Effects of different drying methods on volatile composition of *Melaleuca alternifolia* essential oil

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Abstract. The quality of *Melaleuca alternifolia* essential oil is affected by various drying methods. The aim of this study was to investigate the effect of drying methods on the composition of the essential oil. *Melaleuca alternifolia* essential oil samples subjected to different drying methods were extracted using the methyl tert-butyl ether (MTBE) method and analyzed by gas chromatography-mass spectrometry (GC-MS). In total, 26 volatile compounds, comprising 8 monoterpenes, 6 sesquiterpenes, 4 monoterpenes derivatives, 5 sesquiterpene derivatives, and 3 other derivatives, were identified. We found that the drying methods affected not only the composition of volatile compounds but also the content of different volatiles. The sun-dried essential oil showed the highest amount of volatile compounds, 1895.85 ng/g. Monoterpenes derivatives were a major component in *M. alternifolia* essential oil, especially terpinen-4-ol and 1,8-cineole.

1. Introduction

*Melaleuca alternifolia* is a woody plant of genus *Melaleuca*, which has its origins in coastal areas at 23.5°S in the northern part of the Northern Territory and in other regions in Australia. Currently, 236 known species of *M. alternifolia* exist, of which 230 originated in Australia, and the rest grew in Indonesia, New Guinea, New Caledonia, and Malaysia. *M. alternifolia* essential oil, known as tea tree oil (TTO), is an aromatic transparent liquid obtained after the steam distillation of fresh leaves and branches of *Melaleuca alternifolia*.

It is widely used in healthcare because it exhibits satisfactory broad-spectrum bactericidal ability and is extremely safe [1]. *Melaleuca alternifolia* was introduced in Guangdong, Guangxi, Hainan, Yunnan, and other regions in China in the 1990s and is grown ever since [2]. By the end of the past century, the cultivated area of *Melaleuca alternifolia* in Guangdong Province alone had grown to about 150 hectares, with an essential oil production of 17-20 t. Therefore, research on *Melaleuca alternifolia* essential oil has become increasingly active in China, and Chinese quality standards for the oil have been established [3, 4].

Plant essential oils are volatile aromatic substances extracted from flowers, leaves, stems, roots, or fruits of plants through steam distillation, extrusion, cold maceration, or solvent extraction. These oils have a complex composition, are often composed of numerous compounds with relatively small molecular weight, and are volatile oily liquids at atmospheric temperature. According to their chemical structures, plant essential oils can be divided into aliphatic, aromatic, and terpene...
compounds, their oxygen-containing derivatives such as alcohols, aldehydes, ketones, acids, ethers, esters, and lactones, and their nitrogen-containing and sulfur-containing compounds. Plant essential oils are widely used in the pharmaceutical and food industries and in the manufacture of spices, cosmetics, and agricultural pesticides because of their potential use as a fragrance enhancer, a sterilizing agent, an antiviral agent, and an antioxidant [5, 6].

However, the current research on *Melaleuca alternifolia* mainly focuses on cultivation techniques and applications of its essential oils. Mo et al. [7] found that Murashige and Skoog medium had a good induction effect on the cluster buds of *M. alternifolia*, and 20 g/L glucose could effectively improve the induction rate of the cluster buds of *Melaleuca alternifolia*. Qiu [8] investigated the cutting effect of different cutting substrates, rooting agents, and cutting time duration on the tender shoots of tissue culture seedlings of *Melaleuca alternifolia*. The best cutting effect is observed when yellow subsoil is used as the substrate, and the best cutting time duration are April, May, and mid-October, with the average rooting rate of >95%. Plant essential oils are widely used in agriculture as a preservative. Jing et al. [9] found that 1.25 g/L of TTO can delay the yellowing of banana peel, softening of banana, and degradation of starch in banana pulp, thus maintaining the content of soluble sugar in banana at a high level. In addition, Zhu et al. [10] found that TTO and a chitosan compound solution can preserve bananas. Fumigating fresh strawberries with TTO can effectively prevent spoiling of strawberries and can reduce the weight loss rate of strawberries during storage and transportation [1, 11]. Factors such as cultivation conditions of *Melaleuca alternifolia*, essential oil extraction technology, and leaves with different degrees of maturity can affect the yield and composition of TTO [12-14]. Qiao and Li [15] compared the essential oil components of *Melaleuca alternifolia* with different degrees of maturity and found that the content of essential oil in old leaves is higher than that in young leaves. In addition, the maturity of *Melaleuca alternifolia* leaves affects the content of terpinen-4-ol, which is the most important antifungal component in TTO [16]. Huang et al. [17] found that terpinen-4-ol content in TTO increases while the 1,8-cineole content in TTO decreases with an increase in the distillation pressure.

Therefore, distillation pressure is one of the factors affecting the quality of TTO. Because the composition of *Melaleuca alternifolia* essential oil is affected under various conditions, this study investigated the effect of drying methods on the composition of the essential oil to provide a basis for the development and utilization of *Melaleuca alternifolia*.

2. Materials and methods

2.1. Materials

*Melaleuca alternifolia* plants were provided by Lvye Biotechnology Co., Ltd., Xingning City, Guangdong Province. The annual average temperature and sunshine hours is 21 °C and 2009.8 h, respectively (115.785624 °E, 24.270661 °N). The trees were cultivated about 20 hectares in 2012, and the samples collected in 2018 were subjected to freeze drying, direct sun drying, hot air drying, and vacuum drying for essential oil extraction and volatile component determination.

2.2. Instruments and equipment

The STELLAR Freeze Dryer was provided by Tianmei Scientific Instruments Co., Ltd.; the vacuum dryer was provided by Nanjing Rui’ao Electric Heat Technology Co., Ltd.; the QP-2010-Plus GC-MS was provided by Shimadzu Corporation (Japan); the Rtx-5MS capillary column (30 m × 0.5 µm × 0.32 mm) was provided by Agilent Technologies Inc. (USA).

2.3. Preparation of essential oil

The *Melaleuca alternifolia* essential oil was prepared using the methyl tert-butyl ether (MTBE) method [18]. First, 1.0 g of *Melaleuca alternifolia* sample was accurately weighed and placed in a sample bottle. Next, 10 mL of MTBE solution was added to the bottle for incubation with shaking overnight at room temperature. Then, polar substances and macromolecular compounds such as
chlorophyll were removed from the essential oil sample through a separation column, and the essential oil was placed in a refrigerator at 4 °C to be on standby for use.

2.4. Gas chromatography-mass spectrometry (GC-MS) conditions

2.4.1. GC heating procedure. An initial temperature of 50 °C was maintained for 1 min and was then increased to 260 °C, at a rate of 5 °C/min, and maintained for 10 min. The temperatures at the injection port were 250 °C, and desorption was performed for 5 min. The carrier gas was high-concentration He (99.99%), and 1:50 split injection with a flow rate of 1 mL/min was adopted.

2.4.2. MS conditions. The temperatures at the connection port, ion source, and quadrupole were 280 °C, 230 °C, and 150 °C, respectively, the ionization mode was EI, the electron energy was 70 eV, and whole process scanning was performed.

2.5. Qualitative analysis of components: The component mass spectrometry was searched in NIST/WILEY and analyzed, and then artificial spectrum analysis was carried out by referring to relevant literature to determine various chemical components.

Here, 10 μg/mL iso-butyl benzene was used as the internal standard for repeated (thrice) concentration calculation.

3. Results

3.1. Composition of Melaleuca alternifolia essential oil

A total of 26 volatile substances, including 8 monoterpenes, 6 sesquiterpenes, 4 monoterpen derivatives, 5 sesquiterpene derivatives, and 3 other substances, were detected by gas chromatography-mass spectrometry in Melaleuca alternifolia essential oil treated by different drying methods. Different drying methods have different effects on the composition and content of Melaleuca alternifolia essential oil. After freeze drying, direct sun drying, hot air drying, and vacuum drying, 25, 26, 24, and 26 components, respectively, were detected in the essential oil samples. The relative contents of 1,8-cineole, 4-terpineol, and ethyl phthalate in the Melaleuca alternifolia essential oil prepared by freeze drying were relatively high (460.61, 288.99, and 89.62 ng/g, respectively). Although the contents of 4-terpineol, γ-terpinene, and 1,8-cineole were relatively high in essential oil samples treated by direct sun drying, hot air drying, and vacuum drying, the contents of each component were different when treated by different drying methods. For example, 4-terpineol was the component with the highest contents (> 460.00 ng/g) in essential oil samples treated by different drying methods, while the highest content of 1,8-cineole (519.91 ng/g) was obtained with direct sun drying (Table 1).
Table 1. Volatile compositions of essential oil of *M. alternifolia* treated by different drying methods.

| Volatile components         | Freeze drying | Direct sun drying | Hot air drying | Vacuum drying |
|-----------------------------|---------------|-------------------|----------------|---------------|
| **Monoterpene**             |               |                   |                |               |
| α-Phellandrene              | 3.99±0.22     | 7.26±2.28         | 7.01±0.86      | 9.56±1.53     |
| α-Pine              | 41.38±3.5     | 49.76±11.83       | 36.18±2.28     | 37.17±6.28    |
| β-Pinene               | 15.39±1.39    | 16.57±3.89        | 10.9±0.5       | 9.53±2.05     |
| (+)-2-Carene            | 15.9±2.16     | 28.46±9.03        | 28.36±2.2      | 36.46±5.58    |
| o-Cymene               | 2.63±2.29     | 12.43±10.77       | 22.29±3.92     | 21.03±5.11    |
| β-Ocimene              | 47.03±6.48    | 73.32±67.89       | 61.36±53.02    | 5.13±0.56     |
| γ-Terpine              | 47.47±6.78    | 107.24±26.6       | 95.37±7.51     | 155.82±24.95  |
| Terpinolene            | 2.7±0.47      | 7.57±2.72         | 5.73±5.15      | 12.21±1.51    |
| Sum                     | 176.50        | 302.61            | 267.21         | 286.92        |
| **Sesquiterpenes**       |               |                   |                |               |
| α-Neoclovene           | 1.71±1.65     | 4.36±0.58         | 3.78±0.31      | 4.14±0.84     |
| Isocaryophyllene       | 1.78±1.67     | 3.73±1.9          | 4.11±0.67      | 2.98±0.72     |
| Aromandendrene         | 3.26±2.85     | 11.73±1.58        | 13.00±0.69     | 12.03±1.76    |
| Alloaromandendrene     | 1.01±1.75     | 0.43±0.75         | 3.09±2.75      | 3.88±1.26     |
| α-Bulnesene           | 4.14±7.18     | 9.69±2.74         | 4.23±4.24      | 5.14±4.52     |
| δ-Cadinene            | 9.3±2.48      | 16.77±2.25        | 12.93±0.78     | 13.75±2.46    |
| Sum                    | 21.21         | 46.71             | 41.13          | 41.91         |
| **Monoterpene derivatives** |           |                   |                |               |
| 4-Carvomenthenol       | 85.04±18.65   | 260.26±47.36      | 318.09±26.78   | 243.51±36.8   |
| 3-Caranol              | -             | 0.13±0.22         | -              | 1.61±2.79     |
| α-Terpineol            | 44.53±49.07   | 101.41±10.39      | 70.94±11.89    | 20.29±7.97    |
| 1,8-Cineole            | 460.61±69.84  | 519.94±103.14     | 265.1±18.94    | 150.08±28.07  |
| Sum                    | 794.13        | 1202.1            | 907.57         | 641.52        |
| **Sesquiterpenes derivatives** |         |                   |                |               |
| Spathulenol            | 1.58±1.5      | 6.77±0.99         | 7.29±1.22      | 4.65±0.69     |
| Globulol               | 3.57±3.84     | 11.44±1.19        | 10.03±1.15     | 11.66±1.62    |
| β-Eudesmol            | 20.71±5.87    | 13.63±7.92        | -              | 8.23±2.28     |
| α-Eudesmol            | 1.48±2.37     | 6.99±0.93         | 6.93±1.17      | 6.13±0.8      |
| Cubedol                | 3.22±3.00     | 8.06±0.14         | 5.47±1.33      | 6.03±1.55     |
| Sum                    | 30.56         | 46.88             | 29.72          | 36.70         |
| **Other compounds**    |               |                   |                |               |
| Phytol                 | 10.37±12.31   | 70.24±10.58       | 77.36±13.09    | 29.71±10.14   |
| Octacosanol            | 84.5±78.1     | 184.03±35.4       | 112.15±25.65   | 54.06±21.26   |
| Ethyl phthalate       | 89.62±39.6    | 43.28±23.6        | 54.06±21.26    | 54.61±1.55    |
| Sum                    | 184.48        | 297.55            | 243.58         | 152.41        |

Note: "-" means no detected

3.2. Comparison of the components of essential oil treated by different drying methods

Although there was not much difference in the types of components in *Melaleuca alternifolia* essential oil treated by different drying methods, the relative contents of the essential oil components varied with the drying methods employed. The content of essential oil components obtained by direct sun drying was the highest, reaching 1985.85 ng/g, followed by that obtained with hot air drying (1489.22 ng/g), while those obtained by freeze drying and vacuum drying were only 1206.87 ng/g and 1159.46 ng/g, respectively.

The contents of the eight different monoterpenes detected in *Melaleuca alternifolia* essential oil were different among the samples obtained by different drying methods. The drying method had an
influence on the components of *Melaleuca alternifolia* essential oil and their contents. The monoterpene with the highest content was γ-terpinene, with contents of 47.47 ng/g (freeze drying), 107.24 ng/g (direct sun drying), 95.37 ng/g (hot air drying), and 155.82 ng/g (vacuum drying). The next-most abundant monoterpene was α-pinene at 41.38 ng/g (freeze drying), 49.76 ng/g (direct sun drying), 36.18 ng/g (hot air drying), and 37.17 ng/g (vacuum drying). The contents of β-ocimene varied greatly among samples treated with different drying methods, with the highest content of 73.32 ng/g in the essential oil obtained by direct sun drying, and a content of only 5.13 ng/g was obtained by vacuum drying. The sesquiterpenes detected in *Melaleuca alternifolia* essential oil samples treated by different drying methods included six compounds, among which, aromandendrene and d-cadinene had relatively high contents. Although only four monoterpene derivatives were detected in this study, they are important volatile components in *Melaleuca alternifolia* essential oil. Among them, 4-terpineol and 1,8-cineole had the highest contents, and their contribution to *Melaleuca alternifolia* essential oil was relatively high. The contents of 4-terpineol in the essential oil samples obtained by different drying methods were 288.99 ng/g (freeze drying), 580.62 ng/g (direct sun drying), 571.53 ng/g (hot air drying), and 469.54 ng/g (vacuum drying).

Five categories of essential oil components were detected in *Melaleuca alternifolia* essential oil treated by different drying methods, viz. monoterpenoids, sesquiterpenes, monoterpenic derivatives, sesquiterpene derivatives, and others (Table 2). The absolute and relative contents of different compounds in the essential oil samples obtained by the different drying methods were different. Monoterpenic derivatives are the most important volatile components in *Melaleuca alternifolia* essential oil, with the highest absolute and relative contents. The highest relative content of monoterpenic derivatives in the *Melaleuca alternifolia* essential oil was obtained by direct sun drying (1202.09 ng/g), followed by that obtained by hot air drying (907.58 ng/g). Monoterpenic derivatives had the highest relative contents in the *Melaleuca alternifolia* essential oil obtained by freeze drying and direct sun drying, at 65.80% and 63.41%, respectively. The absolute and relative contents of monoterpenic compounds varied with different drying methods. The lowest absolute content was 176.50 ng/g (obtained by freeze drying, relative content 14.62%), and the relatively high content was 302.61 ng/g (obtained by direct sun drying, relative content 15.96%). The absolute and relative contents of sesquiterpenes and their derivatives were relatively low in essential oil obtained by different drying methods: the absolute contents were all lower than 50 ng/g, and the relative contents were all lower than 4%.

| Table 2. The contents of different volatile profiles of essential oil by different drying methods. |
|---------------------------------------------------------------|
|                  | Freeze drying | Direct sun drying | Hot air drying | Vacuum drying |
|                  | Absolute content/ ng·g⁻¹ | Relative content/ % | Absolute content/ ng·g⁻¹ | Relative content/ % | Absolute content/ ng·g⁻¹ | Relative content/ % |
| Monoterpenes     | 176.50 | 14.62 | 302.61 | 15.96 | 267.21 | 17.94 | 286.92 | 24.75 |
| Sesquiterpenes   | 21.21 | 1.76 | 46.71 | 2.46 | 41.13 | 2.76 | 41.91 | 3.61 |
| Monoterpene derivatives | 794.13 | 65.80 | 1202.09 | 63.41 | 907.58 | 60.94 | 641.52 | 55.33 |
| Sesquiterpene derivatives | 30.56 | 2.53 | 46.88 | 2.47 | 29.72 | 2.00 | 36.70 | 3.17 |
| Others           | 184.48 | 15.29 | 297.55 | 15.69 | 243.58 | 16.36 | 152.41 | 13.15 |
| Total            | 1206.87 | 1895.85 | 1489.22 | 1159.46 |
4. Discussion
Melaleuca alternifolia essential oil has been widely used in the fields of pharmaceuticals, cosmetics, and daily necessities by virtue of its excellent broad-spectrum antimicrobial and antibacterial abilities, strong permeability, and biodegradability [19]. In this study, the MTBE method was used to compare the differences in the contents of Melaleuca alternifolia essential oil components obtained by different drying methods. GC-MS analysis yielded 26 volatile components in five major categories of monoterpenes, sesquiterpenes, monoterpe derivatives, sesquiterpene derivatives, and others; among these, the number of monoterpe compounds was the highest, with a total of 8 detected, followed by sesquiterpene compounds and sesquiterpene derivatives, with 6 and 5 compounds detected, respectively. Terpinen-4-ol and 1,8-cineole are two important components that affect the quality of Melaleuca alternifolia essential oil [2, 20]. [21]Homer et al. found that the 615 Melaleuca alternifolia trees in New South Wales, Australia, can be grouped into 6 different ecotypes based on their main volatile essential oil components, which are mainly terpinen-4-ol, 1,8-eucalyptol, and γ-terpinen. [4] Zhong et al. found that the relative content of 1,8-cineole in tea tree oil obtained from Melaleuca alternifolia introduced from Australia to Yulin, Guangxi was as high as 72.49%. The main components of Melaleuca alternifolia essential oil studied herein were consistent with those obtained in previous studies. Among the 26 volatile components detected in this study, the contents of 4-terpineol, 1,8-eucalyptol, and γ-terpinene were relatively high, with those of 4-terpineol and 1,8-cineole being the highest (Table 1).

The extraction rates of plant essential oils and the types and contents of their components are affected by a series of factors, such as plant cultivation seasons, cultivation conditions, harvesting time, and extraction methods. [22] Zhang and Yuan reported that the leaf position of Melaleuca alternifolia had a relatively significant influence on γ-terpinene and terpinene-4-ol contents: the contents of these two compounds were higher in the essential oil components of lower leaves than those of upper leaves. [15] Qiao and Li compared the difference in the contents of volatile components in the leaves of Melaleuca alternifolia at different maturity levels, and reported that the contents of volatile components were higher in the essential oil from old leaves, and the content of terpinen-4-ol was the highest among those of the volatile components of the essential oil from different types of Melaleuca alternifolia plants. [23] Mo found that the soil water content was negatively correlated with the contents of terpineol and 1,8-cineole in essential oil components of Melaleuca alternifolia leaves. Different drying methods also affect the types and contents of plant essential oil components [24]. Microwave drying of plant materials involves a relatively high internal temperature, which easily damages plant cells, facilitating the release of volatile components [25]; on the other hand, infrared-ray drying will cause oxidation and rearrangement of chemical structures, producing new volatile compounds [26]. Herein we also found that different drying methods will affect the components of Melaleuca alternifolia essential oil and their contents. Herein we found that although the types of Melaleuca alternifolia essential oil components obtained by different drying methods were similar (Table 1), their contents were quite different, especially for terpinen-4-ol, 1,8-cineole, and other components.

5. Conclusion
In this study, a total of 26 volatile components were detected in the essential oil of Melaleuca alternifolia, obtained by different drying methods, among which, the number of different monoterpenes was the highest. By comparing the volatile components of Melaleuca alternifolia essential oil obtained by different drying methods, it is found that although similar types of essential oil components were obtained with different drying methods, the contents of essential oil components obtained by direct sun drying was the highest (1985.85 ng/g), and the contents of terpineol-4-ol or 1,8-cineole were the highest in essential oil obtained from different drying methods of Melaleuca alternifolia, which contribute the most to Melaleuca alternifolia essential oil. This study has shown that drying methods affect the types and components of essential oil from Melaleuca alternifolia.
Basic Scientific Research

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