Original article

Dynamic accumulation of sesquiterpenes in essential oil of Pogostemon cablin

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ABSTRACT

Essential oil produced by patchouli was one of the most important naturally occurring base materials used in the perfume industry, containing various sesquiterpenes. Three different parts (leaves, stems and roots) of Pogostemon cablin (Blanco) Benth., Lamiaceae, were profiled in relation to different maturation phases in this paper, evaluating the variations in content of the major sesquiterpenes in the essential oil. Twelve sesquiterpenes were analyzed by GC-MS throughout the maturity of P. cablin. Patchouli alcohol (37.54%-51.02% in leaves, 28.24%-41.96% in stems and 14.55%-35.12% in roots) was the major sesquiterpene during the maturation of the plant. The average content of several other sesquiterpenes (α-bulnesene, α-guaiene, seychellene, β-humulene and caryophyllene) were higher than 3% among leaves, stems and roots. The content of essential oil, patchouli alcohol, α-bulnesene and several other compounds were highly accumulated at 210 days of maturation after cultivation of P. cablin. Thus, this period was the best moment to exploit the maximum level of these high value-added compounds in P. cablin. Furthermore, our results indicated that the essential oil extracted from leaves of P. cablin has the highest potential to be used in the perfume industry.

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Introduction

Pogostemon cablin (Blanco) Benth., Lamiaceae, common known as patchouli, from southeast Asia has been extensively cultivated in Indonesia, the Philippines, Malaysia, China, and Brazil (Miyazawa et al., 2000; Singh et al., 2002; Wu et al., 2008). Patchouli was introduced into China as early as the Liang Dynasty or potentially before (Wu et al., 2007). Currently, patchouli is widespread in southern China, including Guangdong and Hainan Province. Patchouli is widely used in the traditional Chinese medicine as it offers various types of pharmacological activities, which include removing dampness, relieving summer-heat, exterior syndrome, stopping vomiting and stimulating appetite (Xu et al., 2010). Essential oil produced by patchouli is one of the most important naturally occurring base materials used in the perfume industry (Hasegawa et al., 1992). The composition of patchouli essential oil is unique and complex because it consists of a large number of different sesquiterpenes (Nikiforov et al., 1988), rather than a blend of...
were obtained from a local plant nursery and propagated from Patchouli plants (Pogostemon cablin (Blanco) Benth., Lamiaceae,) contains a large number of other sesquiterpenes such as α-/β-bulnesene (1), α-bulnesene (2), α-guaiene (3), seychellene (4) and so on. Sesquiterpenes are colorless, bitter, relatively stable, lipophilic constituents, biogenetically derived from E,E-farnesyl pyrophosphate following an initial cyclization and subsequent oxidative modifications. In addition to anti-inflammatory, antimicrobial, antiprotozoal, and antitumor properties of sesquiterpenes (Picman, 1986; Galindo et al., 1999; Neerman, 2003), many of these compounds have shown to possess strong phytotoxic activity against several weeds (Pandey, 1996; Duke et al., 1987; Batish et al., 1997, 2002; Fillmoor, 1998; Abdelgaleil et al., 2009).

Due to its uses in perfumery, the demand of patchouli oil is increasing dramatically in the world. Today’s market is supplied with patchouli oil products from different herb producers as the plant is found all around the world (Wu et al., 2013). Therefore, available preparatios of the patchouli oil products may differ significantly in quality depending on a number of factors such as the plant varieties, tissues or organs used, harvesting time (different developmental stages of the plant) and the different and poorly controlled analysis conditions (Bergonzi et al., 2001).

In addition, the geographic location was an important factor affecting the chemical composition and developmental process of the medicinal plant. The plant growth progress and chemical characterization varied under different environmental conditions and cultivation locations. For example, patchouli growth and maturity lasted for thirteen months in Brazil, but only eight months in the Hainan province, China. Blank et al. (2011) reported the change of chemical characterization of the patchouli oil at four different harvest seasons, however, they collected the materials in three month intervals and only the essential oil of leaves was analyzed. In fact, the collection of the materials at intervals of three months could not reflect the details of the accumulation process of chemical composition during the patchouli maturation. Thus, we gathered the leaves, stems and roots of P. cablin at intervals of 15 days after 120 days of culture to analyze the dynamic accumulation of patchouli oil and sesquiterpenes content during the ontogenetic stages. Although the sesquiterpene composition of mature plants have been studied (Chen et al., 2008; Xu et al., 2010), very little information is available regarding the dynamic accumulation of sesquiterpenes during the maturation of P. cablin. The aim of the present study was to carry out a qualitative and quantitative characterisation of sesquiterpenes during the development of P. cablin, and then determine the time at which patchouli oil and sesquiterpenes had maximal accumulation.

Materials and methods

**Plant material**

Patchouli plants (Pogostemon cablin (Blanco) Benth., Lamiaceae,) were obtained from a local plant nursery and propagated from cuttings in Hainan University, Haikou City, Hainan province, China. From January 16 to May 1 in 2014, the leaves, stems and roots of P. cablin at eight different ontogenetic stages were hand-harvested at intervals of 15 days after 120 days of culture (DAC) until complete maturity (225 DAC). Only healthy plants, without any kind of infection or physical damage, were selected. The leaves were air-dried (20 ± 2°C) for one week until constant weight, and the stems and roots were dried at 40°C for three days until constant weights were achieved. They were stored at room temperature. The dried samples were ground using a mill to obtain a coarse powder (40 mesh). The plant of P. cablin was identified and authenticated by Prof. Xiaobo Yang, a plant taxonomist. A voucher specimen (HN201288) was kept at the Key Laboratory of Protection, Development and Utilization of Tropical Crop Germplasm Resources of Ministry of Education, Hainan University, China.

**Extraction of essential oils**

The powdered leaves, stems and roots (100 g) of P. cablin were separately subjected to hydrodistillation for 4 h in a simple laboratory quickfit apparatus, with a 1000 ml steam generator flask, a distilling flask, a condenser and a receiving vessel used to perform the steam distillation. The extracts were dried over anhydrous sodium sulphate, and the resulting essential oils were stored at − 10°C prior to chemical analysis. The dried essential oils were solubilized in n-hexane and filtered through a 0.45 μm Econofilter (Agilent Technologies) prior to injection into the GC-MS system.

**Gas chromatography-mass spectrometry detection (GC-MS)**

GC-MS analyses were performed using an Agilent 6890 gas chromatography instrument coupled to an Agilent 5973 mass spectrometer using Agilent ChemStation software (Agilent Technologies, Palo Alto, CA, USA). Compounds were separated on a 30 m × 0.25 mm i.d. capillary column coated with 0.25 μm film. Helium was used as carrier gas, with a constant flow rate of 1 ml/min. The injector and detector temperature was 250°C. The oven temperature was programmed from 60 to 300°C at a rate of 6°C/min, the final temperature was held constant for 10 min and the transfer line temperature was 250°C. Electron impact mass spectra were measured at acceleration energy of 70 eV. Manual injection of 1.0 μl of the solution of essential oil was performed in the split mode at a 20:1 split ratio. The sesquiterpenes were identified by comparing their relative retention times and mass spectra with the authentic standards. The peaks were also confirmed using NIST Mass Spectral Library. Twelve sesquiterpenes (Table 1) were identified by GC-MS during the ontogenetic stages of P.cablin.

**Data analysis**

The content of sesquiterpene compound (expressed in % of essential oil) was calculated using the following formula; content (%) = PAs/TPA, where PAs = sesquiterpene compound peak area, TPA = total compounds peak areas. Three independent replicates were carried for each sample. Data were subjected to an analysis of variance using a general linear
model and the means were compared using Duncan’s multiple range test (Duncan) at 5% confidence level using SPSS (version 19.0).

Results and discussion

Patchouli plant development and essential oil accumulation

The accumulation of essential oil in P. cablin during maturation was reported in Table 2. Although a gradual increase was found, there were marked differences in oil content at different ontogenetic stages and different parts of the plant. The developmental process of patchouli plant was characterized by changes in size, weight, chemical composition, color, and physical properties of the plant. The results showed that patchouli plant growth and maturity lasted for eight months, and could be divided into four principal phases according to the changes of plant size, color and biomass of the plant: (1st) slow growth period, this phase comprised the first three months (not discussed here), the leaves of the plant were green and the biomass increased slowly in this stage, plant branches were rare in the early but mostly at the end of this stage; (2nd) fast growth period, this phase occurred from the fourth month to the sixth month, the leaves of the plant were green and the biomass had a fast increase in this stage, and the plant branches grew fast and the crown was big at the end of this stage; (3rd) ripe period, this phase occurred at the seventh month, and was characterized by the leaves turning yellow from green, and the biomass and size of the plant increased slowly; (4th) fully ripe period, characterized by parts of the leaves turning orange and a relative stable biomass on the last month. In addition to the developmental process of P. cablin, climatic conditions seem to have an influence on the development of the plant.

Studies on the essential oil accumulation were important to decide the best moment to harvest patchouli. Investigations on the variations of essential oil harvested on different seasons have been carried out for patchouli collected from Gaoyao county (Luo et al., 2000), Wuchuan county (Guo et al., 2002) and Wanning county (Luo et al., 2002). The results described by the authors showed that the influence of seasonality on P. cablin had diversity and complexity. The variations of patchouli oil in different producing areas had different responses to the seasons. For example, as for P. cablin collected from Gaoyao county, the content of patchouli alcohol (5) in the leaf oil was higher in September and October than other months, whereas there were not obvious changes on the contents of patchouli alcohol in stem oil among ontogenetic stages (Luo et al., 2000). Luo et al. (2002) also reported that the contents of patchouli alcohol were higher in July than other months for patchouli collected from Wanning county.

Table 2 shows that patchouli stems have similar essential oil content to that of the roots, but significantly less than that of the leaves from the 2nd to 4th phase. The content of essential oil extracted by steam distillation reached the maximum levels (1.02% in leaves, 0.56% in stems, 0.55% in roots) at the 4th phase. These results were similar to those found by Xu et al. (2010), but different from other reported contents of essential oil (Donelian et al., 2009; Blank et al., 2011). After all, the essential oil production was affected by multiple factors including cultivation pattern (Luo et al., 2000), fertilization (Singh and Rao, 2009), storage time (Yu et al., 2008), extraction methods (Blank et al., 2011; Donelian et al., 2009) and growth environment.

Accumulation of patchouli alcohol

In China, patchouli alcohol (PA) was used as a chemical marker required by law for quality control of P. cablin herb and patchouli oil (China Pharmacopoeia Committee, 2010). In recent years, PA research has attracted significant attention due to its cognition enhancement, learning impairment attenuation, and neuroprotective activities (Huang et al., 2009). PA has also been found to strongly inhibit influenza A replication and weakly inhibit influenza B replication in vitro (Kiyohara et al., 2012).

The composition of PA in the essential oil of P. cablin during maturation was summarized in Fig. 1. Results provided by Fig. 1 revealed that PA had higher quantities in leaves (37.54% to 51.02%) than those in stems (28.24% to 41.96%) and roots
E compounds, Fig. 2 listed the accumulation trends of three sesquiterpenes and often in specialized surface structures such as trichomes. Secondary metabolites could accumulate on or in the leaves, of drug products of the detection of essential oil adulteration and quality control. The maximum accumulation. The qualitative and quantitative stages in which those high value-added compounds had reached their maximum levels at 210 (51.02%) and 180 (41.96%) DAC respectively. Finally, ended by a phase of dramatic decline. Nevertheless, the accumulation of PA in roots presented oscillations alternated with maximums and minimums of PA contents reported a similar accumulation trend in leaves, stems and roots. The highest values of β-humulene, (-)-globulol and β-guaiene were 13.60%; 1.02%; 2.17% in leaves; 6.32%; 0.65%; 1.11% in stems; and 3.71%; 0.24%; 0.78% in roots, respectively. The sesquiterpene profile in leaves, stems and roots was important to choose the optimal materials for the perfumery industry. In fact, roots were seldom used as materials for the extraction of patchouli oil. The sesquiterpenes in roots. Some sesquiterpene synthases were probably responsible for the biosynthesis of more than one sesquiterpene product. For instance, patchoulol synthase, a single sesquiterpene cyclase enzyme, was possibly responsible for the biosynthesis of at least thirteen additional sesquiterpene products (Deguerry et al., 2006). Accumulation patterns among leaves, stems and roots. The sesquiterpene profile in leaves, stems and roots was important to choose the optimal materials for the perfumery industry. In fact, roots were seldom used as materials for the extraction of patchouli oil. The sesquiterpenes in roots.

**Accumulation of several other sesquiterpenes**

Sesquiterpene profile was an important indicator to determine the date in which those high value-added compounds had maximum accumulation. The qualitative and quantitative characterization of sesquiterpenes seemed to be useful for the detection of essential oil adulteration and quality control of drug products of P. cablin. Sesquiterpenes and other volatile secondary metabolites could accumulate on or in the leaves, and often in specialized surface structures such as trichomes. Fig. 2 listed the accumulation trends of three sesquiterpene compounds, β-humulene (6) (Fig. 2a), (-)-globulol (7) (Fig. 2b) and β-guaiene (8) (Fig. 2c). All of them showed similar accumulation trends in leaves, stems and roots. They were characterized by high accumulation in leaves, low accumulation in stems and roots. The highest values of β-humulene, (-)-globulol and β-guaiene were 13.60%; 1.02%; 2.17% in leaves; 6.32%; 0.65%; 1.11% in stems; and 3.71%; 0.24%; 0.78% in roots, respectively. The sesquiterpene profile in leaves, stems and roots was important to choose the optimal materials for the perfumery industry. In fact, roots were seldom used as materials for the extraction of patchouli oil in industry because of little sesquiterpenes in roots.

### Table 2

<table>
<thead>
<tr>
<th>Stage</th>
<th>Leaf colour, state of maturity</th>
<th>Harvest dates</th>
<th>DAC</th>
<th>Volatile oil content (% d/w)</th>
<th>Leaves</th>
<th>Stems</th>
<th>Roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>Fully green; slowly growing</td>
<td>16/01/2014</td>
<td>120</td>
<td>0.50 ± 0.020&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.43 ± 0.021&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.41 ± 0.015&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>2nd</td>
<td>Green; fastly growing</td>
<td>31/01/2014</td>
<td>135</td>
<td>0.53 ± 0.015&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.48 ± 0.015&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.44 ± 0.015&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>3rd</td>
<td>Green-yellow; ripe</td>
<td>17/03/2014</td>
<td>180</td>
<td>0.81 ± 0.015&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.51 ± 0.015&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.47 ± 0.010&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>4th</td>
<td>Green-yellow-orange; fully ripe</td>
<td>16/04/2014</td>
<td>210</td>
<td>1.02 ± 0.010&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.56 ± 0.015&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.55 ± 0.010&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
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</table>

Mean ± SD; For each column, numbers followed by the same letter were not significantly different using Duncan’s multiple range test, p < 0.05; DAC: days after cultivation; % d/w: percentage of dried weight; “-“ means not discussed here.
Figure 1 – Accumulation trends of patchouli alcohol (expressed in g/100g of essential oil) in Pogostemon cablin.

Figure 2 – Accumulation trends of β-humulene(a), (-)-globulol (b) and β-guaiene (c) (expressed in g/100g of essential oil) in Pogostemon cablin.
In a word, our results showed patchouli alcohol (5), α-bulnesene (2), α-guaiene (3), seychellene (4), β-humulene (6) and caryophyllene (11) were major sesquiterpenes whose average contents in leaves, stems and roots were more than 3% in patchouli oil. Similar results have been also found by Tsai et al. (2007). However, trans-farnesol (9), patchouliene (1), (-)-globulol (7), caryophyllene oxide (12), isocaryophyllene (10) and β-guaiene (8) were less present in patchouli oil, their average contents were less than 1.00% in leaves, stems and roots. The results were in accordance with Xu et al. (2010).

In agricultural practice it is difficult to determine the optimum time of harvest if high contents of both sesquiterpenes and biomass are required. Our results indicated that plant materials at different developmental stages showed different sesquiterpenes profiles. Keeping the appropriate harvesting time was imperative for the production of a *P. cablin* herb drug with a satisfying sesquiterpenes profile. When compared all the figures listed, it was not difficult to note that 210 DAC was an important date. At this period, many important compounds attained their maximum values in leaves and roots, such as patchouli alcohol, α-guaiene, α-bulnesene, seychellene, trans-farnesol and isocaryophyllene; and patchouliene in stems and roots. The content of essential oil reached the highest levels in leaves, stems and roots at this moment. Moreover, at the 210 DAC, the ratio of sesquiterpenes to total compounds (sesquiterpenes/total compounds) also reached the maximum in leaves (86.46%) and roots (59.58%).

In summary, the sesquiterpenes were present at all stages of patchouli maturity, whether in leaves, in stems and in roots. The accumulation patterns of these sesquiterpenes were strongly influenced by the development process. During the developmental process, the contents of essential oil, patchouli alcohol, α-bulnesene and several other compounds were highly accumulated at 210 DAC of *P. cablin*. Thus, this date was the best moment to exploit the maximum levels of these high value-added compounds present in patchouli. Furthermore, our results indicated that the essential oil extracted from leaves of *P. cablin* has the greatest potential to be used in the perfume industry due to its content in essential oil; patchouli alcohol and several other sesquiterpenes in leaves were more than those in stems and roots.

**Authors’ contribution**

All authors contributed in collecting the plant sample, running the laboratory work, analysis of data and drafting of the manuscript. All authors participated in drafting the article and critical revision.

**Conflicts of interest**

The authors declare no conflicts of interest.

**Acknowledgements**

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Figure 3 – Accumulation trends of α-guaiene, seychellene, trans-farnesol and isocaryophyllene (expressed in g/100g of essential oil) in leaves (a), stems (b) and roots (c) in Pogostemon cablin.
**Figure 4** – Accumulation trends of α-bulnesene (a), patchoulene (b), caryophyllene (c) and caryophyllene oxide (d) (expressed in g/100g of essential oil) in *Pogostemon cablin*.


