Effects of Different Drying Methods on the Contents of Nine Components and Immunomodulatory Activities of Four Components in *Osmamthus fragrans* Flowers

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Abstract

The effects of drying methods on the contents of four nonvolatile and five volatile components and the immunoregulatory activities of four components in *Osmamthus fragrans* flowers were investigated. In general, microwaving preserved more nonvolatile components than the other methods, while the sun or shade method preserved more volatile components. Nonvolatile components such as salidroside and acteoside and volatile ingredients such as linalool and linalool oxide exhibited better immunoregulatory activity than the other ingredients. Taken together, *O. fragrans* flowers dried by microwaving resulted in the best immunoregulatory activity. This study provides evidence for the optimal drying method for *O. fragrans* flowers as food and medicine.

Keywords

bioactivity, *Osmamthus fragrans*, drying methods, chemical components, immunomodulatory activity

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*Osmamthus fragrans* (Thunb.) Lour., family Oleaceae,¹ is not only a famous aromatic and ornamental plant, but also an excellent edible and medicinal species.² Its flowers can be made into scented tea, wine, and cake.³ In addition, these can be used to treat stomach, liver, and kidney diseases.⁴

The utilization of *O. fragrans* flowers in the treatment of diseases is based on their bioactive constituents, which consist of nonvolatile and volatile components. The nonvolatile compounds mainly include phenylpropanoids, flavonoids, terpenoids, and lignans.⁶ In *O. fragrans* flowers, the phenylpropanoids mainly exist in the form of either β-hydroxyphenylethanol esters or glycosides. Eleven phenylpropanoids, such as acteoside, salidroside, and isoacteoside, have been identified in the aqueous extract of *O. fragrans* flowers.⁷ Phenylpropanoids have antioxidant, anti-melanogenesis, anti-hypoxia, anti-inflammatory, anti-aging, and other biological activities.⁸⁻¹² Six flavonoids were isolated from the ethanol extract of *O. fragrans* flowers, namely, quercetin, rutin, genistin, kaempferol, isorhamnetin, and naringin, which have different anti-inflammatory, DPPH-free radical scavenging, and anti-proliferation activities.¹³ A secoiridoid glycoside (8E-ligstroside) and a coumaric acid analog with a monoterpene moiety (floraosmanol A) inhibit the activity of beta-secretase and nitric oxide (NO) production in lipopolysaccharide-activated RAW264.7 macrophages, respectively.¹⁴¹⁵ Lee and collaborators obtained seven lignans from *O. fragrans* flowers, of which phillygenin has a great effect on protecting liver and preventing NO production.¹⁶¹⁷ In addition, nine phenylphenols with antioxidant properties and 8 monosaccharides were isolated and identified in ethanol extracts of *O. fragrans* flowers.¹⁸¹⁹ The volatile components typically include terpenes, aldehydes, esters, ketones, and alcohols.²⁰ Forty-one volatile organic components were identified from 29 cultivars of *O. fragrans* groups, and the major volatile compounds were alpha-ionone, beta-ionone, linalool, linalool oxide, and geraniol.²¹ A total of 84 volatiles were detected by GC-MS analysis, and among these, terpenoids, ketones, and esters were predominant in the SPME extracts, while aromatics and alcohols were abundant in SDE extracts.²² Eugenol and geraniol were screened as the main active ingredients with potential neuroprotective and anti-tumor effects.²³

Drying is necessary as a means of removing moisture in fresh products to ensure their enzymatic and microbiological...
Different drying methods such as hot air-drying, microwave drying, infrared drying, freeze-drying, and vacuum oven drying have their own unique characteristics and different effects on the chemical components and bioactivities of fresh products. Microwave drying and halogen lamp-microwave drying performed significantly better than traditional hot-air drying in enhancing the quality of black tea, especially the volatile and taste compounds. Freeze-drying results in better physicochemical properties and antioxidant activities of mulberry leaf polysaccharides in comparison with results in better physicochemical properties and antioxidant composition, antioxidant capacity, and sensory quality. The best dried jujube fruits and bitter gourd slice chemical properties are obtained with microwave drying and halogen lamp-microwave drying.

Materials and Methods

Chemicals and Reagents

Acetonitrile and methanol (HPLC-grade) were purchased from Merck (Darmstadt, Germany). Standard products such as salidroside, acteoside, oleanolic acid, and ursolic acid were obtained from the National Institutes for Food and Drug Control (Beijing, China). L(−)-2-Octanol (as internal standard), linalool, linalool oxide, geraniol, α-ionone, and β-ionone were purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). Light petroleum, boiling range of 80 to 120 °C, was purchased from J&K Chemical, Ltd. (Beijing, China). Water of HPLC grade was purified by a reverse osmosis system (Millipore, Ireland). All the reagents of HPLC grade were filtered across a 0.45 μm membrane before the experiment. Dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-dipheny tetrazolium bromide (MTT), trypan, concanavalin A (Con A), penicillin G, and streptomycin sulfate were obtained from Sigma Chemical Co. (St. Louis, MO, USA), and Gibco 1640 medium and fetal bovine serum (FBS) from Gibco Invitrogen Corp. (San Diego, CA, USA). Other reagents and chemicals were of analytical grade.

Plant Material

Fresh O. fragrans flowers were harvested from 3 different O. fragrans groups in our campus (32°33’ N and 117°1’ E), which showed flower colors of almost orange, silver, and gold. These were identified by Dr Li Feng from Shandong University of Traditional Chinese Medicine separately as O. fragrans var. aurantiacus (Batch number: 20191006-1), O. fragrans var. latifolius (Batch number: 20191006-2), and O. fragrans var. thunbergii (Batch number: 20191006-3). For convenience, these three groups will hereafter be called “Aurantiacus,” “Latifolius,” and “Thunbergii.” After drying, the flowers were stored in a glass dryer filled with desiccant silica gel. They were analyzed within a month after drying.

Drying Methods

Shade drying (SHD), sun drying (SUD), quick-line drying (QLD), oven drying (OD), and microwave drying (MD) of fresh flowers from three O. fragrans groups were utilized. SHD and SUD were separately conducted either indoors or in the sun. QLD was performed by placing fresh flowers in the upper glass dryer, while the lower layer was filled with quick lime. OD was operated separately at 40 °C, 50 °C, 60 °C, and 70 °C in a drying oven (DHG-9070B, Shanghai Pei for Experimental Instrument Co., Shanghai, China). MD was performed separately at medium-low power (MLP), medium power (MP), medium-high power (MHP), and high power (HP) in a microwave oven (M1-L213B, Midea Group Co., Foshan, China). All dried O. fragrans flowers were dried until constant weight.

Sample Extraction and Preparation

The dried flowers from three O. fragrans groups were extracted and prepared for HPLC analysis to identify four kinds of non-volatile components. Briefly, 0.1 g of the dried O. fragrans flowers was measured precisely and placed into a 50 ml conical flask with a stopper. Then, 10 ml of methanol was added to the flask, and extraction was performed for 30 minutes under 100 w power and 40 kHz frequency at room temperature using an ultrasonic cleaner (KQ-100 DE, Kunshan Ultrasound Instrument Co., Suzhou, China). The lost weight was compensated by methanol after being cooled to room temperature, and the extracts were passed through a double-layer filter paper. The continuous filtrates above were collected and stored at 4 °C for the subsequent content determination of the four kinds of non-volatile components.

The dried flowers from three O. fragrans groups were extracted and prepared for GC analysis to identify five kinds of volatile components. Briefly, 10 g of the dried O. fragrans flowers was measured precisely and placed into a 500 ml round-bottom flask. Then, 300 ml of deionized water was added to...
the flask. Using a Clevenger apparatus, extraction was performed for 4 hours. Then, 2 ml of L (−)-2-octanol light petroleum solutions with a boiling range of 80 to 120 °C was added to the distilled extracts. The above liquids were thoroughly mixed, and the supernatants were separated and stored at 4 °C for subsequent identification of volatile components. All experiments were performed in triplicate.

**HPLC**

Four kinds of non-volatile components were analyzed by HPLC using an E2695 LC instrument (Waters Instruments Co., MA, USA) with an online degasser, a quaternary pump, a 2489 ultraviolet-visible detector (UVD), and an autosampler. Approximately 10 µL of the HPLC samples was analyzed with a Symmetry C-18 analytical column (250 mm × 4.6 mm, 5 µm, Waters Corp., USA) using a linear gradient with water-phosphoric acid (100:0.05, v/v) as solvent A and acetonitrile as solvent B. The gradient program was as follows: 10% B in 0-8 minutes, 10% to 30% B in 8-22 minutes, 30% to 85% B in 23-36 minutes, and 85% to 100% B in 37-66 minutes. The velocity of flow was 1.0 mL min⁻¹, and the column temperature was kept at 30 °C. Detection was performed at a wavelength of 220 nm in 0-25 minutes and 210 nm in 26-66 minutes.

**Gas Chromatography (GC)**

Five kinds of volatile components of the GC samples were analyzed using a GC-4000A GC instrument (East & West Analytical Instruments Co., Beijing, China) with a nitrogen canister, a flame ionization detector (FID), a hydrogen generator, an air pump, and a 1 µL injection needle. Approximately 1 µL of sample was injected into the GC instrument fitted with an AE.SE-54 nonpolar analytical column (30 m × 0.32 mm, Lanzhou Atech Technologies Co., Lanzhou, China) and with high purity nitrogen gas as carrier using a temperature gradient program. The gradient program was as follows: 50 °C for 2 minutes, 50 to 160 °C at the rate of 2 °C min⁻¹, 160 to 250°C at the rate of 22.5 °C min⁻¹, and 250 °C for 12 minutes. The temperature of the gasification chamber and flame ionization detector was set at 250 °C. The pressure of nitrogen, air, and hydrogen were 0.4, 0.2, and 0.09 MPa, respectively.

**Assessment of Cell Viability**

The cytotoxicity of the relevant chemical components of *O. fragrans* flowers, using various concentrations, was evaluated using an MTT test, with some modifications. Approximately 1 × 10⁵ RAW 264.7 cells were seeded in triplicates into 96-well plates and cultured for 3 hours to allow cell attachment, then cultured with salidroside, acteoside, linalool oxide, linalool, geraniol, α-ionone, and β-ionone at 3.125, 6.25, 12.5, 25, 50, and 100 µg·mL⁻¹ for 24 hours. Subsequently, 20 µL of MTT solution (5 mg·mL⁻¹) was added to each well, and the cells were incubated for 4 hours at 37 °C. The supernatants were discarded, and 150 µL of DMSO was added to each well and homogenized. Absorbance values were measured at a wavelength of 570 nm using an Epoch2 microplate spectrophotometer (BioTek Instruments Co., USA). Cell viability was calculated using the following equation:

\[
\text{Cell viability} (\%) = \frac{A_2}{A_1} \times 100
\]

where \(A_1\) is the absorbance value of the blank control group, and \(A_2\) is the absorbance value of the treatment group.

**Phagocytosis Assay**

The phagocytic activities of RAW 264.7 cells treated with various concentrations of relevant chemical components of *O. fragrans* flowers were examined by neutral red uptake assay. RAW 264.7 cells were seeded at a density of 2.7 × 10⁶ cells per well in 96-well microplates. After incubation for 3 hours, the supernatant was discarded. RAW 264.7 cells were pretreated with salidroside, acteoside, linalool oxide, linalool, and geraniol at different concentrations (3.125, 6.25, 12.5, 25, 50, and 100 µg/mL) in either the presence or absence of LPS (1.0 µg·mL⁻¹) for 24 hours. Then, 100 µL of neutral red solution (0.1%, w/v) was added to each well and incubated for another 30 minutes at room temperature. The supernatants were discarded, the cells washed with phosphate-buffered saline (PBS, 0.01 M, pH 7.4), thrice, and then the cells were lysed for 30 minutes at room temperature by adding 150 µL of cell lysis buffer (1:1 ethanol:acetic acid) and homogenized. The absorbance values were measured at a wavelength of 540 nm using an Epoch2 microplate spectrophotometer and the following equation:

\[
\text{Neutral red phagocytosis rate} (\%) = \frac{B_2}{B_1} \times 100
\]

where \(B_1\) is the absorbance value of the blank control group, and \(B_2\) is the absorbance value of the treatment group.

**Statistical Analysis**

All data were reported as the mean ± standard deviation of 3 samples. Statistical analysis was performed with SPSS 19.0. Significance differences were tested using ANOVA at a significant level (\(P < 0.05\)).

**Results**

Yields and drying time of dried *O. fragrans* flowers with different drying methods.

To avoid moisture interference with the chemical composition content, the yields of dried *O. fragrans* flowers with different drying methods were calculated and compared (Table 1). The yields were calculated using the following equation:

\[
\text{Yield} (\%) = \frac{G_2}{G_1} \times 100
\]
where $C_1$ is the weight of 100 g fresh flowers, and $C_2$ is the weight of 100 g fresh flowers after drying.

The different drying methods had no significant effect on dried flower yield ($P > 0.05$). Also, drying via MD and OD utilized less than 15 minutes and 6 hours, respectively, while SHD, SUD, and QLD spent no less than 3 days. The advantages and disadvantages of these drying methods have also been previously reported.\(^{41}\)

### Effect of Different Drying Methods on Nonvolatile Components of O. Fragrans Flowers

The drying process may be accompanied by changes in chemical composition. The peaks of salidroside, acetoside oleanolic acid, and ursolic acid could be well separated from adjacent peaks and, therefore, the analytical conditions could be used to identify the four components.

The amount of ursolic acid in *Aurantiacus*, *Latifolius*, and *Thunbergii* flowers was apparently below the detection level of *Thunbergii* flowers. Table 3 shows that the highest content of ursolic acid was about 1.78% (g/100 g) in *Latifolius* flowers dried by SUD. In addition, oleanolic acid also existed in the *Latifolius* flowers dried by MD, and their contents initially increased and then decreased with increasing power.

The nonvolatile components in *O. fragrans* flowers are presented in Tables 2–4. Specifically, the highest contents of acetoside were 2.31%, 1.90%, and 2.24% (g/100 g) in *Aurantiacus*, *Latifolius*, and *Thunbergii* flowers, respectively. Similarly, the highest contents of salidroside were 0.87%, 0.19%, and 0.24% (g/100 g) in *Aurantiacus*, *Latifolius*, and *Thunbergii* flowers, respectively. The highest contents of oleanolic acid, acetoside, and salidroside all appeared in *O. fragrans* flowers dried with the MD method from the three *O. fragrans* groups. Taken together, the contents of these three nonvolatile components could be increased by either increasing the temperature (OD) or power (MD). In summary, high temperature such as in OD (60 to 70 °C) and local high temperatures such as MD (MP-HP) are beneficial to the preservation of nonvolatile components.

### Effect of Drying Methods on the Volatile Components of O. Fragrans Flowers

Linalool oxide, linalool, geraniol, α-ionone, β-ionone, and δ-decanolide were the most extensively depleted components after natural drying of *O. fragrans* flowers.\(^{36}\) Therefore, these were selected as the typical constituents for evaluating the effects of different drying methods on the volatile components. GC was used to determine the contents of these compounds, the peaks of which could be separated from the adjacent ones, and utilized in their assay.

There are several findings on the volatile components of *O. fragrans* flowers (Tables 2–4). Specifically, the highest contents of linalool oxide and linalool were 27.93% and 16.64% (mg/100 g) in *Aurantiacus* flowers, respectively, and 57.77% and 31.87% (mg/100 g) in *Thunbergii* flowers, respectively. The highest values were observed in *Aurantiacus* and *Thunbergii* flowers dried using the OD (40 °C) method. The highest content of linalool oxide was 37.31% (mg/100 g) in *Latifolius* flowers subjected to the QLD method, while the highest content of linalool was 10.36% (mg/100 g) in *Latifolius* flowers that underwent the SHD method. The highest contents of geraniol were 17.35%, 29.55%, and 89.93% (mg/100 g) in *Aurantiacus* flowers dried using the OD (40 °C) method and in *Latifolius* and *Thunbergii* flowers with the SHD method, respectively. The highest contents of α-ionone and β-ionone were 94.75% and 2.05% (mg/100 g) in *Aurantiacus* flowers dried using the OD (70 °C) method. The highest content of δ-decanolide was 57.77% (mg/100 g) in *Aurantiacus* flowers.
Table 2. Effects of Drying Methods on the Nonvolatile and Volatile Components of *Auranticacus* Flowers.

| Drying method | Nonvolatile components | Volatile components |
|---------------|------------------------|---------------------|
|               | Salidroside (g/100 g)  | Acteoside (g/100 g) | Oleanolic acid (g/100 g) | Ursolic acid (g/100 g) | Linalool oxide (μg/100 g) | Linalool (μg/100 g) | Geraniol (μg/100 g) | α-Ionone (μg/100 g) | β-Ionone (μg/100 g) |
| OD (40 °C)    | 0.14 ± 0.04            | 0.64 ± 0.03         | —                        | —                       | 27.93 ± 1.24               | 16.64 ± 0.84       | 17.35 ± 1.24       | 11.38 ± 1.02       | 0.51 ± 0.04       |
| OD (50 °C)    | 0.61 ± 0.05            | 0.72 ± 0.04         | —                        | —                       | 18.05 ± 1.98               | 5.22 ± 0.45        | 8.10 ± 0.46        | 32.31 ± 1.35       | 1.15 ± 0.11       |
| OD (60 °C)    | 0.71 ± 0.03            | 0.94 ± 0.06         | —                        | —                       | 10.91 ± 0.95               | 2.78 ± 0.23        | 4.78 ± 0.35        | 25.72 ± 1.54       | 0.84 ± 0.06       |
| OD (70 °C)    | 0.81 ± 0.05            | 1.10 ± 0.06         | —                        | —                       | 9.16 ± 0.86                | 1.35 ± 0.15        | 2.16 ± 0.34        | 17.65 ± 1.41       | 0.56 ± 0.04       |
| MD (MLP)      | 0.68 ± 0.06            | 0.89 ± 0.05         | —                        | —                       | 10.37 ± 0.76               | 3.12 ± 0.26        | 4.07 ± 0.37        | 30.97 ± 2.31       | 0.81 ± 0.06       |
| MD (MP)       | 0.87 ± 0.06            | 2.01 ± 0.07         | —                        | —                       | 13.80 ± 0.87               | 7.41 ± 0.54        | 15.21 ± 0.81       | 32.31 ± 2.33       | 1.05 ± 0.08       |
| MD (MHP)      | 0.77 ± 0.06            | 2.13 ± 0.05         | —                        | —                       | 12.59 ± 0.94               | 11.68 ± 0.75       | 5.27 ± 0.40        | 51.14 ± 2.47       | 1.31 ± 0.11       |
| MD (HP)       | 0.72 ± 0.04            | 2.31 ± 0.12         | —                        | —                       | 24.58 ± 1.35               | 10.48 ± 0.74       | 3.76 ± 0.23        | 25.03 ± 1.32       | 0.71 ± 0.05       |
| SHD           | 0.31 ± 0.04            | 1.09 ± 0.03         | —                        | —                       | 13.57 ± 0.85               | 7.45 ± 0.65        | 11.51 ± 0.86       | 94.75 ± 2.42       | 2.05 ± 0.14       |
| SUD           | 0.39 ± 0.03            | 1.51 ± 0.04         | —                        | —                       | 14.66 ± 0.82               | 5.49 ± 0.41        | 4.47 ± 0.31        | 39.98 ± 2.11       | 1.01 ± 0.08       |

Abbreviations: —, means that the component cannot be detected; HP, high power; MD, microwave drying; MHP, medium-high power; MLP, medium-low power; MP, medium power; OD, oven drying; QLD, quick-lime drying; SHD, Shade drying; SUD, sun drying. Data are presented as mean ± standard deviations (n = 3).

Table 3. Effects of Drying Methods on the Nonvolatile and Volatile Components of *Latifolius* Flowers.

| Drying method | Nonvolatile components | Volatile components |
|---------------|------------------------|---------------------|
|               | Salidroside (g/100 g)  | Acteoside (g/100 g) | Oleanolic acid (g/100 g) | Ursolic acid (g/100 g) | Linalool oxide (μg/100 g) | Linalool (μg/100 g) | Geraniol (μg/100 g) | α-Ionone (μg/100 g) | β-Ionone (μg/100 g) |
| OD (40 °C)    | 0.32 ± 0.03            | 1.30 ± 0.03         | —                        | —                       | 7.07 ± 0.54                | 3.61 ± 0.24        | 15.74 ± 0.87       | 29.22 ± 0.67       | 0.96 ± 0.11       |
| OD (50 °C)    | 0.10 ± 0.02            | 0.90 ± 0.04         | —                        | —                       | 9.35 ± 0.67                | 3.31 ± 0.27        | 7.99 ± 0.63        | 20.13 ± 0.81       | 0.50 ± 0.06       |
| OD (60 °C)    | 0.17 ± 0.04            | 1.62 ± 0.05         | —                        | —                       | 27.10 ± 1.21               | 8.18 ± 0.51        | 16.33 ± 0.55       | 17.59 ± 0.83       | 0.52 ± 0.04       |
| OD (70 °C)    | 0.17 ± 0.03            | 0.96 ± 0.04         | —                        | —                       | 32.64 ± 1.36               | 8.67 ± 0.43        | 15.48 ± 0.46       | 29.92 ± 1.31       | 0.65 ± 0.02       |
| MD (MLP)      | 0.16 ± 0.04            | 1.10 ± 0.04         | 0.65 ± 0.02              | —                       | 21.09 ± 1.24               | 7.01 ± 0.56        | 12.09 ± 0.33       | 23.85 ± 1.04       | 0.71 ± 0.04       |
| MD (MP)       | 0.16 ± 0.03            | 1.55 ± 0.05         | 0.78 ± 0.03              | —                       | 16.05 ± 0.98               | 6.62 ± 0.40        | 9.74 ± 0.37        | 16.95 ± 0.91       | 0.28 ± 0.02       |
| MD (MHP)      | 0.19 ± 0.04            | 1.47 ± 0.06         | 1.32 ± 0.04              | —                       | 13.34 ± 0.86               | 9.30 ± 0.57        | 13.78 ± 0.41       | 49.93 ± 1.58       | 1.11 ± 0.21       |
| MD (HP)       | 0.19 ± 0.06            | 1.90 ± 0.04         | 1.06 ± 0.05              | —                       | 13.11 ± 0.81               | 5.19 ± 0.42        | 10.32 ± 0.54       | 12.92 ± 0.64       | 0.38 ± 0.04       |
| SHD           | 0.02 ± 0.004           | 1.06 ± 0.03         | 0.37 ± 0.01              | —                       | 20.93 ± 1.03               | 10.36 ± 0.84       | 25.55 ± 1.21       | 35.38 ± 1.31       | 0.55 ± 0.03       |
| SUD           | 0.14 ± 0.03            | 1.11 ± 0.07         | 1.78 ± 0.07              | —                       | 27.35 ± 1.14               | 8.43 ± 0.53        | 15.13 ± 0.66       | 72.63 ± 1.65       | 1.51 ± 0.14       |
| QLD           | 0.07 ± 0.006           | 1.89 ± 0.08         | —                        | —                       | 37.31 ± 1.64               | 5.83 ± 0.54        | 17.13 ± 0.53       | 5.94 ± 0.36        | 0.25 ± 0.03       |

Abbreviations: —, means that the component cannot be detected; HP, high power; MD, microwave drying; MHP, medium-high power; MLP, medium-low power; MP, medium power; OD, oven drying; QLD, quick-lime drying; SHD, Shade drying; SUD, sun drying. Data are presented as mean ± standard deviations (n = 3).
speaking, the contents of linalool oxide, linalool, and geraniol were high in the O. fragrans groups. In addition, the contents of α-ionone were high in Aurantiacus and Latifolius flowers, while that of β-ionone was high in Thunbergii flowers. The high temperature used in OD (50 to 70 °C) and local high temperature such as MD were not beneficial to the preservation of volatile components. In contrast, low temperature such as in OD (40 °C), SHD, SUD, and QLD were beneficial in this respect.

**Effect of Chemical Components of O. Fragrans Flowers on Cell Viability of RAW 264.7 Cells**

The MTT test was used to determine the number of viable cells and cell viability by assessing the activity of mitochondrial succinate dehydrogenase of live cells. The MTT colorimetric method has a very wide range of applications in cell proliferation and differentiation and is related to immunological experiments in cell metabolism. Figure 1 shows that the survival rates of RAW 264.7 cells were basically above 90% after the disposition of salidroside, acteoside, linalool oxide, and linalool within the concentration range of 3.125–50 µg·mL⁻¹. However, the survival rates of the cells decreased to less than 50% after the disposition of geraniol, α-ionone, and β-ionone in the concentration range of 50–100 µg·mL⁻¹. As these three compounds showed significant cytotoxic effects, they were not selected for the next phagocytosis assay.

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**Effect of relevant chemical components of O. fragrans flowers on the phagocytic activity of RAW 264.7 cells**

The phagocytosis of macrophages is the first step in an immune response, and increasing phagocytic activity is characteristic of activated macrophages. Thus, the phagocytic activities of RAW 264.7 cells treated with various concentrations of relevant chemical components were investigated by a neutral red uptake assay. When the tissues and organs undergo pathological changes, macrophages can eliminate antigens through intracellular cytotoxicity. Phagocytosis is a key indicator of evaluating macrophage activities, and activated macrophages help initiate specific defense mechanisms by recruiting other immune cells and play a critical role in the immune system.

**Table 4. Effects of Drying Methods on the Nonvolatile and Volatile Components of Thunbergii Flowers.**

| Drying method | Nonvolatile components | Volatile components  |
|---------------|------------------------|----------------------|
|               | Salidroside (g/100 g)  | Acteoside (g/100 g)  |
|               | Oleanolic acid (g/100 g) | Ursolic acid (g/100 g) |
|               | Linalool oxide (μg/100 g) | Linalool (μg/100 g)  |
|               | Geraniol (μg/100 g)    | α-Ionone (μg/100 g)  |
|               | β-Ionone (μg/100 g)    |                     |
| OD (40 °C)    | 0.15 ± 0.04            | 0.19 ± 0.04          |
| OD (50 °C)    | 0.17 ± 0.05            | 0.17 ± 0.05          |
| OD (60 °C)    | 0.19 ± 0.06            | 0.24 ± 0.02          |
| OD (70 °C)    | 0.20 ± 0.06            | 0.22 ± 0.03          |
| MD (MLP)      | 0.07 ± 0.03            | 0.08 ± 0.03          |
| MD (MP)       | 0.09 ± 0.03            | 0.10 ± 0.04          |
| MD (MHP)      | 0.10 ± 0.04            | 0.22 ± 0.02          |
| MD (HP)       | 0.05 ± 0.03            | 0.37 ± 0.04          |
| SHD           | 0.06 ± 0.06            | 0.42 ± 0.03          |
| SUD           | 0.08 ± 0.06            | 0.42 ± 0.03          |
| QLD           | 0.08 ± 0.06            | 0.42 ± 0.03          |

Abbreviations: — means that the component cannot be detected; HP, high power; MD, microwave drying; MHP, medium-high power; MP, medium power; OD, oven drying; QLD, quick-lime drying; SHD, Shade drying; SUD, sun drying.

Data are presented as mean ± standard deviations (n = 3).
could increase the phagocytosis of RAW 264.7 cells compared with the control cells (Figure 2A). Linalool oxide could also increase the phagocytosis at 3.125-6.25 µg·mL⁻¹, but not at 12.5-100 µg·mL⁻¹. Linalool did not increase the rate of phagocytosis. Therefore, salidroside and acteoside had more abilities of enhancing the phagocytic activity of RAW 264.7 cells than linalool oxide and linalool.

Phagocytosis of pathogens is an important function of macrophages, which participate not only in activating the adaptive immune system, but also in immune regulation. The phagocytic rate was significantly suppressed (P < 0.05) when the cells were exposed to LPS after pretreatment with salidroside, acteoside, linalool oxide, and linalool at 3.125-100 µg·mL⁻¹ (Figure 2B). The phagocytic rates (P < 0.05) in LPS-stimulated RAW 264.7 cells through treating with salidroside, acteoside, linalool oxide, and linalool at 3.125-100 µg·mL⁻¹ were lower than those (155.1 ± 5.6%) of LPS-stimulated RAW 264.7 cells. However, linalool oxide and linalool had obvious advantages over salidroside and acteoside in reducing the phagocytic activity of LPS-stimulated RAW 264.7 cells.

Effects of the Drying Method for O. Fragraeus Flowers on Cell Viability and Phagocytic Activity

Based on the earlier results, geraniol, α-ionone, and β-ionone induced poor cell proliferation and viability. Salidroside and acteoside possessed notable effects on enhancing the phagocytic activity of natural RAW 264.7 cells. Salidroside, acteoside, linalool oxide, and linalool all had remarkable advantages in reducing the phagocytic activity of LPS-stimulated RAW 264.7 cells. Therefore, salidroside and acteoside exhibited two-way immunoregulation of phagocytic cells. However, the contents of linalool oxide and linalool, only in the order of mg, were lower than salidroside and acteoside, in the order of g per 100 g of fresh O. fragrans flowers. Therefore, salidroside and acteoside played the main part in regulating the activities of phagocytic cells in the immune system. The total content of salidroside and acteoside in “Aurantiacus,” “Latifolius,” and “Thunbergii” was the highest with the MD method in HF. Therefore, the rapid drying method represented by microwave drying maintained the medicinal properties of O. fragrans flowers, and thus should be advocated. In contrast, the unique fragrance of O. fragrans flowers, which is important in the food industry, is retained by traditional drying methods such as shade drying and lime drying.

Discussion

Calculating dried flower yields is employed in determining water content and other chemical components in bitter gourd slices of different groups and subjected to various drying methods. MD had the highest drying efficiency among the 5 studied drying methods. The local high temperature that is involved in MD had the more rapid and greater function of inactivating a large amount of enzymes and preserving glycosides than OD. SHD, SUD, QLD, and OD at low temperature and MD at low power retained enzyme activity and caused the hydrolysis of salidroside. Thus, enzyme content and activity play an important role in the nonvolatile components of fresh O. fragrans flowers. In addition, most of the volatile components of O. fragrans flowers have a low boiling point. Therefore, temperature in the drying process of O. fragrans flowers is the dominant factor affecting the content of volatile components, which has been confirmed in the present research. The contents of volatile components are also influenced by product characteristics such as physical structure (density and porosity), plant species, and their localization in the plant. Drying methods also play an important role in the preservation of nonvolatile components.

Immunomodulation is a process of adapting the immune system to obtain the optimal response via either stimulation or suppression of the immune reaction. Immunotherapy has already been applied to cancer therapy. O. fragrans flowers, as a natural medicine and food, should be comprehensively investigated. RAW 264.7 macrophages are often used to evaluate the immunomodulatory activity of chemical substances. The cytotoxic effects of salidroside, acteoside, linalool oxide, linalool, geraniol, α-ionone, and β-ionone were determined using the MTT assay to ensure the safety of these ingredients. Phagocytosis of RAW 264.7 macrophages was determined using the neutral red phagocytosis experiment to assess their immunomodulatory activities.

Conclusions

O. fragrans flowers from three O. fragrans groups, namely, “Aurantiacus,” “Latifolius,” and “Thunbergii” were dried using 5 different methods. The non-volatile and volatile components such as salidroside, acteoside, linalool oxide, and linalool were determined.
The different characteristics appeared in the effects of drying methods on the non-volatile and volatile components of various *O. fragrans* flowers. The effects of the former quantitative components on the cell viability and phagocytic activity of normal and LPS-stimulated RAW 264.7 cells were investigated. Four components, salidroside, acteoside, linalool oxide, and linalool, all showed good immunoregulatory effects. Considering their different contents, the best drying method was dependent on the total salidroside and acteoside content. Microwave drying and oven drying at high temperature were beneficial for the preservation of the bioactive nonvolatile components for enzymes inhibition and protection of glycosides. Other drying methods, such as shade drying, sun-drying, quick-lime drying, and oven-drying at low temperature were beneficial for the preservation of fragrant volatile components. This research provides evidence for the selection of the appropriate drying method for *O. fragrans* flowers as food and medicine.

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