Optimization of microwave hydro-distillation of lemongrass leaves (Cymbopogon nardus) by response surface methodology

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Abstract. Essential oil of lemongrass (Cymbopogon nardus) or Citronella Oil is widely used in industry, especially pharmaceuticals and perfumes. The microwave assisted method was successfully applied in extracting fragrant essential oils on a laboratory scale of extraction, namely Microwave Hydrodistillation (MHD). MHD is a combination of hydrodistillation with microwave for extraction. The purpose of this research was to study several effects parameters (microwave power, material size, and extraction time) on the yield of Citronella Oil obtained by the MHD method and to find optimal conditions using response surface methodology (RSM). The results showed that the smaller size of the material, the lower the yield of oil obtained. The longer extraction time, results in higher yields was. While the greater the power, the higher the yield produced. The highest results obtained for extraction using the MHD method are the microwave power at 600 W, material size of 0.5 cm, and extraction time is 90 min in resulting a yield of 1.817%. Chemical Analysis of Citronella Oil was the highest component that analyzed by gas chromatography-mass spectrometry (GC-MS) technology.

1. Introduction
Essential oils are also known as ethereal oils produced from several plant varieties with several extraction methods. The oil is volatile at room temperature without component decomposition, has a bitter taste, good odor and a distinctive odor according to the type of plant, generally soluble in organic solvents and insoluble in water. Essential oils are desirable commodities in various industries such as in the perfume, cosmetics, pharmaceutical and food, beverage and beverage industries and can also be used as natural insecticides [1]. We know that the mega-biodiversity centers, Indonesia produces 40 types of 80 types of essential oils traded on the world market. They have entered the world's essential markets, namely patchouli, lemongrass, cloves, ginger, nutmeg, pepper, cinnamon, sandalwood, jasmine, vetiver, cananga, cajuput, and kemukus. Lately, many farmers have turned to lemongrass oil because of the large market demand and easy cultivation. In addition, fragrant lemongrass is widely developed because it has relatively easy growing conditions, making it easy to cultivate for wide-scale development on various types of soil [2]. Scented lemongrass (Cymbopogon nardus) belongs to the family of grasses (Gramineae) which produces essential oils known as ”of Java” [3]. And lemongrass fragrant is a plant that has many benefits, including as a producer of essential oils. Widely used by the pharmaceutical industry, soap fragrances, detergents, fragrances, sweets and beverage industries [4, 5]. Other benefits of Citronella Oil include antifungal agents [6] and antibacterial activities [7, 8].
Extraction of essential oils contained in fragrant lemongrass on an industrial scale is currently carried out by steam distillation. The selection of this distillation system is because the material used is in the form of leaves and stems so that the essential oils are better, the distillation is the shorter and the distilled material does not burn. However, in this system there are weaknesses, namely the results obtained cannot be optimal and the time taken is longer about 7 hours. Fragrant lemongrass oil produced by distillation of leaves and stems, it has an oil yield ranging from 1.03-1.52%, contains active citronella between 44.92 - 85.73% [9]. Various methods can be used to extract essential oils from plant materials, such as hydro-distillation (HD), steam distillation, soxhlet, and distillation extraction simultaneously. However, the molecules of it are sensitive to heat because of chemical compounds. This extraction method can cause the loss of several volatile compounds, low efficiency, long extraction time, degradation of compounds or unsaturated esters through thermal or hydrolytic effects and toxic solvent residues in extracts. To overcome this problem, several new green technologies for extracting essential oils have been developed by researchers [10-15]. These techniques usually use less solvents and energy, such as supercritical fluids, ultrasonic and microwave extraction. The microwave process has been successfully applied for extracting essential oils on a laboratory scale. Microwave-assisted hydrodistillation (MHD) and solvent-free microwave extraction (SFME) appear to be important industries because of simple technique, economic configuration and high product yield [16].

In extracting Citronella Oil fragrant with the MHD method there are several variables that affect oil yield including Size of material, microwave power, extraction time, and set constant variable in ratio of ingredients to solvents [15-20]. The aim of this study was to study several effect parameters (material size, extraction time and microwave power) on the results of fragrant Citronella Oil obtained by the MHD method and to find optimal conditions using response surface methodology (RSM). Response surface methodology is an important technique by statistical method for optimizing and improving the design of process condition. Besides that, the main purpose of RSM from this study usually to evaluate and obtain an interaction parameter that produces the highest response of the extraction process.

2. Materials and Methods
2.1 Materials
The materials were lemongrass leaves (Cymbopogon nardus) that obtained from Pacet Mojokerto, East Java, Indonesia and distilled water as a solvent in the distillation process. The chemicals required for this research were obtained from PT. Brataco Chemicals (Surabaya branch).

2.2 Methods
2.2.1 Microwave Hydro-Distillation. This study applied microwave hydro-distillation for Citronella Oil extraction. EMM2308X, Electrolux microwave oven was used. It has a maximal power of 800 W and a wavelength frequency of up to 2450 MHz. Its dimensions referred to 48.5 cm x 37.0 cm x 29.5 cm. It was modified by drilling a hole at its top. A 1000 mL round bottom flask connected to Clevenger condenser through the hole was placed inside. The hole was closed with a PTFE to prevent the entry of any contaminants (Figure 1).

2.3 Experimental Procedure
The extraction work carried out consisted of the following steps. The plant material was packed in the distiller and then water was added in a sufficient amount. The samples extracted strung together with Clevenger apparatus. The preheating was measured from the moment the microwave was turned on till that of the evaporation beginning. The steam which came out from the extraction chamber was condensed by a condenser apparatus and transported to a separator, where the oil separated automatically from the water. The yield was followed every 10 min after preheating. The process continued until reaching the maximum extraction time of 150 min.
2.4 Response Surface Methodology (RSM)

The Response Surface Methodology (RSM) is a group of methods that used to evaluate the variables and the practices involved in develop to optimize for extraction process [23]. The functioning environment of Citronella Oil extraction was developed by Box-Behnken Design (BBD). It used the Design-Expert 10 software package (Trial version; State-Ease Inc., Minneapolis, USA) applied for 17 experiments in the optimization of three variables (Table 2): the microwave power (A), material size (B) and extraction time (C). Each of these variables was at its low and high level: A (400 W; 600 W; 800 W), B (0.5 cm; 1 cm; 1.5 cm) and C (30 min; 60 min; 90 min). This is shown in Table 1. The regression analysis was performed in order to estimate the response function as a second order as follows:

\[
Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j
\]

where \( \beta_0, \beta_i, \beta_{ii} \) and \( \beta_{ij} \) are regression coefficients in the linear, quadratic, cubic, and interaction terms, severally; \( X_i \) and \( X_j \) are the independent variables.

| Factors        | Name          | Units | Low  | High |
|----------------|---------------|-------|------|------|
| A              | Microwave power| W     | 400  | 800  |
| B              | Material size | cm    | 0.5  | 1.5  |
| C              | Extraction time| min   | 30   | 90   |

2.5 Chemical Analysis of Citronella Oil Components

The components of the oil obtained from citronella plant were identified by a coupled gas chromatography- mass spectrometry (GC-MS) analysis. It was performed with Agilent 6980N gas chromatograph and Agilent 5973 mass spectrometric detector. The column has a length of 30 m, an internal diameter of 0.25 µm, a film thickness of 0.32 mm contained 5% phenylmethylsiloxane. It was introduced to the chromatography column HP5. The injection port and the interface were held at 230°C and 280°C, respectively. The temperatures were read between 50°C and 280°C at a rate of 10°C per minute. Helium was used as a carrier gas. Most of the components were identified by the mass spectra.
Table 2. Box-behnken design (BBD) matrix and response for extraction yield.

| Run | Microwave power (W) | Material size (cm) | Extraction time (min) | Observed | Predicted | Residual |
|-----|---------------------|-------------------|----------------------|----------|-----------|----------|
| 1.  | 600                 | 0.5               | 90                   | 1.817    | 1.7500    | 0.0659   |
| 2.  | 400                 | 1                 | 90                   | 0.675    | 0.7269    | -0.0519  |
| 3.  | 600                 | 1.5               | 30                   | 0.952    | 1.0200    | -0.0659  |
| 4.  | 400                 | 1.5               | 60                   | 0.880    | 0.8477    | 0.0323   |
| 5.  | 400                 | 1                 | 30                   | 0.467    | 0.4334    | 0.0336   |
| 6.  | 800                 | 0.5               | 60                   | 1.459    | 1.4900    | -0.0323  |
| 7.  | 600                 | 1                 | 60                   | 1.163    | 1.1600    | 0.0042   |
| 8.  | 600                 | 0.5               | 30                   | 1.246    | 1.2700    | -0.0196  |
| 9.  | 800                 | 1                 | 90                   | 1.054    | 1.0900    | -0.0336  |
| 10. | 800                 | 1.5               | 60                   | 0.945    | 0.9310    | 0.0140   |
| 11. | 400                 | 0.5               | 60                   | 0.996    | 1.0100    | -0.0140  |
| 12. | 600                 | 1                 | 60                   | 1.181    | 1.1600    | 0.0222   |
| 13. | 600                 | 1                 | 60                   | 1.142    | 1.1600    | -0.0168  |
| 14. | 800                 | 1                 | 30                   | 0.689    | 0.6371    | 0.0519   |
| 15. | 600                 | 1.5               | 90                   | 1.296    | 1.2800    | 0.0196   |
| 16. | 600                 | 1                 | 60                   | 1.197    | 1.1600    | 0.0382   |
| 17. | 600                 | 1                 | 60                   | 1.111    | 1.1600    | -0.0478  |

3. Result and Discussion

3.1 Effects of Single parameter

3.1.1 Effect of the microwave power. In this study, the preheating has a significant effect on the yield and the extraction time, especially at the beginning of the process. Preheating is the process through which the materials and the solvents in a distiller are irradiated at the initial temperature until solvent evaporation starts. The initial temperature value is equal to 27°C, while that of evaporation amounts to 115°C. Table 1 presents the duration of the preheating in case of different distiller volumes and shows that the higher the microwave power used, the shorter the heating time is [25].

3.1.2 Effect of the material size. That extraction of catechins is disconnected when it comes to use small particle size which causes enlarger surface area and increasing the contact of particles. from the research, get the best results to produce the highest yield when in the size of 0.5 cm. However, when the sample particle size is more than it, with a decrease in particle size in the sample, the extraction of essential oil from Citronella Oil decreases. Perhaps the smaller particle size is easy to absorb the target compound [24], the target compound will experience a reduction in the diffusion process from sample to solvent.

3.1.3 Effect of the extraction time. Based on result, the best time is to be at 90 minutes. In general, the longer the extraction time is needed, the higher the results obtained [15]. The results of this study, support the extraction results with the influence of extraction time will increase yield in the extraction process.
3.2 Analysis of variance from ANOVA

Table 3. Analysis of variance (ANOVA) for response to evaluate of significant factors.

| Sources of variations | Sum of Squares | df  | Mean Square | F-value | p-value |
|------------------------|----------------|-----|-------------|---------|---------|
| Model                  | 1.5500         | 9   | 0.1719      | 49.92   | < 0.0001 significant |
| A-Microwave power      | 0.1593         | 1   | 0.1593      | 46.28   | 0.0003 |
| B-Material size        | 0.2610         | 1   | 0.2610      | 75.81   | < 0.0001 |
| C-Extraction time      | 0.2768         | 1   | 0.2768      | 80.38   | < 0.0001 |
| AB                     | 0.0396         | 1   | 0.0396      | 11.50   | 0.0116 |
| AC                     | 0.0062         | 1   | 0.0062      | 1.79    | 0.2228 |
| BC                     | 0.0129         | 1   | 0.0129      | 3.74    | 0.0943 |
| A²                     | 0.5089         | 1   | 0.5089      | 147.80  | < 0.0001 |
| B²                     | 0.2821         | 1   | 0.2821      | 81.94   | < 0.0001 |
| C²                     | 0.0340         | 1   | 0.0340      | 9.88    | 0.0163 |
| Residual               | 0.0241         | 7   | 0.0034      |         |         |
| Lack of fit            | 0.0196         | 3   | 0.0065      | 5.75    | 0.0621 not significant |

The acceptable response showed that the data can be evaluated to obtain a good model of the statistical result. The model coefficients are represented by constant terms, linear coefficients for independent variables, coefficients of quadratic terms, and cubic terms. Then, the determination coefficient (R²), the adjusted coefficient of determination (Adj-R²) and adequate precision are used to evaluate the adequacy of the model; this model is valid when the value of p <0.05; lack of fit p >0.05 (table 4); and R² >0.98 (see in table 4) [25]. Then, summary statistics suggest to selecting the quadratic model for this extraction process, which is model selected by Design-Expert.

Table 4. Fit statistics from ANOVA for the extraction process.

| Insignificant factors | Response |
|-----------------------|----------|
| Standard deviation    | 0.0587   |
| Mean                  | 1.070    |
| Coefficient of variance % (CV) | 5.460  |
| R²                    | 0.9847   |
| Adjusted R²           | 0.9649   |
| Predicted R²          | 0.7962   |
| Adeq Precision        | 29.2809  |

3.3 Effect of interaction parameters

A good way to explain the effect of each parameter in the experimental data under investigation is to produce a surface response plot of the equation. The response surface was plotted using Design-Expert software (version 10.0.4.1) to evaluate the effect of a single parameter and their interactions for the Citronella Oil. The yield of Lemongrass Oil is influenced by microwave power, material size ratio and extraction time are presented in Figures 1 and 2.

In figures 1a and 2a, when 3-D response surface plots and contour plots were developed for Citronella Oil yields with varying microwave power, material size, and extraction time. Citronella Oil yields have improved markedly due to increased microwave power and have almost peaked at moderate microwave power. The highest extraction yield occurred in microwave power at 600 W.
Figure 2. Response surface plots showing the interaction effect of the microwave power and material size (AB) (a), microwave power and extraction time (AC) (b), and material size and extraction time (BC) (c) on the essential oil for extraction yield.

Again, in figure 2b and 3b, when the response surface plot and the contour plot were showed for the recovery of **Citronella Oil** with varying microwave power and extraction time, it can be seen that maximum recovery of **Citronella Oil** could be achieved when microwave power and extraction time were 600 W and 90 min, respectively. Furthermore, it can be seen that maximum recovery of **Citronella Oil** can be achieved when extraction time were around 90 min, respectively.

It can be resulted that optimal extraction conditions of **Citronella Oil** was microwave power 600 W and extraction time was 90 min (Figure 3). Based on optimized condition, the predicted yield of the **Citronella Oil** was 1.817%.
Figure 3. Contour plots showing the effect of the AB interaction (a), AC interaction (b) and BC interaction (c) on the Citronella Oil for extraction yield.

3.4 Model Adequacy Checking
In addition, it is necessary to check the model installing to ensure that it provides an adequate estimate for a real process. Excepting the model shows an adequate match, continuing with the investigation and optimization of the response surface installed tends to produce inadequate or confusing results. Residues from the least squares fit show an important role in assessing the adequacy of the model. By adding a residual normal probability plot, a check is made for the normality assumption, as given in Figure 4. Normal assumptions are accepted when the residual plot is approached along a straight line. Besides that, figure 5 presents a plot of the residue versus the predicted response. The general impression is that the residue is scattered randomly on the screen, showing that the variance of the original observations is constant for all Y values. Both plots are satisfactory, so we conclude that the empirical model is sufficient to describe Citronella Oil results with response surfaces.

3.5 Verification and Validation of the model
Based on parameters respon, to compare between the predicted model and actual data, rechecking experiment was carried out using several extraction conditions with several parameters. Values obtained from actual experiments, indicate the validity of the RSM model, because the model was significant (p <0.05) (Table 3). The strong correlation between actual and predictions confirm that the response model is sufficient to reflect for expecting extraction conditions so that it could be optimized in this extraction.
3.6 GC-MS analysis

The results of the component analysis have been presented in table 5. The biggest component detected is citronella of 40.54%. Similar results were also supported by research conducted by Toledo et. al (2016) [26]. Citronella Oil is an anti-parasitic agent, antifungal activities, a strong antibacterial agent and anti-mosquito [27].

Table 5. Chemical compounds contained in the essential oil of dried leaves of Cymbopogon nardus obtained by microwave hydrodistillation.

| Chemical                          | % of total | Quality |
|----------------------------------|------------|---------|
| Citronella                       | 40.54      | 98      |
| Trans-Geraniol                   | 17.39      | 86      |
| β-citronellol                    | 15.88      | 98      |
| d-limonene                       | 1.60       | 99      |
| Isopulegol                       | 0.35       | 98      |
| cis-2,6-Dimethyl-2,6-octadiene   | 0.98       | 97      |
| Eugenol                          | 0.45       | 98      |
| Delta-3-Carene                   | 0.46       | 96      |
| α.-cubebene                      | 1.84       | 95      |
| Delta-cardine                   | 0.80       | 98      |
| Torreyyol                        | 0.85       | 97      |
| Naphthalene                        | 3.95 | 96 |

4. Conclusions

The box-behnken response surface (BBD) has established to be adequate for estimating the effects of three independent parameters that are microwave power, material size, and extraction time on Citronella Oil yield and to predict optimal conditions in extraction process. The experimental data resulted that the term in quadratic model of three independent variables (microwave power, material size, and extraction time) has generally significant effect on the response value. Based on analysis of variance (ANOVA) and agreement of experimental and predictive results, it can be concluded that the resulting model is suitable for simulating Citronella oil-aided extraction with the help of a microwave
with a combination of variables studied. The optimal conditions are microwave power at 600 W, material size in 0.5 cm and extraction time is 90 minutes. In this optimized condition, the predicted yield of extract oil is 1.817%.

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