Characterization of volatile compounds in ten Piper species cultivated in Hainan Island, South China

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ABSTRACT

The volatile compounds of 10 Piper species cultivated in Hainan Island, China, have been investigated. Eighty compounds were profiled after headspace–solid phase microextraction (HS–SPME) with gas chromatography–mass spectrometry (GC–MS). Mean Bray–Curtis similarity value was only 22.78 ± 1.98% among the different Piper species. The volatile compounds were largely grouped as hydrocarbons, aldehydes, alcohols, acids, ketones, esters, and phenols. The main compounds comprised benzaldehyde, cinnamaldehyde, β-caryophyllene, ocimene, linalool, myrcene, cubebene, terpinene, linalool, α-caryophyllene, β-elemene, and germacrene. Principal component analysis revealed that Piper laetispicum, Piper longum, Piper hainanense, Piper betle, and Piper flaviflorum were characterized by high contents of β-caryophyllene, α-caryophyllene, germacrene, and β-pinene. Piper puberulum and Piper cathayanum were associated with high contents of linalool, myrcene, and germacrene D. On the other hand, Piper pseudofuligineum and Piper retrofractum were related with high contents of ocimene. Finally, Piper auritum was associated with high content of cinnamaldehyde. Volatile profiling of Piper species by HS–SPME–GC–MS and the interrelationship investigated among the volatiles can be used as a roadmap for future resource utilization or biotechnological applications.

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Introduction

Piper is the largest genus in the family Piperaceae, which is distributed throughout the tropical regions, with major concentrations occurring in Central and South Americas, Caribbean, Africa, Asia, and some Pacific Islands.¹,² The aroma emitted by its leaf/fruit/root has caused Piper species to be widely utilized as flavoring ingredient in cooking.³–⁵ Among Piper species, Piper nigrum is characterized as the “king of spice” and is incredibly popular since ancient times; its yields can reach 461,452 t, and the corresponding profits may exceed 100 billion dollars.⁶ Piper laetispicum, P. nigrum, Piper tuberculatum, Piper retrofractum, Piper longum, Piper hainanense, and Piper puberulum have been applied in folk medicine for invigorating the circulation, reducing stasis, detumescence, and as analgesics.⁷ In accordance with the “Flora of China”⁸, many Piper species, such as Piper betle, P. hainanense, Piper hancei, and Piper Yunnanense, present high economical and ethnobotanical benefits given their use as spices, vegetation, fragrance, and traditional medicines.

Aroma is one of the essential components of spice and medicine quality. Aroma is also one of the essential factors that attract consumers. Compared with other spices, such as saffron⁹, chili...
peppers\textsuperscript{[10]}, and thyme\textsuperscript{[11]}, several studies have focused on volatile aroma compounds in \textit{Piper} species although some investigations have shown that \textit{Piper} species are remarkably rich in volatile compounds, of which more than 270 compounds have been identified.\textsuperscript{[12,13]} However, most of such studies only focused on the analysis of volatile compounds released from \textit{Piper nigrum}.\textsuperscript{[14,15]} Limonene, α-pinene, D-3-carene, β-pinene, 4-carene, terpinolene, α-copaene, β-caryophyllene, α-caryophyllene, and D-elemene have been identified as important compounds to the overall flavor of \textit{Piper nigrum} and its essential oil.\textsuperscript{[14–18]} Recently, several studies reported that aroma is specific to species and exhibits unique potential flavor characteristics.\textsuperscript{[19,20]} Topul et al.\textsuperscript{[21]} concluded that \textit{Piper aduncum} contains significantly higher amounts of dillapiole than \textit{Piper gibbilimbum} species, whereas \textit{P. gibbilimbum} contains more gibbilimbols than \textit{P. aduncum}.

In this study, headspace–solid phase microextraction (HS–SPME) combined with gas chromatography–mass spectrometry (GC–MS) was applied to study the characteristic volatiles of 10 \textit{Piper} species for food and flavor. This study essentially focused on comparing aroma composition and content, particularly among \textit{Piper} species that have not been extensively studied. These plants include Chinese local species and a number of species from other countries in order to acquire information for further breeding programs, cultivation, and finding new food sources.

**Materials and methods**

**Plant materials**

All fresh \textit{Piper} leaves were obtained from the Germplasm Repository of Black Pepper of the Spice and Beverage Research Institute, CATAS, Hainan Province, China. Approximately 20 g of raw leaves was collected at 8:00–10:00 a.m., on August 8–10, 2016, depending on 10 different \textit{Piper} species, \textit{P. betle}, \textit{P. auritum}, \textit{P. retrofractum}, \textit{P. hainanense}, \textit{Piper pseudofuligineum}, \textit{P. laetispicum}, \textit{Piper flaviflorum}, \textit{Piper cathayanum}, \textit{P. puberulum}, and \textit{P. longum}. Following collections, the leaves were moisturized and transported to the laboratory immediately. In all experiments, leaves were sliced by using a knife into similarly thin slices to enable their placement in the headspace bottle (volume: ~5 mL).

**Extraction of volatile compounds**

HS–SPME was used to extract the volatile compounds and evaluate their concentrations. For each extraction sample, 1.5 g of leaf slices was weighted and placed in a 5-mL capped SPME vial and allowed to stand for 30 min at ambient room temperature ((22 ± 3)°C). Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fibers were applied in this study for assaying, and film thickness was 50/30 μm (Supelco, Bellefonte, PA, USA). Through manual penetration of the silicone septum, SPME products can be inserted into the vial. Then, after 40 min, the fibers will be exposed to the headspace of sliced leaves. After extraction, the operator of SPME device will set the needle to 0.5 cm for GC injector and then desorbed the fiber for 10 min. During the experiment, each step of sampling or analysis was finished in triplicate. This study also used empty bottles as control for analysis.

**GC–MS conditions**

Applications of 7890A/5975C GC–MS system (Agilent Technologies, USA), coupled with a DB-5MS column, were carried out under the following conditions: temperature of line heater of MS transfer of 280°C, injector temperature of 250°C, and those of corresponding operations should be carried out in split-less mode. The initial temperature of the oven should be set at 50°C and kept for 5 min. Afterward, the temperature was increased as follows: 50–80°C at the rate of 10°C/min, 80–100°C at the rate of 2°C/min, 100–140°C at the rate of 8°C/min, 140–200°C at the rate of 6°C/min, and
200–280°C at the rate of 15°C/min. Helium was applied as carrier gas at a flow rate of 1.0 mL/min. The mass spectrometer Agilent 5975C was operated at 70 eV in electron ionization mode. Source temperature was set to 230°C, the quadrupole temperature was set to 150°C, and the mass range m/z 30–500 amu was conducted in a full-scan mode.

Volatile compounds were identified according to their linear retention indices (LRIs) and by comparing their mass spectra with the MS database via the NIST 2014 library, Volatile Compounds in Food 15.1, and Pherobase (database of pheromones and semi-chemicals). We also compared the fragmentation patterns in the mass spectra with those reported in the literature. LRIs were determined via alkane series (C7–C30). Compounds were quantified as area percentages of total volatiles.

Statistical analysis

Bray–Curtis similarity (BCS), an indicator of the composition similarity between different species, was applied to identify whether these species were the same. This index not only incorporates the existence of components but also incorporates their relative contents. Analysis of volatile compounds verified in the 10 species of *Piper* was carried out by principal component analysis (PCA) via the R package (2.12.2) under Windows environment.

Results and discussion

**Volatile compounds of different *piper* species**

| *Piper* species | Hydrocarbons | Alcohols | Aldehydes | Esters | Acids | Ketones | Phenols | Other Compounds |
|-----------------|--------------|----------|-----------|--------|-------|---------|---------|-----------------|
| *P. betle*      | 20           | 6        | 2         | 2      | 1     | 1       | 1       | 31              |
| *P. auritum*    | 14           | 3        | 2         | 1      | 0     | 0       | 0       | 21              |
| *P. retrofractum* | 21           | 4        | 1         | 1      | 1     | 1       | 3       | 21              |
| *P. hainanense* | 10           | 5        | 1         | 1      | 0     | 0       | 0       | 21              |
| *P. pseudofuligineum* | 20          | 2        | 2         | 0      | 0     | 0       | 0       | 20              |
| *P. laetispicum* | 20           | 2        | 1         | 0      | 0     | 0       | 0       | 20              |
| *P. flaviflorum* | 20           | 2        | 2         | 1      | 0     | 0       | 0       | 20              |
| *P. cathayanum* | 20           | 2        | 2         | 1      | 0     | 0       | 0       | 20              |
| *P. puberulum*  | 20           | 2        | 2         | 1      | 0     | 0       | 0       | 20              |
| *P. longum*     | 20           | 2        | 2         | 1      | 0     | 0       | 0       | 20              |

*P. betle* exhibited a major complex volatile profile. This plant consisted of 31 compounds, among which 20 were hydrocarbons (cubebene: 9.36%, β-caryophyllene: 8.54%, myrcene D: 6.27%), 6 were alcohols (leaf alcohol: 3.74%), 2 were acids (acetic acid: 4.16%, and benzoic acid: 2.14%), 2 were esters (leaf acetate: 4.11% and (Z)-2-pentenyl ester: 0.50%), and 1 was aldehyde (benzaldehyde: 23.56%).

In *P. hainanense*, 21 components were detected (Table 1). The main compounds can be grouped as follows: 10 hydrocarbons (β-elemene 10.49%, β-pinene 8.37%, (E)-β-farnesene 7.23%, and α-pinene 7.14%), 5 aldehydes (leaf aldehyde 0.69%, benzaldehyde 0.26%), 1 acid (acetic acid 0.14%), and 1 ester (methyl benzoate 0.13%).

In *P. retrofractum*, 21 compounds were detected (Table 1): 14 hydrocarbons (ocimene: 15.6%, linalool: 12.9%, α-caryophyllene: 9.61%, and germacrene D: 7.15%), 4 alcohols (linalool: 12.9% and *trans*-hex-3-en-1-ol: 1.78%), 1 ester (octyl salicylate: 0.34%), 1 phenol (catechol: 2.48%), and 1 ketone (2-octanone: 0.29%).

As for *P. laetispicum*, 20 components were detected with a strong majority of hydrocarbons (76.9%). Other compounds, such as alcohols (9.04%), ketones (1.22%), and aldehydes (0.83%), were also detected (Table 1). The most abundant compound was β-caryophyllene, accounting for 26.15% of the total GC peak area, followed by germacrene (15.28%), α-caryophyllene (9.98%), nerolidol (8.84%), and α-pinene (6.09%).
### Table 1. Volatile compounds identified in 10 different species of *Piper* using HS–SPME–GC–MS.

| Peak | RT* (min.) | LRI * | Compounds | Pre | PAU | Pre | PHA | PPS | PLA | PFL | PCA | PPU | PLO |
|------|------------|-------|-----------|------|-----|------|-----|-----|-----|-----|-----|-----|-----|
|      |            |       | Acids     |      |     |      |     |     |     |     |     |     |     |
| 1    | 1.839      | <800  | Acetic acid | 4.16 ± 0.160 | –  | –  | 0.14 ± 0.015 | –  |     |     |     |     |     |
| 2    | 19.411     | 1280  | Ethanedioic acid | –  | 0.49 ± 0.021 | –  |     | –  |     |     |     |     |     |
| 3    | 13.098     | 1116  | Benzoic acid | 2.14 ± 0.072 | –  | –  |     |     |     |     |     |     |     |
|      |            |       | Esters    |      |     |      |     |     |     |     |     |     |     |
| 4    | 5.869      | 897   | Acetic acid (Z)-2-pentenyl ester | 0.5 ± 0.046 | –  | –  |     |     |     |     |     |     |     |
| 5    | 8.147      | 986   | Leaf acetate | 4.11 ± 0.017 | –  | –  |     |     |     |     |     |     |     |
| 6    | 30.456     | 1753  | Octyl salicylate | –  | 3.49 ± 0.145 | –  |     |     |     |     |     |     |     |
| 7    | 11.294     | 1072  | Methyl benzoate | –  | –  |     | 0.13 ± 0.012 |     |     |     |     |     |     |
| 8    | 11.519     | 1078  | Leaf alcohol | 2 ± 0.027 | 12.9 ± 0.902 | 1.1 ± 0.031 |     |     |     |     |     |     |
| 9    | 30.452     | 1753  | Isopentyl salicylate | 0.25 ± 0.040 |     |     |     |     |     |     |     |     |     |
| 10   | 31.842     | 1834  | 2-Pentanol | 0.89 ± 0.179 | 0.38 ± 0.025 | 0.86 ± 0.026 |     |     | 0.24 ± 0.040 |     |     |     |
|      |            |       | Alcohols  |      |     |      |     |     |     |     |     |     |     |
| 11   | 3.481      | <800  | 2-Pentanol | 0.89 ± 0.179 | 0.38 ± 0.025 | 0.86 ± 0.026 |     |     | 0.24 ± 0.040 |     |     |     |
| 12   | 4.878      | 842   | Leaf alcohol | 3.74 ± 0.111 |     |     |     |     |     | 9.66 ± 0.546 |     |     |
| 13   | 9.059      | 1015  | Cinene | 0.58 ± 0.044 |     |     |     |     |     |     |     |     | 2.53 ± 0.066 |
| 14   | 4.915      | 844   | trans-Hex-3-en-1-ol | 1.78 ± 0.046 |     |     |     |     |     |     |     |     |
| 15   | 11.519     | 1078  | Linoleal | 2 ± 0.027 | 12.9 ± 0.902 | 1.31 ± 0.155 | 1.62 ± 0.224 | 0.2 ± 0.015 | 0.76 ± 0.045 | 1.46 ± 0.046 | 2.25 ± 0.056 |
| 16   | 2.573      | <800  | Cyclopentanol | 0.16 ± 0.017 |     |     |     |     |     |     |     |     |
| 17   | 2.517      | <800  | 1-Penten-3-ol | 2.09 ± 0.040 | 0.49 ± 0.015 |     |     |     |     |     |     |
| 18   | 5.103      | 855   | Octanol | 0.29 ± 0.030 |     |     |     |     |     |     |     |
| 19   | 7.041      | 943   | Benzyl alcohol | 2.54 ± 0.068 |     |     |     |     |     |     |
| 20   | 24.833     | 1486  | 4-Methylbenzyl alcohol | 0.33 ± 0.078 |     |     |     |     |     |     |
|      |            |       | Hydrocarbons |      |     |      |     |     |     |     |     |     |     |
| 21   | 25.687     | 1523  | Nerolidol | 8.84 ± 0.215 | 8.07 ± 0.099 | 1.38 ± 0.017 | 0.88 ± 0.020 | 8.69 ± 0.494 | 1.38 ± 0.053 |     |
| 22   | 5.080      | 853   | n-Hexyl alcohol | 1.18 ± 0.015 |     |     |     |     |     |     |     |     |
| 23   | 12.080     | 1092  | Lavandulol | 28.69 ± 0.494 |     |     |     |     |     |     |
| 24   | 1.367      | <800  | Cyclohexanol | 1.38 ± 0.053 |     |     |     |     |     |     |

(Continued)
| Peak | RT* (min.) | LRI * | Compounds | PBE | PAU | PRE | PHA | PPS | PLA | PFL | PCA | PPU | PLO |
|------|------------|-------|------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 36   | 23.78      | 1443  | Myrcene    | 6.27 ± 0.012 1.24 ± 0.010 | 6.27 ± 0.012 1.24 ± 0.010 | 1.67 ± 0.029 |
| 37   | 24.77      | 1484  | Cadinene   | 0.92 ± 0.046 0.12 ± 0.015 | 0.4 ± 0.015 | 6 ± 0.353 | 1.03 ± 0.015 4.08 ± 0.089 |
| 38   | 24.28      | 1463  | Bicyclogermacrene | 4.61 ± 0.025 0.76 ± 0.011 | 0.71 ± 0.047 |
| 39   | 23.94      | 1450  | Cubaene    | 9.36 ± 0.025 | 0.12 ± 0.015 |
| 40   | 8.28       | 991   | α-Phellandrene | 0.92 ± 0.046 0.12 ± 0.015 | 0.4 ± 0.015 | 6 ± 0.353 | 1.03 ± 0.015 4.08 ± 0.089 |
| 41   | 8.60       | 1002  | α-Terpineene | 1.89 ± 0.01 |
| 42   | 9.97       | 1038  | Terpinene  | 5.11 ± 0.0305 0.64 ± 0.051 | 0.08 ± 0.015 | 0.2 ± 0.025 |
| 43   | 10.99      | 1064  | Terpinene  | 5.11 ± 0.0305 0.64 ± 0.051 | 0.08 ± 0.015 | 0.2 ± 0.025 |
| 44   | 22.69      | 1499  | Germacrene D | 0.36 ± 0.010 | 0.72 ± 0.038 |
| 45   | 23.21      | 1420  | Farnesene  | 0.19 ± 0.031 |
| 46   | 24.19      | 1460  | Germacrene D | 0.65 ± 0.025 1.24 ± 0.021 | 7.15 ± 0.565 | 5.71 ± 0.188 0.47 ± 0.010 1.13 ± 0.036 |
| 47   | 24.55      | 1475  | (E)-α-Farnesene | 0.4 ± 0.015 | 0.21 ± 0.017 0.41 ± 0.025 |
| 48   | 25.70      | 1524  | α-Farnesene | 0.13 ± 0.011 |
| 49   | 9.17       | 1017  | trans-β-ocimene | 4.67 ± 0.345 6.53 ± 0.596 | 0.41 ± 0.021 0.24 ± 0.023 |
| 50   | 24.13      | 1457  | α-Selinene  | 1.76 ± 0.078 | 0.39 ± 0.038 408 ± 0.030 |
| 51   | 24.22      | 1461  | trans-Isolimonone | 0.73 ± 0.006 | 0.21 ± 0.017 0.41 ± 0.025 |
| 52   | 24.29      | 1464  | β-Humulene  | 1.35 ± 0.093 | 0.29 ± 0.025 |
| 53   | 24.53      | 1474  | Bisabolene  | 0.81 ± 0.038 | 0.8 ± 0.044 |
| 54   | 23.48      | 1431  | Calarene   | 0.28 ± 0.003 | 0.21 ± 0.017 |
| 55   | 4.90       | 843   | 2,4-Hexadiene | 0.11 ± 0.021 1.78 ± 0.012 | 0.11 ± 0.020 0.24 ± 0.026 |
| 56   | 7.34       | 955   | β-Terpilene | 0.18 ± 0.012 | 0.18 ± 0.012 |
| 57   | 24.28      | 1464  | Germacrene  | 0.76 ± 0.025 | 15.28 ± 0.600 | 3.2 ± 0.086 6.8 ± 0.130 | 0.08 ± 0.021 |
| 58   | 7.47       | 960   | β-Pinene   | 8.37 ± 0.512 11.95 ± 0.839 | 4.15 ± 0.061 6.08 ± 0.106 6.1 ± 0.025 4.39 ± 0.095 |
| 59   | 26.14      | 1543  | (E)-β-Farnesene | 7.23 ± 0.584 | 15.28 ± 0.600 | 3.2 ± 0.086 6.8 ± 0.130 | 0.08 ± 0.021 |
| 60   | 7.36       | 955   | Sabineine  | 1.26 ± 0.199 | 0.17 ± 0.006 10.46 ± 0.115 |
| 61   | 9.98       | 1038  | β-Phellandrene | 0.11 ± 0.020 0.24 ± 0.026 |
| 62   | 21.27      | 1347  | Copaene    | 4.56 ± 0.106 |
| 63   | 22.90      | 1407  | (+)-Aromadendrene | 0.58 ± 0.032 0.43 ± 0.029 |
| 64   | 4.97       | 1012  | Cyclohexene | 2.72 ± 0.020 5.72 ± 0.087 14.26 ± 0.072 1.36 ± 0.031 |
| 65   | 11.23      | 1070  | perillene  | 6.63 ± 0.026 | 1.91 ± 0.020 |
| 66   | 9.52       | 1026  | 3-Carene   | 5.88 ± 0.031 |
| 67   | 19.40      | 1281  | Valencen   | 5.88 ± 0.031 |
| 68   | 7.00       | 941   | Sabinene   | 0.11 ± 0.020 0.24 ± 0.026 |

(Continued)
| Peak | RT* (min.) | LRI * | Compounds      | PBE | PAU | PRE | PHA | PPS | PLA | PFL | PCA | PPU | PLO |
|------|------------|-------|----------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| **Phenols** |           |       |                |     |     |     |     |     |     |     |     |     |     |
| 69   | 19.407     | 1280  | Catechol       |     |     |     |     |     |     |     |     |     |     |
|      |            |       | Phenols        | 69  |     |     |     |     |     |     | 69  |     |     |
| 70   | 19.074     | 1270  | Cinnamaldehyde |     |     |     |     |     |     |     |     |     |     |
|      |            |       | Aldehydes      | 70  |     |     |     |     |     |     |     |     |     |
| 71   | 4.813      | 838   | Leaf aldehyde  | 70  |     |     |     |     |     |     |     |     |     |
| 72   | 21.001     | 1336  | Benzaldehyde   | 70  |     |     |     |     |     |     |     |     |     |
| 73   | 16.373     | 1188  | Decanal        | 70  |     |     |     |     |     |     |     |     |     |
| 74   | 30.122     | 1736  | Palmitaldehyde | 70  |     |     |     |     |     |     |     |     |     |
| 75   | 23.929     | 1449  | 2-Methyl-2-pentenal | 70 |     |     |     |     |     |     |     |     |     |
| 76   | 21.001     | 1337  | Benzaldehyde   | 70  |     |     |     |     |     |     |     |     |     |
| 77   | 24.664     | 1479  | Piperonylaldehyde | 70 |     |     |     |     |     |     |     |     |     |
| 78   | 3.933      | <800  | Leaf aldehyde  | 70  |     |     |     |     |     |     |     |     |     |
| **Ketones** |           |       |                |     |     |     |     |     |     |     |     |     |     |
| 79   | 21.28      | 1270  | 2-Octanone     |     |     |     |     |     |     |     |     |     |     |
| 80   | 19.071     | 1270  | 2-Nonanone     |     |     |     |     |     |     |     |     |     |     |

Notes: PBE: *P. betle*; PAU: *P. auritum*; PRE: *P. retrofractum*; PHA: *P. hainanense*; PPS: *P. pseudofuligineum*; PLA: *P. laetispicum*; PFL: *P. flaviflorum*; PCA: *P. cathayanum*; PPU: *P. puberulum*; PLO: *P. longum*.

*RT: Retention time (minutes).
*LRI: Line retention index.
The volatile compound profile of *P. pseudofuligineum* comprised 16 components (Table 1): 11 hydrocarbons (β-pinene: 11.95%, α-pinene: 6.73%, cinene: 4.64%, and myrcene: 2.76%), 3 esters (diethyl phthalate: 0.42% and isopentyl salicylate: 0.25%), and 2 alcohols (linalool: 1.62% and 4-methylbenzyl alcohol: 0.33%).

*P. flaviflorum* contained major alcohol compounds. The plant was composed of 24 components (Table 1), among which 7 were alcohols (lavandulol: 28.69%, leaf alcohol: 9.66%, nerolidol: 8.07%). Other compounds, such as hydrocarbons (30.95%), acids (3.49%), ketones (4%), and aldehydes (0.21%), were also detected.

*P. cathayanum* possessed major hydrocarbons compounds (Table 1). The plant consisted of 19 components, among which 15 were hydrocarbons (70.57%). The most abundant compound was myrcene, accounting for 16.06% of the total GC peak area, followed by β-caryophyllene (13.23%), sabinene (10.46%), germacrene (6.8%), and β-pinene (6.1%). Other compounds, such as aldehydes (2.03%), ester (1.1%), and alcohols (0.88%), were also detected.

Similar to *P. cathayanum* species, *P. puberulum* also featured major hydrocarbons compounds (Table 1). The plant was composed of 14 components, among which 12 were hydrocarbons (86.03%). The most abundant compound was myrcene, accounting for 34.92% of the total GC peak area, followed by β-caryophyllene (22.51%), cyclohexene (14.26%), β-elemene (4.82%), and trans-β-ocimene (4.08%). Other compounds, such as alcohols (1.46%) and aldehydes (0.47%), were also detected.

In *P. longum*, 21 components were identified (Table 1): 16 hydrocarbons (52.78%), 3 alcohols (4.94%), and 1 ketone (3.2%). The most abundant compound was β-caryophyllene, accounting for 27.7% of the total GC peak area, followed by β-pinene (4.39%), cadinene (4.08%), α-pinene (3.06%), and 2-nonenone (3.2%).

Diverse volatile compounds were observed in the different *Piper* species. In accordance with Varughese et al. [22] and Srivastave et al. [23] the total profile of volatile compounds in the 10 *Piper* species revealed the predominance of esters, hydrocarbons, aldehydes, and alcohols. Volatile
assemblages differed considerably among the different Piper species (BCS = 22.78% ± 1.98%, range: 3.15–58.83%, n = 45 comparisons, Table 2). Volatile compositions of *P. laetispicum* were more similar to those of *P. longum* (BCS = 58.83%) than those of *P. retrofractum* (35.67%), *P. hainanense* (34.53%), *P. cathayanum* (34.47%), *P. puberulum* (31.90%), *P. flaviflorum* (30.39%), *P. pseudofulgineum* (20.07%), and *P. betle* (17.94%). *P. auritum* showed significant dissimilarity to the other nine *Piper* species (BCS = 4.38% ± 0.12%, range: 3.15%–6.09%, n = 9 comparisons), especially to that of *P. hainanense* (3.15%). This pattern indicated high diversity in volatile compounds of *Piper* at the species level.

Relative abundance of different classes of volatile compounds in different *piper* species

Compositions and relative contents of volatiles in the different *Piper* species varied significantly (Table 3). Forty-four hydrocarbons were identified, and these hydrocarbons accounted for 20.10–86.03% of the total volatiles. The sum of hydrocarbons in *P. retrofractum*, *P. pseudofulgineum*, *P. laetispicum*, *P. cathayanum*, and *P. puberulum* was significantly high at over 60% (Table 3). However, *P. auritum* presented low hydrocarbon content (20.1%). Some hydrocarbons, such as β-caryophyllene, myrcene, ocimene and β-pinene, are linked to pleasant woody, fruit, fragrance of flowers, and pine odor, respectively.²⁴⁻²⁹ In this study, β-caryophyllene, myrcene, ocimene, and β-pinene were the major hydrocarbon compounds, accounting for more than 50% of the total hydrocarbons. *P. puberulum* yielded the highest β-caryophyllene, myrcene, and ocimene contents at 22.51 ± 0.36%, 34.92 ± 0.36%, and 0.94 ± 0.03%, respectively, followed by *P. pseudofulgineum* at 2.32 ± 0.10%, 2.76 ± 0.12%, and 31.48 ± 0.55% (Table 1). However, in *P. auritum*, contents of these compounds were very low, with mean content of 0.45 ± 0.029% in myrcene and 1.88 ± 0.031% in β-caryophyllene.

Fourteen alcohols were detected in the 10 *Piper* species. The highest alcohol content was observed in *P. flaviflorum* (50.18%), showing significant differences from the remaining *Piper* species, whereas no alcohol was detected in *P. auritum*. Alcohols are described to possess “fruity”

| Table 2. The Bray–Curtis similarity values (%) among different species of *Piper*. |
|-------------------|---|---|---|---|---|---|---|---|---|---|
| PBE   | 100 | PAU | 5.77 | 100 | PRE | 17.94 | 4.93 | 100 | PHA | 21.48 | 3.15 | 22.02 | 100 | PPS | 12.41 | 3.20 | 26.70 | 39.04 | 100 | PLA | 17.93 | 3.91 | 35.67 | 34.53 | 20.07 | 100 | PFL | 18.45 | 3.30 | 12.47 | 22.33 | 16.09 | 30.39 | 100 | PCA | 16.94 | 5.78 | 26.48 | 35.01 | 18.96 | 34.47 | 27.17 | 100 |
| PPU | 19.80 | 3.34 | 34.46 | 30.50 | 12.25 | 31.90 | 15.60 | 46.07 | 100 | PLO | 25.97 | 6.09 | 38.93 | 38.55 | 22.29 | 58.83 | 27.14 | 34.94 | 41.58 | 100 |

Notes: PBE: *P. betle*; PAU: *P. auritum*; PRE: *P. retrofractum*; PHA: *P. hainanense*; PPS: *P. pseudofulgineum*; PLA: *P. laetispicum*; PFL: *P. flaviflorum*; PCA: *P. cathayanum*; PPU: *P. puberulum*; PLO: *P. longum*.

| Table 3. Relative abundance of different classes of volatile compounds in 10 *Piper* species. |
|-------------------|---|---|---|---|---|---|---|---|---|---|
| PBE   | 6.30 | 0.49 | 0.00 | 0.14 | 0.00 | 0.00 | 3.49 | 0.00 | 0.00 | 0.00 |
| PAU | 4.61 | 0.00 | 0.34 | 0.13 | 1.52 | 0.00 | 0.00 | 1.10 | 0.00 | 0.00 |
| PRE | 9.46 | 0.00 | 15.55 | 5.00 | 1.95 | 9.04 | 50.18 | 0.88 | 1.46 | 4.94 |
| PHA | 46.63 | 20.10 | 64.59 | 59.65 | 63.53 | 76.9 | 30.95 | 70.57 | 86.03 | 52.78 |
| PPS | 0.00 | 0.00 | 2.48 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PLA | 0.00 | 9.46 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PFL | 0.47 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| PCA | 23.56 | 78.06 | 15.55 | 5.00 | 1.95 | 9.04 | 50.18 | 0.88 | 1.46 | 4.94 |
| PPU | 0.00 | 0.00 | 0.29 | 0.00 | 0.00 | 1.22 | 4.00 | 0.00 | 0.00 | 0.00 |
| PLO | 90.56 | 98.65 | 83.25 | 69.59 | 67 | 87.99 | 88.83 | 74.58 | 87.96 | 61.82 |

Notes: PBE: *P. betle*; PAU: *P. auritum*; PRE: *P. retrofractum*; PHA: *P. hainanense*; PPS: *P. pseudofulgineum*; PLA: *P. laetispicum*; PFL: *P. flaviflorum*; PCA: *P. cathayanum*; PPU: *P. puberulum*; PLO: *P. longum*. 
For alcohols, lavandulol, linalool, and 2-pentanol are described to feature “fruity,” “floral” notes. These compounds also contribute to the fruity and floral odors in fine Piper. In the current study, contents of 2-pentanol were significantly higher in P. betle and P. hainanense species than in the remaining studied plants. P. retrofractum presented high levels of linalool, accounting for 12.9% of the total compounds. However, linalool contents of P. auritum and P. cathayanum were very low.

Aldehyde compounds of the 10 Piper species contributed 0.00–78.06% of the total volatile content. As major components, aldehydes can result from lipid oxidation or self-oxidation and degradation of fatty acids. The sum of aldehydes was significantly higher in P. auritum than notes. For alcohols, lavandulol, linalool, and 2-pentanol are described to feature “fruity,” “floral” notes. These compounds also contribute to the fruity and floral odors in fine Piper. In the current study, contents of 2-pentanol were significantly higher in P. betle and P. hainanense species than in the remaining studied plants. P. retrofractum presented high levels of linalool, accounting for 12.9% of the total compounds. However, linalool contents of P. auritum and P. cathayanum were very low.

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the other *Piper* plants. Cinnamaldehyde and benzaldehyde were detected as the major aldehyde compounds, accounting for more than 90% of the total aldehydes. The relative content of cinnamaldehyde in *P. auritum* was very high, reaching 77.98% (Table 1). Cinnamaldehyde, which is the main component of volatile oil of the traditional Chinese medicine cinnamon, features a special cinnamon flavor.\textsuperscript{[30,31]} Cinnamaldehyde exerts pharmacological effects, including antipyretic, analgesic, antibacterial, and antiviral properties.\textsuperscript{[32]}

The current study detected 7 esters, 3 acids, 2 ketones, and 1 phenol compound in 10 *Piper* species. Volatiles such as 2-nonanone (ketone) and leaf acetate (ester) are described to manifest a fresh and sweet odor and fruity taste.\textsuperscript{[10]} *P. flaviflorum* contained the highest 2-nonanone content (4.00%), followed by *P. longum* (3.20%) and *P. laetispicum* (1.22%). Leaf acetate was present only in *P. betle* and *P. cathayanum* species (Table 1). No significant differences in the total contents of acids, esters, and phenols were detected among *P. laetispicum*, *P. puberulum*, and *P. longum*.

**PCA of volatile compounds**

PCA was used to analyze the data for the 80 volatile compounds obtained from 10 *Piper* species. The first three components of PCA explained 37.67%, 12.16%, and 10.35% of variation, explaining ~60.19% of combined variance (Fig. 2). As shown in Fig. 2a, the 10 *Piper* species can be divided into 4 groups based on their position in the score scatter plot of PCA. The first group was laid on the positive side of PC 1 and included *P. laetispicum*, *P. longum*, *P. hainanense*, *P. betle*, and *P. flaviflorum* species, which were characterized as possessing high concentrations of β-caryophyllene, α-caryophyllene, germacrene, and β-pinene. *P. auritum* species sighted on the negative side of PC 1 formed the second group. *P. auritum* was characterized by extremely high cinnamaldehyde content. The third group contained *P. puberulum* and *P. cathayanum*, which were on the negative side of PC 2 and were characterized as species with high concentrations of myrcene, linalool, and germacrene D. The fourth group contained *P. pseudofuligineum* and *P. retrofractum*, which were on the positive side of PC 2 and were characterized as species with high concentration of ocimene. The remaining 71 volatiles, which include general components, (E)-β-farnesene, α-farnesene, benzyl alcohol, leaf aldehyde, copaene, and trans-β-ocimene, and relatively rare volatiles, such as 4-methylbenzyl alcohol, cineole, and 2-nonanone, showed no relationship with the first 3 components.

**Conclusion**

Volatiles composition and content depended largely upon *Piper* species background. A total of 80 volatile compounds were identified, and the predominant volatile compounds comprised hydrocarbons and aldehydes, followed by alcohols, acids, ketones, esters, and phenols. *P. laetispicum*, *P. longum*, *P. hainanense*, *P. betle*, and *P. flaviflorum*, were characterized by abundant β-caryophyllene, α-caryophyllene, germacrene, and β-pinene; *P. puberulum* and *P. cathayanum* were characterized by high concentrations of linalool, myrcene, and germacrene D; *P. pseudofuligineum* and *P. retrofractum* were associated with extremely high contents of ocimene. Specifically, *P. auritum* featured high content of cinnamaldehyde. The volatile components of *Piper* not only had been used as flavor ingredients but also had contained terpenoids with biological activities. This research showed significance for the comprehensive utilization of *Piper* germplasm and provides an important reference for further research.

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