Optimization of Microwave-assisted Extraction of Essential Oil from Lavender Using Response Surface Methodology

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Abstract: A microwave-assisted hydrodistillation (MAHD) method was investigated for extraction of essential oils from lavender. The essential oil extracts at optimized MAHD conditions was compared with hydrodistillation (HD). Response surface methodology coupled with Box-Behnken design was applied to optimize the parameters for MAHD. The optimized MAHD conditions were 500 W microwave power, 17 mL/g liquid-to-solid ratio and 40 min microwave time. The ANOVA results revealed that microwave time had the greatest impact on the essential oil yield followed by liquid-to-solid ratio and microwave power. Under the MAHD optimized conditions, the essential oil yield was 3.19%, approximating the predicted yield (3.20%). MAHD was superior in terms of saving energy and extraction time (40 min, compared to 120 min in HD). The essential oil analyzed by GC-MS, presented 39 compounds constituting 98.37% and 97.51% of the essential oils obtained through MAHD and HD, respectively. No obvious differences were found in composition between MAHD oil and HD oil. Antimicrobial study showed that the lavender essential oil exhibited broad-spectrum antibacterial activity and the MAHD oil showed a higher antimicrobial activity than the HD oil. This study revealed that MAHD could be a good method for extracting essential oil in lavender and other aromatic plants.

Key words: lavender essential oil, microwave-assisted hydrodistillation, hydrodistillation, response surface methodology, antimicrobial activity

1 Introduction

Lavender (Lavandula angustifolia) belongs to the family labiatae (Lamiaceae) and is one of the most widely cultivated essential oil crops in the world. Traditionally, the lavender provides several important essential oils to the fragrance industry, including shampoos, shower gels, perfumes, soaps, skin lotions and other cosmetics. In food manufacturing, lavender essential oil is employed in baked goods, ice cream, beverages, candy, and chewing gum. Meanwhile, a great range of medical uses of lavender essential oil has also been reported including antispasmodic, sedative, antihypertensive, antiseptic, healing and anti-inflammatory properties. In addition, lavender essential oil is effective against the growth of a wide range of microorganisms. It is evident that lavender is one of the most important plants in the mint family (Lamiaceae) from therapeutic as well as economic aspects. In recent years, lavender is widely cultivated in Xinjiang of China. The chemical composition of lavender essential oils is complex. Before such compositions can be analyzed, they have to be extracted from the matrix. Extraction is a very important analytical step in the separation and identification of essential oils from plant materials prior to chromatographic determination. Various extraction techniques are employed to the extraction of essential oils from plants, include hydrodistillation (HD), steam distillation (SD), ultrasound assisted extraction, Soxhlet extraction method, and supercritical fluid extraction. However, these methods have several shortcomings, including low extraction efficiency, long extraction time, degradation of some volatile compounds, and low reproducibility. Microwave-assisted extraction can significantly improve the extraction efficiency and reduce the extraction time. In addition, microwave-assisted extraction can also be performed at a low temperature to avoid degradation of some volatile compounds.

Abbreviations: HD, hydrodistillation; MAHD, microwave-assisted hydrodistillation; GC-MS, chromatography-mass spectrometry; RSM, response surface methodology; BBD, box-behken design; SEM, scanning electron microscopy; DIZ, diameter of inhibition zone; MIC, determination of the minimum inhibitory concentration; DMSO, dimethyl sulfoxide; LB, Luria-Bertani; ANOVA, Analysis of variance; RSD, relative standard deviation.

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Accepted June 19, 2018 (received for review January 29, 2018)

Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online
http://www.jstage.jst.go.jp/browse/jos/ http://mc.manuscriptcentral.com/jjocs
high energy expense and toxic solvent residuals in the products. Supercritical fluid extraction provides a rapid and selective technique for extracting essential oils from plant materials. Nevertheless, the operational complexity and high cost of the apparatus has limited its exploitation\textsuperscript{13, 14}. These disadvantages have led to the consideration of new techniques for essential oil extraction. Recently, the application of microwave heating to the analysis of essential oils has attracted widespread attention and microwave-based techniques are an appropriate alternative to conventional extraction methods\textsuperscript{15–17}. The advantages of using microwave heating in comparison with conventional extraction methods include a shorter extraction time, faster energy transfer, higher extraction yield, and lower energy consumption, while maintaining a high quality of extract. Microwave-assisted hydrodistillation (MAHD) is an advanced hydrodistillation (HD) technique that uses microwave energy and water to extract the target compounds from plant materials. The water was used as a solvent for absorbing the microwave energy, which increases the temperature and pressure; this, in turn, causes target compounds to be very rapidly transferred from the matrix materials to the water phase. There are a large number of reports concerning the application of MAHD to the extraction of essential oil from various kinds of plant materials\textsuperscript{18, 19}. However, due to the many factors that influence MAHD, optimization of the process parameters is required to retain the maximum extraction of essential oils.

Response surface methodology (RSM) is a collection of mathematical and statistical method that can be used to optimize multifaceted processes and determine the effects of multiple variables and their interactions\textsuperscript{20–22}. In the present work, extraction of essential oil from lavender was optimized using MAHD method. Response surface methodology (RSM) based on Box–Behnken design (BBD) was applied to investigate and optimize the process parameters such as microwave power, liquid-to-solid ratio and microwave time on the maximum yield of essential oil from lavender by MAHD method. The chemical composition of the essential oil was analyzed by gas chromatography-mass spectrometry (GC-MS). Additionally, the antibacterial activity of the essential oil extracted under optimal conditions was investigated. Furthermore, the results were compared with those of hydrodistillation as a conventional extraction method.

2 Materials and methods
2.1 Materials and reagents
Lavender flowers were collected from Xinjiang of China in July 2017. The flowers were dried under ambient conditions on a large screened tray and stored at 4°C in airtight bags until further use. Analytical grade sodium chloride and dimethyl sulfoxide (DMSO) were purchased from Tianjin Zhi Yuan Chemical Reagent Co., Ltd. (Tianjin, China). Analytical grade sodium sulfate anhydrous was purchased from Tianjin Hongyan Chemical Reagent Factory (Tianjin, China). The antimicrobial activity of the lavender essential oil samples was tested against five different microorganisms provided by China Center for Type Culture Collection (Wuhan, China). Three bacteria were Staphylococcus aureus (CCTCC AB 91093), Bacillus subtilis (CCTCC AB 90008) and Actinomycyes viscosus (CCTCC AB 99001). Two fungal were Aspergillus niger (CCTCC AF 91006) and Penicillium sp. (CCTCC AF 93302). The cultures of microorganisms were maintained on Luria-Bertani (LB) agar or Luria-Bertani (LB) broth.

2.2 Microwave-assisted hydrodistillation (MAHD)
MAHD extraction has been performed in a XH-100A microwave laboratory oven (Beijing Xianghu Science and Technology Development Co., Ltd. China). It is a multimode microwave reactor 2.45 GHz with a maximum delivered power of 1000 W. Temperature was monitored by an external infrared sensor. A Clevenger system located at the outside of the microwave cavity continuously condensed the distillate. For each extraction, 30 g of lavender samples and water of specified volume were added into a flask. The flask was setup within the microwave oven cavity, and directly connected to the Clevenger apparatus through a hole at the top of microwave oven. The MAHD extraction parameters were microwave power (400-700 W), extraction time (10-40 min) and liquid-to-solid ratio (6-22 mL/g), where the influence of each parameter was investigated in single-factor experiments (Supplement Table S1). Each trial was carried out in triplicate and a standard deviation was calculated. After extraction, the essential oil was collected, dehydrated with anhydrous sodium sulfate and kept at 4°C until being analyzed. Each trial was carried out in triplicate and a standard deviation was calculated. Extraction yield was calculated according to equation (1):

\[
\text{Yield of essential oil (\%) } = \frac{\text{Mass of extracted essential oil}}{\text{Mass of dried material}}
\]

(1)

2.3 Hydrodistillation (HD)
A quantity of 30 g of lavender was subjected to hydrodistillation with a Clevenger-type apparatus according to the previous research\textsuperscript{23} and extracted with 540 mL of water for 120 min (until no more essential oil was obtained). The essential oil was collected, dehydrated with anhydrous sodium sulfate, and stored at 4°C for further analysis. Each extraction was performed three times. The extraction yield was calculated according to equation (1).

2.4 Experimental design of MAHD
Response surface methodology (RSM) using three-factors
three-levels Box-Behnken design (BBD) approach was launched to investigate the individual and interactive effects of process variables to extract the essential oil from lavender flowers using MAHD. Based on the single-factor experimental results (Supplement Table S2), the three independent variables were microwave power ($X_1$: 400-600 W), liquid-to-solid ratio ($X_2$: 10-18 mL/g) and microwave time ($X_3$: 20-40 min), while the yield of essential oil was selected as the dependent variable. Table 1 shows the arrangement of the BBD in this research, a total of 17 different combinations including 5 replicates at the centre point were employed to fit the full second-order polynomial equation model. The general equation is expressed as the equation (2). Where $Y$ is the predicted essential oil yield, $\beta_0$, $\beta_i$, $\beta_{ii}$ and $\beta_{ij}$ are the regression coefficients for linearity, quadratic and interactive terms respectively, while $X_i$ and $X_j$ are levels of the independent variables and $\varepsilon$ is the residual associated to the experimental.

$$Y = \beta_0 + \sum_i \beta_i X_i + \sum_{i=1}^2 \sum_{j=i+1}^2 \beta_{ij} X_i X_j + \varepsilon \quad (2)$$

In order to more intuitively reflect response surface characteristics, the regression coefficients were applied to fitting model to generate response surface in three-dimensional plots and contour maps. The independent variables were coded as $-1$ (low value), $0$ (central point) and $+1$ (high value), respectively. The relevant mathematical equations were presented as the follows equation (3):

$$X = \frac{X_i - X_0}{\Delta X_i} \quad (3)$$

Where $X$ is the coded value of $X_i$, $X_0$ is the value of independent variable at the centre point and $\Delta X_i$ is the step change.

### 2.5 Gas chromatography-mass spectrometry (GC-MS) analysis

The GC-MS analyses were performed with an Agilent model 7890B gas chromatograph (Agilent Technologies Inc., USA) coupled to an Agilent model 5977A Mass Spectrometry (Agilent Technologies Inc., USA). Essential oil was separated on HP-INNOWax capillary column ($60 \text{ m} \times 0.25 \text{ mm} \times 0.5 \text{ µm}$ film thickness), and 0.4 µL samples were injected in split mode (ratio 40:1). The column oven temperature was programmed with an initial temperature of 50°C for 2 min, heated at a rate of 3°C/min to 190°C, and then heated at 10°C/min to 250°C and held at 250°C for a further 10 min. The injection temperature was 250°C. Helium was used as the carrier gas with a flow rate of 1 mL/min. All data were obtained by collecting the full-scan mass spectra within the scan range of 30-500 amu. Kovats indices were calculated for all volatile compounds by use of a homologous series of C$_{17}$-C$_{24}$ n-alkanes on the HP-INNOWax column. Identification of compounds was based on computer matching with the NIST 14 library and comparisons of retention indices (RI) with previously reported in

| Run | Microwave power ($X_1$ W) | Liquid-to-solid ratio ($X_2$ mL/g) | Microwave time ($X_3$ min) | Extraction yield of essential oil (%) |
|-----|----------------|-----------------|-----------------|-------------------------------|
| 1   | 400           | 10              | 30              | 2.41                          |
| 2   | 400           | 18              | 30              | 2.52                          |
| 3   | 600           | 10              | 30              | 2.43                          |
| 4   | 600           | 18              | 30              | 2.72                          |
| 5   | 500           | 10              | 20              | 2.47                          |
| 6   | 500           | 18              | 20              | 2.52                          |
| 7   | 500           | 10              | 40              | 2.73                          |
| 8   | 500           | 18              | 40              | 3.16                          |
| 9   | 400           | 14              | 20              | 2.02                          |
| 10  | 600           | 14              | 20              | 2.38                          |
| 11  | 400           | 14              | 40              | 2.72                          |
| 12  | 600           | 14              | 40              | 2.88                          |
| 13  | 500           | 14              | 30              | 3.10                          |
| 14  | 500           | 14              | 30              | 2.99                          |
| 15  | 500           | 14              | 30              | 2.96                          |
| 16  | 500           | 14              | 30              | 2.95                          |
| 17  | 500           | 14              | 30              | 3.04                          |
the negative control. All the tests were repeated in triplicate. The diameter of the inhibition zone was measured with a caliper in mm. A microbial suspension without essential oil for 24 h for bacteria, and at 28°C for fungal. A microbial suspension without essential oil was employed as a positive control while DMSO served as the negative control. All the tests were repeated in triplicate.

2.6 Scanning electron microscopy (SEM) observation

Scanning electron microscopy (SEM) images of lavender flowers were obtained for the untreated samples as well as for those samples after MAHD (for 40 min) and HD (for 120 min). The samples were attached on the specimen holder with aluminum tape and then all of them sputtered with gold in a sputter coater for SEM observation. All the samples were examined with a Hitachi SU8010 scanning electron microscopy (Hitachi Ltd., Japan) under high vacuum condition at an accelerating voltage of 20 kV and at a working distance of 10 mm.

2.7 Antibacterial assays

2.7.1 Diameter of inhibition zone (DIZ)/determination

The diameter of inhibition zone (DIZ)/values of the lavender essential oil against five different microorganisms were determined by the filter paper disc diffusion assay. The bacterial suspensions were adjusted with sterile saline to a concentration of $1.0 \times 10^8$ CFU/mL. The prepare experimental plates were determined as the formerly described with slightly modifications. At first, 0.2 mL bacterial suspensions were added to the 20 mL LB agar. Afterward, the agar solutions were vortexed for 15 s in order to perfectly disperse the bacterial suspension in the culture medium. At last, the uniform agar solution was poured into Petri dishes of 90 mm diameter at a rate of 20 mL per box and was cooled and solidified on the bench. Filter paper discs (6 mm in diameter) were individually impregnated with 10 μL essential oil then placed onto the surfaces of the inoculated plates. The incubated was performed at 37°C for 24 h for bacteria, and at 28°C for 24 h for fungal. The diameter of the inhibition zone was measured with a caliper in mm. A microbial suspension without essential oil was employed as a positive control while DMSO served as the negative control. All the tests were repeated in triplicate.

2.7.2 Determination of the minimum inhibitory concentration (MIC)

The determination of the MIC of the essential oil, which reflects the concentration that completely inhibits the growth of microorganisms were determined by the method of broth macrodilution. The microorganism suspensions were adjusted with sterile saline to a concentration of $1.0 \times 10^8$ CFU/mL. Dilution series were made in a concentration range from 0.03 to 2 mg/mL of the essential oil in sterile test tubes containing LB broth medium. Using a vortex, each tube was vigorously stirred in order to perfectly disperse the lavender essential oil in the culture medium. Then, 30 μL of the tested microorganism suspension (about $10^5$ CFU/mL) were inoculated into the test tube. The MIC was defined as the lowest concentration of the essential oil at which the microorganism did not exhibit visible growth after 24 h of incubation at 37°C for bacteria and at 28°C for fungal. A microbial suspension without essential oil was employed as a positive control while DMSO served as the negative control. All the tests were repeated in triplicate.

2.8 Statistical analysis

All experiments were carried out in triplicate, and the mean of essential oil yields were used for statistical analysis. Analysis of variance (ANOVA), multi-regression analyses, and significance test were performed using Design-Expert version 8.0.6.1 (State-Ease, Inc., Minneapolis, MN, USA) statistical package.

3 Results and discussion

3.1 Model and fitting the model using response surface methodology (RSM)

MAHD parameters including microwave power, microwave time and liquid-to-solid ratio were optimized by RSM using BBD to achieve the maximum yield of lavender essential oil. The experimental conditions and the results of 17 runs were shown in Table 1. The observed data was analyzed by analysis of variance (ANOVA) and the results were shown in Table 2. Significance of the developed model equations was evaluated by their corresponding p-values and F-value. The lower p-value ($p < 0.0001$) of the model demonstrated that, the developed model was highly significant. The F-value of the model was estimated to be 30.80 specifying that the model was significant. There was only a 0.01% chance that a model F-value of this size could occur due to statistical noise. In this model, the F-value for lack-of-fit was estimated to be 2.01 implying that the lack-of-fit was not significant relative to the pure error. There was a 25.54% chance that a lack-of-fit F-value of this size could occur due to statistical noise. The determination coefficient ($R^2$) meant the proportion of the total variation in the response expected by the model. In this study, the determination coefficient ($R^2$) was 0.9754 suggesting a 97.54% match between the predicted and experimental data. Meanwhile, the value of adjusted determination coefficient ($\text{Adj } R^2 = 0.9437$) was also very high, indicating a high significance of the model developed through experimental data. The smaller the coefficient of variance (C.V.%) was, the more reliable the model would get. The coefficient of variance value of 2.75% showed that, the deviations between experimental and predicted values are low and also showed a high degree of precision and reliability of the conducted experiments. The adequate precision was used to measure the signal to noise ratio and the ratio greater than 4 is desirable. In this present study, the adequate precision was found to be 19.138, which indicates the best fitness of the developed model. From the ANOVA summary,
the model was accurate and applicable. As shown in Table 2, the independent variables (X₁, X₂, and X₃), cross product coefficient (X₁X₂) and quadratic terms (X₁², X₂², and X₃²) significantly affected the essential oil yield of lavender (p < 0.05). The extraction yield value could be expressed by the following second order polynomial equations equation (4):

\[
Y = 3.01 + 0.093X_1 + 0.11X_2 + 0.26X_3 + 0.045X_1X_2 - 0.050X_1X_3 + 0.095X_2X_3 - 0.35X_1^2 - 0.13X_2^2 - 0.15X_3^2
\]

(4)

Where Y is the essential oil extraction yield, X₁ is the microwave power, X₂ is the liquid-to-solid ratio, X₃ is the microwave time. Experimental data were analysed using Design-Expert software (Trial Version 8.0.6.1) and fitted to a second order polynomial regression model containing the coefficient of linear, quadratic, and three factors interaction effects. ANOVA was used to analyze the model for significance and suitability. According to the model above, the optimal experimental conditions for the highest essential oil yield were microwave power of 511.05 W, liquid-to-solid ratio of 17.13 mL/g, and microwave time of 40 min. Considering the feasibility of operation, the experimental conditions was modified to microwave power of 500 W, liquid-to-solid ratio of 17 mL/g, and microwave time of 40 min. The maximum predicted yield of lavender essential oil was 3.20. Verification experiments were performed three times under the optimized conditions and the mean extraction yield was 3.19 with a relative standard deviation (RSD) of 0.0071. The predicted values were very close to the actual values, indicating the models established were reasonable and reliable.

3.2 Analysis of response surface

Three-dimensional profiles of multiple non-linear regression models were employed to investigate the linear and quadratic effects as well as the interaction effects between microwave power (X₁), liquid-to-solid ratio (X₂), and microwave time (X₃) on the extraction yield of lavender essential oil (Fig. 1a-c). The response surface methodology (RSM) was employed to explore the variables that affect the microwave extraction and this approach enables the overall number of experiments and possible interactions between

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**Table 2** ANOVA of response surface quadratic model for extraction yield of essential oil from lavender.

| Variables   | Sum of squares\(b\) | Df\(c\) | Mean square\(d\) | F-value\(e\) | P-value\(f\) |
|-------------|---------------------|---------|------------------|-------------|-------------|
| Model       | 1.54                | 9       | 0.17             | 30.80       | <0.0001     |
| X₁          | 0.068               | 1       | 0.068            | 12.36       | 0.0098      |
| X₂          | 0.097               | 1       | 0.097            | 17.47       | 0.0041      |
| X₃          | 0.55                | 1       | 0.55             | 99.50       | <0.0001     |
| X₁X₂        | 8.100E-003          | 1       | 8.100E-003       | 1.46        | 0.2658      |
| X₁X₃        | 0.010               | 1       | 0.010            | 1.81        | 0.2210      |
| X₂X₃        | 0.036               | 1       | 0.036            | 6.52        | 0.0380      |
| X₁²         | 0.53                | 1       | 0.53             | 95.24       | <0.0001     |
| X₂²         | 0.076               | 1       | 0.076            | 13.65       | 0.0077      |
| X₃²         | 0.100               | 1       | 0.100            | 18.02       | 0.0038      |
| Residual    | 0.039               | 7       | 5.540E-003       |             |             |
| Lack of fit | 0.023               | 3       | 7.767E-003       | 2.01        | 0.2554      |
| Pure error  | 0.015               | 4       | 3.870E-003       |             |             |
| R²          | 0.9754              |         |                  |             |             |
| Adj R²      | 0.9437              |         |                  |             |             |
| C.V. %      | 2.75                |         |                  |             |             |
| Adep Precision | 19.138            |         |                  |             |             |
| Cor Total   | 1.57                | 16      |                  |             |             |

\(\text{a} \) Coefficients refer to the model.
\(\text{b} \) The sum of squares between the average values and the overall mean.
\(\text{c} \) Degree of freedom.
\(\text{d} \) Sum of the squared divided by degree of freedom.
\(\text{e} \) Test for comparing term variance with residual variance.
\(\text{f} \) Probability of the observed F-value.
the variables to be considered. Figure 1a describes the mutual effects between microwave power and liquid-to-solid ratio on the yields of lavender essential oil extracted at fixed microwave time (30 min). As shown in Fig. 1a, when increasing microwave power from 450 to 550 W together with an increase of liquid-to-solid ratio from 10 to 18 mL/g, extraction yield was not further enhanced. When the microwave power was increased, the yield of essential oil isolated increased at first, but began to decrease when the power was further increased. This indicates that an increase in power accelerated the mass transfer ratio until a certain value, thus increasing the extraction yield. However, when the power was higher than 500 W, the yield was decreased. This may be due to the fact that excessively high microwave power could cause thermal decomposition of volatiles. Figure 1b describes the interactive effect of microwave power and microwave time at fixed liquid-to-solid ratio (14 mL/g). Extraction time is one of the key factors that influence the extraction efficiency. In order to select a proper time to enhance the extraction yield of essential oil, the extraction was carried out at different times (20-40 min). It was observed that the extraction yield of lavender essential oil increased significantly with the increase of extraction time at a fixed power. It did not continue to significantly increase until the extraction time was over 38 min. The essential oil yields could improve with the increase of microwave power, but further increase of microwave power was together with decrease of oil yields. Figure 1c shows the mutual effects between liquid-to-solid ratio and microwave time on the essential oil yields extracted at given microwave power (500 W). An increase in extraction yield was observed by increasing the liquid-to-solid ratio. For increased liquid-to-solid ratio from 10 to 18 mL/g together with an increase of extraction time from 35 to 40 min, the extraction yield reaches a peak value. Liquid-to-solid ratio is an important parameter that influences the extraction yield of essential oil. Although large volumes of solvent could obtain higher extraction yields, but it also could lead to a difficult procedure and an unnecessary waste of the solvent. For small volumes of solvent, incomplete extraction will be obtained. Therefore, selecting an appropriate liquid-to-solid ratio was very important. The yield of essential oil increased with increasing liquid-to-solid ratio was probably due to the fact that, solvent (water) can efficiently absorb microwave energy and leads to increase the release of essential oil into the solvent. According to the analysis of ANOVA and three-dimensional response surface among the three independent variables, the major factor that affects the yield of essential oil was the microwave time, subsequently was the liquid-to-solid ratio and microwave power.

3.3 Analysis of microscopic changes

In order to study the structural alteration during the dif-
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3.4 Chemical composition of the essential oil

With the optimal conditions, the essential oil compounds in lavender were extracted by MAHD followed by analyzing with GC-MS. The HD method was also studied, and compared to the MAHD method. The GC-MS total ion chromatograms of the essential oils obtained by MAHD and HD are shown in Fig. S1a and S1b, respectively (Fig. S1 in supplemental). Through the retrieval of computer and manual analysis, qualitative and quantitative differences in the components of the essential oils extracted by MAHD and HD from lavender flowers are represented in Table 3. The number of replicates was three. The ultimate yield of essential oils obtained from lavender was 3.23% by HD (120 min) and 3.19% by MAHD (40 min). Among 39 compounds identified in lavender essential oil, representing 98.37% and 97.51% of the total oils obtained by MAHD and HD, respectively. The results showed that the essential oil of lavender is a complex mixture of mainly monoterpenes (e.g. linalool, and linalyl acetate) and a few monoterpenes (e.g. trans-β-ocimene) as minor oil constituents.

Using the MAHD method, volatile compounds were identified. The major volatile compounds in essential oil of lavender extracted by MAHD are linalool (32.90%), linalyl acetate (27.00%), lavandulyl acetate (10.90%), trans-β-ocimene (4.21%), and α-terpineol (3.51%). Using HD, compounds were identified in the essential oil from the lavender. The major volatiles found in HD oil are linalool (30.50%), linalyl acetate (22.80%), lavandulyl acetate (11.20%), α-terpineol (5.52%) and trans-β-ocimene (3.82%). The essential oils isolated by MAHD and HD contained the same dominant components, including linalool, linalyl acetate and lavandulyl acetate, but in different relative amounts. Comparing the essential oil samples extracted by means of MAHD and HD no remarkable difference was observed with regards the identified compounds. However, the concentrations of compounds varied greatly with the extraction method. Among the major differences were the much higher content of linalool and linalyl acetate in MAHD oils compares with HD oils. The results may indicate that linalool and linalyl acetate was partly decomposed during hydrodistillation. In this application, microwave irradiation highly accelerated the extraction process, but without causing considerable changes.
Table 3  Chemical compositions of lavender essential oils by GC-MS analysis.

| No. | Compounds                        | Molecular formula | RI°        | RI°        | Content (%) |
|-----|---------------------------------|-------------------|------------|------------|-------------|
|     |                                 |                   | HD MAHD    | HD MAHD    |             |
| 1   | Camphene                        | C_{10}H_{16}      | 1084 1065  | 0.08 ± 0.00| 0.10 ± 0.01 |
| 2   | β-Myrcene                       | C_{10}H_{16}      | 1178 1170  | 0.61 ± 0.00| 0.38 ± 0.01 |
| 3   | d-Limonene                      | C_{10}H_{16}O     | 1220 1212  | 0.29 ± 0.00| 0.24 ± 0.01 |
| 4   | Eucalyptol                      | C_{10}H_{16}O     | 1231 1223  | 0.72 ± 0.08| 1.01 ± 0.03 |
| 5   | trans-β-Ocimene                 | C_{10}H_{16}      | 1252 1258  | 3.82 ± 0.01| 4.21 ± 0.03 |
| 6   | β-Ocimene                       | C_{10}H_{16}      | 1270 1266  | 1.68 ± 0.01| 1.52 ± 0.03 |
| 7   | 3-Octanone                      | C_{10}H_{16}      | 1275 1261  | –          | 0.09 ± 0.01 |
| 8   | Acetic acid, hexyl ester        | C_{6}H_{12}O_{2}  | 1289 1282  | 0.27 ± 0.00| 0.40 ± 0.01 |
| 9   | β-Cymene                        | C_{10}H_{14}      | 1296 1278  | 0.11 ± 0.01| 0.14 ± 0.01 |
| 10  | Terpinolene                     | C_{10}H_{16}      | 1308 1290  | 0.26 ± 0.01| 0.15 ± 0.01 |
| 11  | 1-Pentylallyl acetate           | C_{10}H_{16}O     | 1395 1379  | 2.53 ± 0.00| 2.73 ± 0.01 |
| 12  | (3E,5Z)-1,3,5-Undecatriene       | C_{10}H_{16}      | 1434 1424  | 0.44 ± 0.01| 0.41 ± 0.01 |
| 13  | Butanoic acid, hexyl ester      | C_{10}H_{16}O     | 1468 1458  | 0.58 ± 0.00| 0.53 ± 0.01 |
| 14  | cis-Linalool oxide              | C_{10}H_{16}O     | 1490 1462  | 0.27 ± 0.01| 0.40 ± 0.02 |
| 15  | 1-Octen-3-ol                    | C_{10}H_{16}O     | 1497 1471  | 0.64 ± 0.02| 0.57 ± 0.07 |
| 16  | trans-Linalool oxide (furanoid) | C_{10}H_{16}O     | 1573 1566  | 30.50 ± 0.18| 32.90 ± 0.83 |
| 17  | Linalool                        | C_{10}H_{16}O     | 1581 1565  | 22.80 ± 0.51| 27.00 ± 0.15 |
| 18  | Linalyl acetate                 | C_{10}H_{16}O     | 1608 1597  | 0.37 ± 0.03| 0.39 ± 0.01 |
| 19  | α-Santalene                     | C_{10}H_{16}      | 1617 1603  | 0.15 ± 0.02| 0.15 ± 0.01 |
| 20  | α-Bergamotene                   | C_{10}H_{16}      | 1619 1597  | 0.39 ± 0.03| 0.38 ± 0.01 |
| 21  | L-Bornyl acetate                | C_{10}H_{16}O     | 1632 1617  | 11.20 ± 0.76| 10.90 ± 0.14 |
| 22  | Lavandulyl acetate              | C_{10}H_{16}O     | 1637 1637  | 0.76 ± 0.07| 1.28 ± 0.01 |
| 23  | Terpinen-4-ol                   | C_{10}H_{16}O     | 1643 1628  | 2.33 ± 0.17| 1.78 ± 0.03 |
| 24  | Caryophyllene                   | C_{10}H_{16}      | 1685 1668  | 1.07 ± 0.03| 1.02 ± 0.01 |
| 25  | cis-β-Farnesene                 | C_{10}H_{16}O     | 1714 –     | 0.11 ± 0.01| –            |
| 26  | 1,4,7-Cycloundecatriene, 1,5,9,9-tetramethyl- Z,Z,Z- | C_{10}H_{16} | 1715 1692  | –          | 0.09 ± 0.01 |
| 27  | Humulene                        | C_{10}H_{16}      | 1723 1690  | 0.63 ± 0.02| 0.60 ± 0.01 |
| 28  | Crypton                         | C_{6}H_{12}O      | 1731 1718  | 5.52 ± 0.05| 3.51 ± 0.02 |
| 29  | α-Terpineol                     | C_{10}H_{16}O     | 1750 1733  | 1.68 ± 0.00| 0.98 ± 0.02 |
| 30  | Neryl acetate                   | C_{10}H_{16}O     | 1753 –     | 0.69 ± 0.01| 0.62 ± 0.01 |
| 31  | β-Copaene                       | C_{10}H_{16}      | 1780 1765  | 2.58 ± 0.01| 1.75 ± 0.08 |
| 32  | Geranyl acetate                 | C_{10}H_{16}O     | 1833 1804  | 0.30 ± 0.02| 0.28 ± 0.01 |
| 33  | Cuminaldehyde                   | C_{10}H_{16}O     | 1877 1864  | 0.15 ± 0.01| 0.12 ± 0.01 |
| 34  | p-Cymen-8-ol                    | C_{10}H_{16}O     | 1885 –     | 0.18 ± 0.01| –            |
| 35  | Cyclohexene, 2-ethenyl-1,3,3-trimethyl- | C_{10}H_{16} | 2015 –     | 1.88 ± 0.26| 0.52 ± 0.24 |
| 36  | Geraniol                        | C_{10}H_{16}O     | 2050 2011  | 1.51 ± 0.03| 0.93 ± 0.04 |
| 37  | Caryophyllene oxide             | C_{10}H_{16}O     | 2199 2192  | 0.28 ± 0.01| –            |
| 38  | t-Cadinol                       | C_{10}H_{16}O     | 2199 –     | –          | 0.14 ± 0.01 |
| 39  | Bicyclo[4.4.0]dec-1-ene,2-isopropyl-5-methyl-9-methylene- | C_{10}H_{16} | 1714 –     | 0.11 ± 0.01| –            |

Total compounds (%) 97.51 98.37
Total oxygenated compounds (%) 85.63 87.29
Total extraction time (min) 120 40
Yield (%) 3.23 ± 0.12 3.19 ± 0.04

a Kovats indices to n-alkanes (C_{5}-C_{24}) on an HP-INNOWax column.
b Kovats indices from previous studies.
in the essential oil composition. Therefore, microwave does not involve in any deterioration of the extracted components and it can be introduced as a safe method for the extraction of essential oils.

3.5 Cost, energy and environmental ecology
Hydrodistillation (HD), a conventional extraction method for essential oil, was implemented to make a contrast with MAHD. Under the optimized conditions, MAHD process was performed. The result that the essential oil yields of MAHD for 40 min (3.19 ± 0.04%) was comparable to those of HD for 120 min (3.23 ± 0.12%). At the extraction time of 40 min, MAHD resulted in a similar essential oil recovery to that obtained by HD after 120 min. No significant differences were found in essential oil yields between MAHD and HD. Additionally, MAHD result in significant saving in the extraction time. Unlike the classical conductive heating methods, microwaves can heat the entire sample almost simultaneously and at a higher rate. Therefore, MAHD was more effective than the HD on the extraction of essential oil. The energy required to perform the extraction, considering the total periods of full extractions, was 0.33 kWh for MAHD and 0.70 kWh for HD. According to previous research, the calculated quantity of carbon dioxide (CO₂) released to the atmosphere is dramatically higher in the case of HD (566.32 g CO₂/g of essential oil) than for MAHD (277.59 g CO₂/g of essential oil). The reduced cost of extraction is clearly advantageous for the proposed MAHD method in terms of energy and time. From these results, the MAHD was an efficient, environmentally friendly and energy-saving extracted method for the extraction of lavender essential oil.

3.6 Antimicrobial activity
The essential oils obtained through HD and MAHD were tested against five different microorganisms, involving three bacteria (Staphylococcus aureus, Bacillus subtilis and Actinomycetes viscosus) and two fungal (Aspergillus niger and Penicillium sp.). The DIZ values of the essential oil determined by paper disc diffusion method are shown in Table 4. As can be seen in Table 4, the essential oils exhibited obvious antibacterial activities against all tested microorganism. The DIZ values of lavender oil by MAHD and HD against five different microorganisms were in the range of 9.3-10.2 mm and 9.0-10.5 mm, respectively. In contrast, the MAHD oil showed higher antimicrobial activity against all the tested microorganisms than HD extract except Penicillium sp. The MIC of different lavender essential oils against five microorganisms are also shown in Table 4. The MIC values of the essential oil determined by broth macrodilution method. The MIC values ranged from 0.125 to 0.250 mg/mL for oils obtained by the MAHD method and from 0.125 to 0.250 mg/mL for those by the HD method. According to the value of MIC, MAHD oil was most effective against Actinomycetes viscosus (0.125 mg/mL) and HD oil was most effective against Penicillium sp. (0.125 mg/mL), respectively. The negative control (1% DMSO) showed no antibacterial effect on any tested microorganisms. The essential oil isolated from lavender using different methods showed different antimicrobial activity. Generally, the antibacterial properties of essential oils are closely associated with their most abundant components therein. It can be seen from Table 3 that linalool and linalyl acetate were the predominant components of lavender essential oil. However, studies investigating the relationship between the biological activity and the chemical composition of lavender have found no correlation between the percentage of the predominant components and the antimicrobial activity. Thus, the activity could be attributed to the presence of minor components. Comparing the essential oil samples extracted by means of MAHD and HD no remarkable difference was observed with regards the identified compounds. However, the concentrations of compounds varied greatly with the extraction method. Therefore the lavender essential oil extracted by different methods had different antimicrobial activity suggesting that the antimicrobial activity is not the result of these compounds but may be also related to the concentrations.

| Tested Microorganisms | Zone of inhibition (mm) | MIC (mg/mL) |
|-----------------------|-------------------------|------------|
| **Bacteria**          |                         |            |
| Staphylococcus aureus | 9.2 ± 0.3               | 9.3 ± 0.6  | 0.250 | 0.250 |
| Bacillus subtilis     | 9.8 ± 0.3               | 10.1 ± 0.8 | 0.250 | 0.250 |
| Actinomycetes viscosus| 9.3 ± 0.6               | 10.2 ± 0.3 | 0.250 | 0.125 |
| **Fungal**            |                         |            |
| Penicillium sp.       | 10.5 ± 0.7              | 9.3 ± 0.5  | 0.125 | 0.250 |
| Aspergillus niger     | 9.0 ± 0.0               | 9.3 ± 0.6  | 0.250 | 0.250 |
| DMSO                  | 6.0 ± 0.0               | 6.0 ± 0.0  |        |    |
of compounds. In addition, it was also speculated that a synergistic effect between the major and minor components of the essential oil contributed to the antibacterial activity. These results demonstrated that MAHD produced lavender essential oil was relatively more active than HD oil. Therefore, MAHD could be a good alternative method to extract essential oil for antimicrobial experiment.

4 Conclusions

The MAHD technique has been compared with the conventional HD method, for extraction of essential oil from lavender. A multivariate optimization strategy using RSM based on BBD was carried out to optimize effective parameters on the performance of MAHD. The optimum extraction conditions were as follows: extraction time of 40 min, microwave power of 500 W and liquid-to-solid ratio of 17 mL/g. Microwave time had the greatest impact on the essential oil yield of lavender followed by liquid-to-solid ratio and microwave power. MAHD was found to be highly effective enabling a substantial reduction in extraction time (40 min for MAHD against 120 min for HD), providing an essential oil with yields similar to those of traditional HD. GC-MS results indicated that there were no significant differences between the constituents of lavender essential oil obtained through MAHD and those obtained through HD. Antimicrobial study showed that the lavender essential oil exhibited broad-spectrum antibacterial activity and the MAHD oil showed a higher antimicrobial activity than the HD oil. SEM micrographs confirmed that microwave technique efficiently promoted the release of essential oil by breaking down the cell structure of lavender flower. MAHD can be considered as a green technology that offers significant advantages over conventional hydrodistillation: shorter extraction times with similar yields, considerable energy saving, environmentally friendly technique, and lower cost. Consequently, this method appears to be a good alternative for the extraction of essential oil from lavender for their applications in the food, cosmetic industries, and pharmaceutical.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 21565024).

Conflict of interest

The authors declare no conflict of interest.

Supporting Information

This material is available free of charge via the Internet at http://dx.doi.org/ios.67.10.5650/jos.ess.18019

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