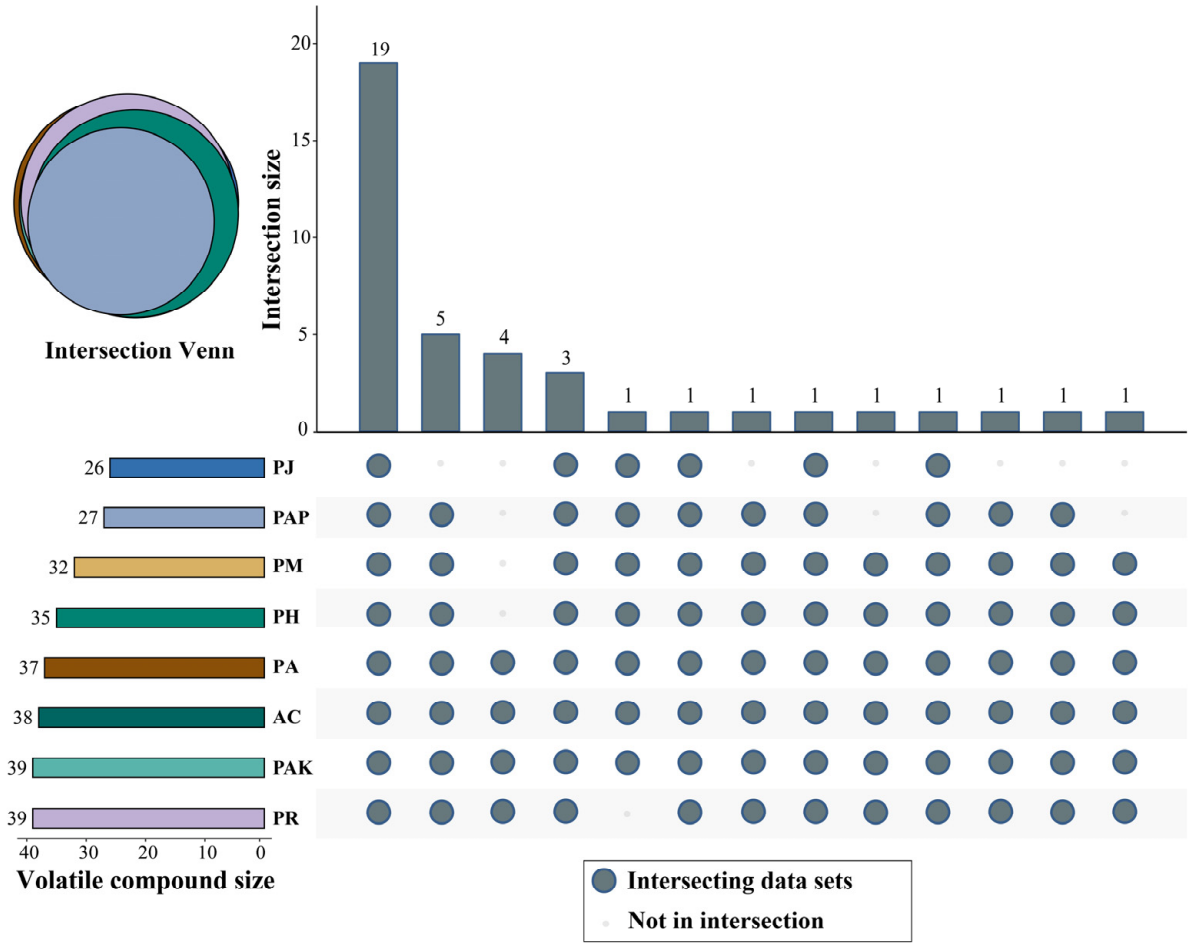

Figure 3. UpSet version showing the unique and shared different VOCs of eight bamboo species leaves.

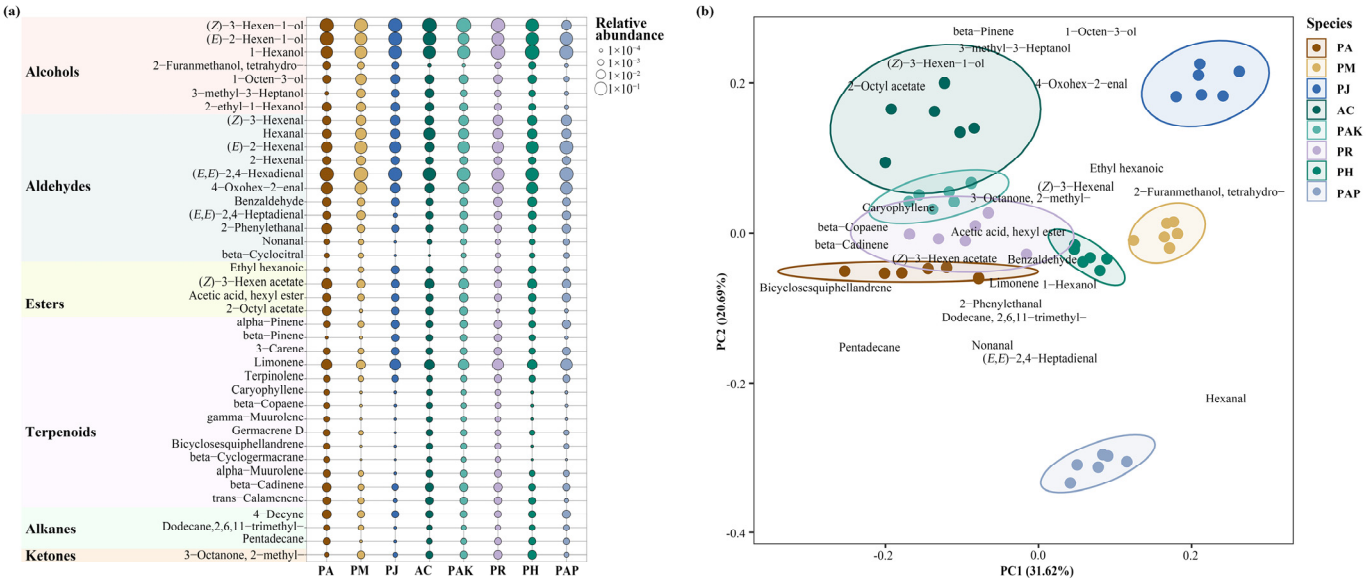

Figure 4. VOCs among eight different bamboo species, (**a**) relative abundance and (**b**) composition structure difference.

3.2. Differences in Volatile Organic Compounds among Leaves of Different Bamboo Species

To investigate the relationship between bamboo species and volatile organic components, and better express the associated compounds, principal component analysis was conducted on VOCs among different bamboo leaf species. The analysis revealed that the contribution of the PCA1 axis was 31.62%, while that of the PCA2 axis was 20.69%, collectively explaining 52.31% of the total variance in the data ($p < 0.01$). The samples exhibited distinct group clustering, indicating significant differences in leaf VOCs among species, and a representative classification (Figure 4b). *P. amarus*, *A. chienouensis*, *P. amabilis*, and *Ph. rubromarginata* were noticeably clustered together, closely associated with sesquiterpene compounds such as Caryophyllene, beta-Copaene, and beta-Cadinene. In contrast, *P. juxianensis* and *P. amabilis* were relatively distant from other species.

3.3. Odor-Active Compounds in Leaves of Different Bamboo Species

Within the realm of aroma components, our study identified 24 key constituents that positively impact the sensory attributes of bamboo leaves, comprising six alcohol types, nine aldehydes, three lipids, and six terpenes, notably including (*Z*)-3-Hexen-1-ol, (*Z*)-3-Hexenal, Ethyl hexanoic, alpha-Pinene, Limonene, and so on (Table 2). *P. amarus* and *Ph. hirtivagina* have 22 OAVs, and *P. maculatus* 21 OAVs, *P. juxianensis* 20 OAVs, *A. chienouensis* 23 OAVs, *Pseudosasa amabilis* (McClure) P. C. Keng 19 OAVs, *Pseudosasa amabilis* (McClure) Keng f. and *Ph. rubromarginata* 24 OAVs. Notably, the significant difference between the highest and lowest OAV values spans three orders of magnitude, underscoring significant variations in the contributions of different aroma components to the overall bamboo leaf profile. To explore the differential grouping and similarity of the OAV aroma components of leaves of different bamboo species in Table 2, cluster analysis was performed on the aroma components of eight bamboo leaves (Figure 5). The results showed that the eight species were divided into two categories. The OAVs of *Ph. hirtivagina*, *Ph. rubromarginata*, and *Pseudosasa amabilis* are grouped into one category with similar aroma components. While the OAVs of *A. chienouensis*, *P. juxianensis*, *P. amarus*, and *P. maculatus* are in another category.

1-Hexanol and Benzaldehyde have higher OAV values in *Ph. rubromarginata* and *Ph. hirtivagina*, contributing significantly to their primary aroma profiles, characterized by fruity aroma. Terpinolene is significantly higher in *Ph. rubromarginata* and presents a grassy aroma. The main aroma is contributed by Nonanal, beta-Cyclocitral, 2-ethyl-1-Hexanol, and the overall aroma is fatty. Among them, (*Z*)-3-Hexen-1-ol, acetic acid, and hexyl ester have a greater connection with *Pseudosasa* (McClure) Keng f. Ethyl hexanoic and beta-Pinene make significant contributions to *P. juxianensis*, resulting in an overall pleasant aroma. 1-Octen-3-ol, (*Z*)-3-Hexenal are the predominant contributors in *A. chienouensis*, leading to an overall appealing aroma. Hexanal, (*E*)-2-Hexen-1-ol, 2-Phenylethanal, (*Z*)-3-Hexen acetate play a substantial role in *P. amarus* and presents an overall fragrance; (*E*, *E*)-2,4-Heptadienal, (*E*)-2-Hexenal, 2-Hexenal have a more substantial impact on the aroma composition of *P. maculatus*.

Table 2. Aroma vitality value (OAV) of eight bamboo species leaves.

Category	Compound	Odor	Odor Threshold [a] ($\mu g \cdot g^{-1}$)	OAV [b]							
				PA	PM	PJ	AC	PAK	PR	PH	PAP
Alcohols	(*Z*)-3-Hexen-1-ol	Grassy	0.07	2155.83 ± 245.20 bc	1672.27 ± 459.84 c	2233.64 ± 1146.80 bc	3188.90 ± 1430.67 b	4605.07 ± 1868.03 a	2045.13 ± 692.70 bc	1024.87 ± 460.10 c	-
	(*E*)-2-Hexen-1-ol	Green leafy	6.7	19.67 ± 5.34 a	6.79 ± 1.70 cd	14.31 ± 5.30 b	12.99 ± 4.60 b	3.51 ± 1.96 d	6.85 ± 1.37 cd	7.99 ± 2.87 c	7.73 ± 1.12 c
	1-Hexanol	Fruity	0.5	71.95 ± 20.02 d	91.85 ± 32.56 cd	180.96 ± 134.71 cd	109.46 ± 20.00 cd	158.80 ± 79.27 cd	354.18 ± 63.50 a	339.90 ± 85.01 ab	214.94 ± 42.92 bc
	1-Octen-3-ol	Mushroom	0.01	-	451.65 ± 129.67 a	197.27 ± 85.68 b	432.11 ± 79.11 a	90.57 ± 16.86 c	70.76 ± 12.44 c	61.30 ± 7.89 c	-
	3-methyl-3-Heptanol	Fruity	0.0078	633.66 ± 206.70 ab	779.70 ± 158.99 a	532.36 ± 151.68 ab	419.87 ± 634.84 bc	349.14 ± 183.53 bc	129.67 ± 26.90 c	112.54 ± 30.99 c	-
	2-ethyl-1-Hexanol	Fruity	1.28	2.31 ± 0.51 c	6.49 ± 1.77 ab	5.93 ± 1.87 ab	2.83 ± 1.50 c	7.62 ± 4.16 a	5.89 ± 2.34 ab	4.52 ± 2.12 bc	7.96 ± 1.36 a
Aldehydes	(*Z*)-3-Hexenal	Leafy	0.00025	18,357.07 ± 7952.21 d	84,226.81 ± 36,812.96 b	24,895.54 ± 10,041.07 cd	127,377.68 ± 49,034.13 a	49,957.97 ± 23,302.60 c	34,823.21 ± 13,718.08 cd	23,560.04 ± 7879.90 cd	20,994.50 ± 5265.83 d
	Hexanal	Green	0.0045	3842.65 ± 752.21 d	14,203.32 ± 4521.09 b	5562.55 ± 3712.06 cd	1212.71 ± 451.16 d	9391.24 ± 1334.29 bc	5722.75 ± 2642.87 cd	9372.77 ± 1635.10 bc	20,562.26 ± 4551.17 a
	(*E*)-2-Hexenal	Leafy	0.017	178.78 ± 80.24 b	352.86 ± 25.46 a	167.36 ± 70.92 b	89.88 ± 28.23 bc	144.94 ± 26.25 bc	115.39 ± 51.81 bc	71.80 ± 8.16 c	138.73 ± 54.81 bc
	2-Hexenal	Grassy	0.03	5405.40 ± 985.01 b	7257.87 ± 1144.90 a	4681.08 ± 1204.33 b	3012.93 ± 825.62 c	4311.26 ± 1599.38 b	2128.03 ± 555.91 c	2740.95 ± 244.59 c	4407.82 ± 1019.72 b
	Benzaldehyde	Fruity	0.35	10.24 ± 1.49 a	5.10 ± 0.88 b	5.76 ± 0.95 b	2.99 ± 1.30 b	5.55 ± 1.61 b	11.64 ± 3.62 a	13.36 ± 6.47 a	4.35 ± 2.41 b
	(*E*, *E*)-2,4-Heptadienal	Fruity, fatty	0.056	36.33 ± 9.27 b c	70.10 ± 10.37 a	-	23.24 ± 8.42 c	33.60 ± 4.92 bc	28.74 ± 5.39 bc	32.87 ± 15.31 bc	45.03 ± 12.59 b
	2-Phenylethanal	Floral	0.004	2478.43 ± 670.91 a	465.32 ± 98.66 bc	305.34 ± 88.06 c	312.03 ± 63.84 c	692.62 ± 121.53 b	301.01 ± 57.88 c	344.23 ± 82.06 c	699.77 ± 163.07 b
	Nonanal	Orange peel	0.001	402.13 ± 131.24 bc	339.38 ± 85.80 cd	-	180.37 ± 29.68 d	613.04 ± 214.53 a	281.11 ± 27.18 cd	310.32 ± 51.37 cd	529.31 ± 265.20 ab
	beta-Cyclocitral	Fatty	0.005	49.02 ± 7.60 ab	58.15 ± 12.77 ab	-	-	57.97 ± 18.53 ab	47.53 ± 19.48 b	47.03 ± 17.57 b	69.20 ± 26.15 a
Esters	Ethyl hexanoic	Fruity	0.001	455.91 ± 230.80 c	403.52 ± 140.51 c	2350.61 ± 835.01 a	900.52 ± 497.92 bc	1491.27 ± 801.31 b	1148.75 ± 563.56 b	1289.84 ± 381.10 b	462.41 ± 190.99 c
	(*Z*)-3-Hexen acetate	Fruity	0.056	215.43 ± 88.04 a	50.35 ± 17.49 bc	36.18 ± 11.95 c	103.29 ± 25.56 b	192.80 ± 102.44 a	21.98 ± 12.60 c	79.24 ± 41.37 bc	47.30 ± 21.30 bc
	Acetic acid, hexyl ester	Fruity	0.002	1220.19 ± 480.37 ab	429.47 ± 254.60 b	721.21 ± 257.54 b	519.48 ± 56.61 b	2019.75 ± 1435.77 a	526.82 ± 178.53 b	673.22 ± 196.23 b	1699.64 ± 932.48 ab
Terpenes	alpha-Pinene	Cedar	0.006	142.83 ± 99.78 b	111.74 ± 78.92 b	389.11 ± 178.20 a	146.30 ± 73.97 b	313.57 ± 125.88 a	347.03 ± 178.21 a	123.46 ± 64.23 b	384.39 ± 241.29 a
	beta-Pinene	Cedar, woody	0.14	-	-	11.44 ± 3.70 a	6.07 ± 2.59 b	7.92 ± 1.81 ab	11.09 ± 3.75 a	3.86 ± 1.95 b	-
	3-Carene	Cedar, orange	0.77	1.33 ± 0.62 b	<1	2.37 ± 0.93 a	1.10 ± 0.56 b	1.46 ± 0.77 ab	2.42 ± 0.73 a	<1	2.44 ± 1.70 a
	Limonene	Orange	0.01	1655.93 ± 446.82 c	546.50 ± 264.46 d	1985.03 ± 769.69 bc	991.58 ± 303.45 d	1862.48 ± 712.15 c	2631.19 ± 487.22 ab	795.17 ± 343.21 d	2886.35 ± 825.88 a
	Terpinolene	Cedar, woody	0.2	3.27 ± 0.97 ab	1.46 ± 0.63 b	3.22 ± 1.16 ab	1.87 ± 0.69 b	2.98 ± 1.27 b	5.63 ± 2.34 a	2.61 ± 1.28 b	3.98 ± 1.84 ab

[a] Odor detection threshold determined in water as a fixed value by references. [b] Odor activity value (aqueous concentration/odor threshold) represents mean ± standard deviation (S.D.) ($n = 6$). Significant differences among the species are indicated by different letters ($p < 0.05$).

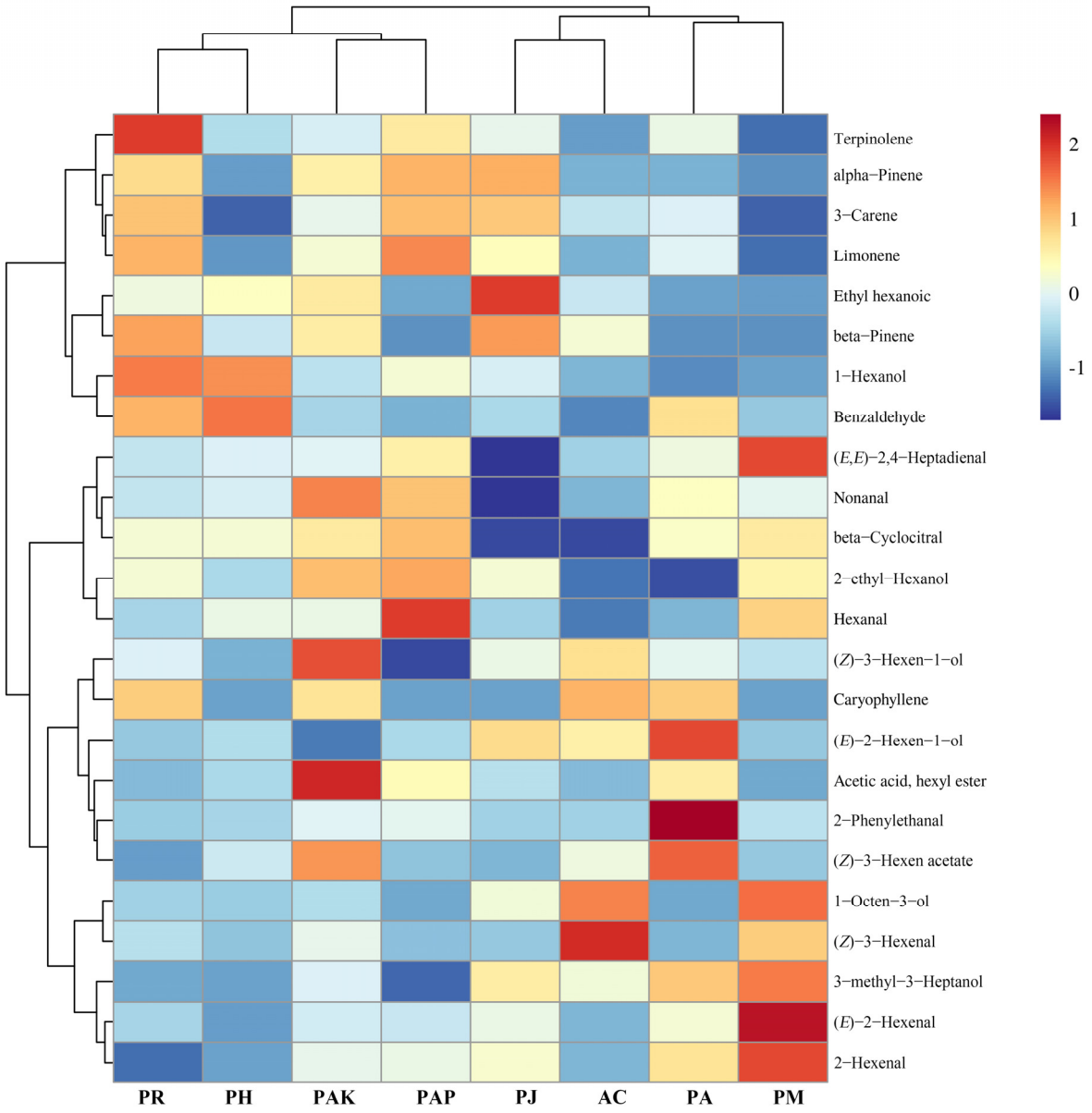

Figure 5. Hierarchical clustering with heatmap showing the differences in the OAV abundance among eight bamboo species leaves.

4. Discussion

4.1. Characterization of Volatile Organic Compounds in Leaves of Different Bamboo Species

Compared with the previous studies on VOCs from bamboo plants, most of them focused on the spatial and temporal variations in isoprene released by plants in the regional atmosphere [32]. Our study focuses on the healthcare effects of different bamboo plants, and the volatile extraction methods were different from environmental science, so isoprene was not detected in this study, as in the same method using HS-SPME-GCMS [21]. It could be found that the eight analytes in each sample had significant diversity. The commonality of the leaves of different bamboo species is that the dominant groups consisted of alcohols and aldehydes, which is similar to the results of previous studies [18,33]. The eight bamboo species involved in this study belonged to four genera, i.e., three species in the genus *Pleioblastus*, two in *Pseudosasa*, two in *Phyllostachys*, and one in *Acidosasa*. It was found that the VOCs from the four genera showed intergeneric similarities. Relatively the most VOCs of genus *Pleioblastus* were (Z)-3-Hexen-1-ol and 2-Hexenal. 1-Hexanol was the most abundant in *Phyllostachys*, with low 2-Hexenal compared to other genera. PCA showed

significant differences in VOC among different bamboo species. Chitiva et al. [34] similarly found that environmental factors influenced the genetic and chemical diversity of bamboo species, so that even bamboo species growing in the same area may have VOC diversity. Although the number of compounds determined is relatively small compared to other tree species, we found that in addition to the known alcohols, many compounds have not been studied in the previous studies of bamboo, such as 2-Phenylethanal, which is an aromatic aldehyde and a component of many natural essential oils [35]. Ethyl hexanoic and 2-Octylacetate for esters, and the more well-known terpenes, monoterpene 3-Carene, polyterpene Terpinolene, Germacrene D, Bicyclosesquiphellandrene, beta-Cyclogermacrane, trans-Calamenene, which have been proven to have functions such as being soothing and acting as an antioxidant by the medical field [36,37]. Therefore, the analysis of VOCs in bamboo leaves can not only provide chemical information but also help the healthcare potential development.

4.2. Characterization of Odor-Active Compounds in Leaves of Different Bamboo Species

Based on OAV analysis, we identified 24 components with aroma contribution (Figure S1). Compared with previous studies, we believe that the olfactory sensory value of bamboo plants is underestimated. Bamboo leaves have the advantage of a large number of natural active compounds. However, only a few studies use bamboo leaves to develop fragrance and modify the aroma of cigarettes. Flowering plants in botany have been used in industries such as the preparation of artificial flavors [38,39]. Alcohols, aldehydes, esters, and terpenes were dominant among the odor-active compounds that contribute to the strong smell of different bamboo leaves. These compounds can be roughly divided into three groups, grassy and leafy aromas represented by (*Z*)-3-Hexen-1-ol, (*E*)-2-Hexen-1-ol, (*Z*)-3-Hexenal, (*E*)-2-Hexenal; fruity and floral aromas represented by 1-Hexanol, 3-methyl-3-Heptanol, 2-ethyl-1-Hexanol, (*E*, *E*)-2,4-Heptadienal; and cedar aromas represented by alpha-Pinene, beta-Pinene, and terpinolene. The study found that *P. amarus* and *P. maculatus* of genus *Pleioblastus* have a stronger bias towards the common grassy aroma of bamboo, while *P. amabilis* and *Ph. rubromarginata* released a more noticeable pine and cypress scent, with the potential to develop the health benefits of terpenes. The study found that *P. amarus* in bamboo plants is more biased towards common leaf aroma. The *Pseudosasa amabilis* (McClure) P. C. Keng and *Ph. rubromarginata* have a more obvious pine and cypress aroma and have the potential to develop the health functions of terpenes.

Relevant research shows that among the five senses of human beings, smell is the only one that directly connects the brain with the external environment. The brain areas that process smell are closely related to emotions. The sense of smell affects and regulates human emotions and is a lubricant for the development of human society [40]. Ranasinghe et al. [41] believe that odors can affect human psychological and physiological states. Certain odors can regulate emotions and cognition, reduce stress, and have positive significance in the treatment of psychological problems [42]. (*Z*)-3-Hexen-1-ol, the dominant compound of bamboo leaves in this study, is a compound commonly known as leaf alcohol. It has a green, grassy, and slightly woody smell, and is often used in blends with floral essential oils such as balsam geranium oil, and clove, to create fresh, natural fragrances reminiscent of freshly cut grass or leaves. These leafy fragrances reduce the amplitude value of the alpha waves in the human brain, thus reducing feelings of depression [43]. The health benefits of terpenes are becoming more visible to the medical community. In addition to the commonly believed smell of pine and cypress, terpenes can be emotionally relaxing and contribute to mental health [44]. 3-Carene was found to be anti-anxiety and enhance sleep effects [37]. Terpinolene, which is closely related to *Ph. rubromarginata*, is a bioactive compound with significant pharmacological activities, such as antifungal properties, and can improve memory complications and disorders in combination with exercise [45]. 2-Phenylethanal, the key aroma component in *P. amarus*, and acetic acid and hexyl ester in *Pseudosasa amabilis* (McClure) Keng f. are aldehydes and esters, which were studied on making raw materials for flavors, edibles commonly [46], and can also be used in plant essential oils and function

in aromatherapy. The diversity of VOCs indicates that the bamboo species has a unique aroma profile, implying the potential of utilizing VOC profiles as identification markers for bamboo species. More practically, it may contribute to its unique sensory characteristics and potential applications in various industries, and the study can encourage sustainable business in the recreation and tourism industry.

5. Conclusions

In this study, we conducted a qualitative and semi-quantitative analysis of the VOCs present in the leaves of eight bamboo species that have not been previously studied and characterized the main aroma components of different bamboo using odor-active analysis. A total of 40 VOCs were identified, the commonality of different bamboo species was that the dominant groups consisted of alcohols and aldehydes. PCA analysis showed a clear classification with two groups. The compounds such as 2-Phenylethanal and terpinolene, not previously reported in other bamboo species, were found due to intergeneric similarity and rich diversity. The aroma of bamboo leaves is a combination of grassy, fruity, and piney notes by 24 VOCs. There are significant differences in leaf OAV among bamboo species. The key aroma from *Pleioblastus amarus* is leaf alcohol, which contributes to the grassy scent, while the piney aroma is dominant in *Pseudosasa amabilis* and *Phyllostachys rubromarginata*. Our findings supplement the available data on bamboo species in subtropical regions and provide a basis for a sustainable model for bamboo forest management to develop landscape horticultural healing through the healthcare benefits of bamboo. Future studies should include empirical and medical validation studies such as olfactory experiments and sensory evaluations to better address human health needs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10040394/s1, Figure S1: OAV abundance among eight bamboo species leaves.

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