GC-MS Analysis of Volatile Components in Partridge Tea (Mallotus obongifolius)

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Abstract. Partridge tea is one of the famous local herbal teas of Hainan Island, China. In the present study, headspace solid-phase microextraction and gas chromatography-mass spectrometry were combined to determine and analyze the volatile components in the red and green leaves of partridge tea. Seventeen volatile components were identified in the red young leaves. The olefins, alkanes, and alcohols accounted for 71.24%, 1.1%, and 0.54%, among which the main components were cyclohexene (22.50%), humulene (18.73%), and α-guaiene (8.78%), respectively. Twenty volatile components were identified from red mature leaves, including 34.74% olefins, 6.14% esters, and 3.11% acids. Eighteen volatile components were identified from green young leaves, among which olefins (70.52%), alkanes (4.32%), and alcohol (0.89%) were the major components. Nineteen volatile components were identified from green mature leaves, among which the olefins, esters, and acids were the major components with the contents of 46.04%, 6.38%, and 1.37%, respectively. Results showed that the major volatile components of partridge tea were olefins, in which cyclohexane was the most abundant. The contents of volatile components between red leaves and green leaves had notable differences, which might be useful for germplasm identification of partridge tea.

China is one of the major tea-producing nations in the world, and partridge tea is one of the famous herbal teas of Hainan Island due to its many health benefits. It has been reported that partridge tea has antithrombotic-sclerotic effects (Liu et al., 2011). Mallotus species can be used as herbal tea because they all have important antioxidant properties (Tistaert et al., 2012). Tea is reported to contain nearly 4000 bioactive compounds, of which one-third is a large group of plant chemicals that includes polyphenols (Tarig et al., 2010). Other compounds are alkaloids (caffeine, theophylline, and theobromine), amino acids, carbohydrates, proteins, chlorophyll, volatile organic compounds (chemicals that readily produce vapors and contribute to the odor of tea), fluoride, aluminum, minerals, and trace elements (Cabrera et al., 2003). Polyphenols found in tea are mostly flavonoids (Sumpio et al., 2006). The polyphenols catechins are thought to be responsible for the health benefits that have traditionally been attributed to tea, especially green tea (Cabrera et al., 2006). The most active and abundant catching in green tea is epigallocatechin-3-gallate (EGCG). Black tea contains much lower concentrations of these catechins than green tea (Wu and Yu, 2006). Oolong tea contains a mixture of simple polyphenols, such as catechins and complex polyphenols (Mukhtar and Ahmad, 2000). Black, green, and oolong teas are all good sources of vitamin C.

Generally, the extraction of volatile components from EGCG depend to a great extent on steam refining (SD) and soluble extraction (SE) at both logical and preparative dimensions. In any case, there have been rare studies on the correlation of volatile components from EGCG by various extraction strategies. Solid phase microextraction (SPME) was progressively quick, sensitive, and solvent-free contrasted with conventional techniques (Li et al., 2007; Lv et al., 2012). SPME was first having been widely adopted in air, water, soil, and sustenance examination (Roberts and Milo, 2000). Ordinarily, analyses are extracted from a vaporous or a fluid example by ingestion in direct-inundate SPME (DI-SPME) or adsorption on headspace SPME (HS-SPME) with a thin polymer covering being fixed to the strong surface of a fiber in an infusion needle (Pragt, 2007). Because HS-SPME is nonpolluting to fiber-contrasted and DI-SPME, it was eventually chosen over the SD and SE methods. Using gas chromatography-mass spectrometry (GC-MS) has the advantage of empowering compound-recognizable proof by comparing the mass spectra of the samples with those of authentic standards from the National Institute of Standards and Technology and contrasting the retention indices with those announced by a past accessible investigation.

Tea is one of the most popular beverages in the world because of its taste and numerous health benefits. Partridge tea, a famous local tea with numerous favorable characteristics from Hainan Island of China, has elements of strengthening the gallbladder (Li et al., 2014), relieving pain, and enhancing immunity (Liu et al., 2008, 2011; Yan et al., 2012). It has been admired by the literati of the past and is known as Gastrodiae lucidum grass in various tea items (Yan and Li, 2016). Although many studies have revealed volatile components in tea, the research on volatile components in the leaves of partridge tea is scarce. Therefore, in the present study, HS-SPME followed by GC-MS was conducted to determine the volatile components in the red and green leaves of partridge tea.

Materials and Methods

Plant materials and sample preparation. Young leaves (YL) and Mature leaves (ML) of red and green varieties (Fig. 1) were collected from Hainan University, China. Each ground leaf sample (2 g) was quickly placed in a 20-mL headspace vial and embedded into a prematured extraction for extraction and adsorption. After 30 min extracting time, the sample was inserted into the GC-MS injection port. The analytes were thermally desorbed in the splitless mode at 250 °C for 10 min. Each sample was run in three replicates.

GC-MS analysis. An Agilent 7890A gas chromatograph coupled with an Agilent 5975C mass spectrometer was used to perform the volatile analysis (Agilent Technologies, Santa Clara, CA). Samples were analyzed on a HP-5MS (30 m × 250 μm × 0.25 μm). Helium (99.999% purity) was used as the carrier gas at a rate of 1 mL/min. The oven temperature was held at 40 °C for 4 min, increased to 80 °C at 5 °C/min, and then increased to 260 °C at 10 °C/min, and maintained for 10 min. The mass spectrometer was operated in an electron impact (EI) mode at 70 eV. Ion source temperature was at 230 °C, quadrupole 150 °C. The scan range was 35 to 800 m/z in a full scan acquisition mode.

Data analysis. The mass spectrometry data obtained by GC-MS was matched and searched by computer and mass spectrometry library, and the significant mass spectrometry information was checked to choose the pinnacle number with similarity ≥96. The base peak, mass proportion, and relative abundance
were analyzed. The chemical structure and name of the aroma substances represented by each peak were affirmed separately, and the relative rate was investigated by the peak region standardization technique; the proportion of the peak territory of every unstable part of the absolute peak zone shows the overall level of the components. The major volatile components in different leaf samples and their relative contents (%) are listed in Table 1.

**Results**

**Analysis of volatile components.** The GC-MS chromatograms of the red leaves and green leaves from partridge tea are shown in Fig. 2. A total of 25 volatile components were identified in various leaf samples (Table 1).

In red young leaves, 17 volatile components were identified, and the major components were olefins (71.24%), alkanes (1.1%), and alcohols (0.54%). As for alkenes, caryophyllene (22.50%), humulene (18.73%), and α-guaiene (8.78%) were the most abundant. Among the identified alkenes, 8-isopropenyl-1,5-dimethyl-cyclodeca-1,5-diene (1.10%) was detected as the major alkane. Among the alcohols, geraniol (0.54%) was the most abundant.

In red mature leaves, 20 volatile components were identified, and the major components were olefins (34.74%), esters (6.14%), and acids (3.11%). The main volatile components for alkenes were squalene (5.22%), caryophyllene (3.82%), and d-cadinene (3.88%). The predominant components for esters were methyl hexadecanoic (2.96%) and methyl stearate (1.42%).

In green young leaves, 18 volatile components were identified, and the major components were olefins (70.52%), alkanes (46.04%), esters (6.38%), and acids (1.37%). As for alkenes, the main components were caryophyllene (10.76%), α-selinene (4.49%), and eremophilen (4.29%). Methyl hexadecanoic (3.04%) and methyl stearate (1.60%) were identified as the major esters.

**Relative proportion of principal volatile components.** The correlation of volatile components is shown in Fig. 3. Red leaves and green leaves had slightly different levels of volatile components. The contents of olefin volatile components in the red and green young leaves were notably higher than in the red and green mature leaves. The contents of olefins in green young leaves (70.52%) and red young leaves (71.24%) were similar. The olefin content in green mature leaves (46.04%) was slightly higher than that in the red mature leaves (34.74%). The contents of alcohols and acids in the red leaves and green leaves were similar.

**Relative proportion of volatile compounds.** A comparison of partridge volatile components is shown in Table 1. There were obvious differences in the contents of volatile components caryophyllene, humulene, α-guaiene, and α-bulnesene between red young and red mature leaves. Differences in other unstable segments were not evident (Fig. 4A).

The volatile components caryophyllene, humulene, α-guaiene, α-bulnesene, α-copaene,
and α-cubebene in green young leaves were higher than those in green mature leaves, suggesting that the major volatile components in green leaves are caryophyllene, α-guaiene, α-bulnesene, α-copaene, and α-cubebene (Fig. 4B).

Differences in the relative content of caryophyllene, humulene, α-copaene, α-cubebene, and δ-cadinene between the red young leaves and the green young leaves were clear, whereas the differences of other volatile components were not evident (Fig. 4C).

As for the red and green mature leaves, noticeable differences were observed in the contents of caryophyllene, α-guaiene, eremophilene, squalene and n-hexadecanoic acid. The variation in the contents of other volatile components was not apparent between red and green mature leaves (Fig. 4D).

Discussion

Nowadays, the investigation of the volatile components from plants and their action has expanded. The combination of a best partition procedure (GC) with the best identification method (MS) made GC-MS a perfect skill for qualitative analysis for volatile and semivolatile bioactive compounds (Grover and Patni, 2013). The analysis found that the volatile components of partridge tea leaves include a variety of valuable chemical components; for example, caryophyllene has antianxiety and antitumor actions (Medeiros et al., 2007; Sabulal et al., 2006) humulene offers significant relief from cough, removing phlegm and preventing asthma effects (Legault and Pichette, 2007); cadinene remove phlegm (Calleja et al., 2013); and...
Fig. 4. The relative proportion of 25 compounds. (A) Red leaves. (B) Green leaves. (C) Young leaves. (D) Mature leaves.

In the current study, 17 volatile compounds were identified in the red young leaves of partridge tea. The major components were olefins, alkanes, and alcohols (Guo and Lin, 2005; Li et al., 2015; Zhao et al., 2018), among which caryophyllene, humulene, and α-guaiene were the most abundant. Twenty volatile components were identified from red mature leaves, including olefins, esters, and acids. The main components were squalene, d-cadinene, and caryophyllene. Eighteen volatile components were identified from green young leaves, among which olefins, alkanes, and alcohol were predominant. The main components, respectively, were α-copaene, caryophyllene, and α-cubebene. Twenty volatile components were identified from green mature leaves. Among the identified components, olefins, esters, and acids were detected as the major components. The main components, respectively, were caryophyllene, eudesma-3,11-

diene(8CI), and eremophilene (Chu et al., 2018; Li et al., 2018; Lin et al., 2001).

There are a few differences in the volatile elements of the young leaves and mature leaves; the plant material used was of a single variety, and the procedures were identical; however, numerous elements affect the volatile elements of partridge tea, including soil and season, even with identical plant material. Other variations may additionally affect volatile compounds. The major volatile components in partridge tea are olefins, and caryophyllene was identified as the major olefins. Furthermore, obvious differences were observed in the contents of some volatile components between red and green leaves as well as between young and mature leaves, which could be attributed to the identification of the varieties (red and green) and leaf maturation (young and mature).

To explore all endogenous components and avoid the high-temperature destruction of certain compounds throughout hydrodistillation, solvent extraction followed by GC-MS allowed analysis of the chemical constituents with superior results and has been verified to be an economical methodology for extraction of volatile parts and effective chemotaxonomy, which can be an auxiliary to phylogenetic systematics.

In conclusion, the volatile composition of young and mature leaves in red and green varieties of partridge tea were analyzed. The olefins, alkanes, and alcohols and their derivatives were the compounds found in highest quantities. With horizontal assessment of the materials among plant material, some samples were characterized by means of excessive concentrations of specific compounds that could be further exploited and applied to germplasm evaluation.

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α-cubebene has antioxidant activity and can be used as a food preservative (Wang et al., 2007). Element injection has high efficiency and low toxicity, can inhibit a variety of tumor cells, induces tumor cell apoptosis and differentiation, and improves the body’s immunity, among other characteristics (Li et al., 2010; Lin, 1977); squalene reduces the toxicity of carcinogens (Nakagawa et al., 1985), improves the efficiency of the body's discharge of theophylline and alkaloids (Kim and Karadeniz, 2012), antioxidation, moisturizing, and other effects (Huang et al., 2009). In the experimental results, the percentage of such substances in the mature leaves is not high, which may be related to the shading of the leaves. Yang et al. (2012) found that volatile fatty acid derivatives and phenylpropanoid/benzene ring volatile is significantly improved; aroma components of terpenes and glycosides did not change significantly.
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