The role of pre-treatment in enhancing yield and antioxidant activity of lemongrass (Cymbopogon citratus) essential oil

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Abstract. Sample preparation before distillation is an important step in producing high yield and good quality of lemongrass (Cymbopogon citratus) essential oil. In order to improve the production process and to provide information as reference for further practical applications, the effects of sample pre-treatment (with and without microwave heating) and distillation time (3, 4, and 5 h) were studied. In addition, the samples types (dried stem and dried leaf) were compared. Results of the study suggested that the highest antioxidant activity of lemongrass essential oil (86.33%) was obtained from lemongrass dried stem that were heated in microwave and distilled for 4 h with yield of 2.307%. While the highest yield of lemongrass essential oil produced (2.63%) was obtained from lemongrass dried stem that were heated in microwave and distilled for 5 h with antioxidant activity of 69.67%. Based on the results, it can be concluded that lemongrass stem contains higher essential oil compare to that of the leaf and microwave heating before distillation gave higher yield and better quality of the product.

1. Introduction
Lemongrass (Cymbopogon citratus) is a plant from the family Graminaceae/Poaceae. This plant grows in tropical and sub-tropical climates area in Southeast Asia and Africa [1]. Lemongrass is rich in bioactive compounds, such as; alkaloids, flavonoids, tannins, and essential oils which have many benefits, especially in the fields of food, agriculture, pharmacy and health. Those various ingredients indicate that lemongrass has considerable antibacterial activity. In general, the use of lemongrass can be obtained from its bioactive compounds such as citral at 65 - 85% of the total essential oils components from lemongrass extraction [2,3]. In addition, citral is also proven to have anti-fungal activity that can control post-harvest disease in oranges [4,5].

Lemongrass essential oil is generally obtained through distillation process, the oil is yellowish with a distinctive aroma of lemon (citral). Extraction of essential oil is an attempt to separate essential oils from the original plant or plant parts. The essential oils are found on the inside of the glandular cells. Essential oils can only be separated from plant cells when water vapour or other solvents reach the oil container which in turn, carries the oil to evaporate simultaneously. Therefore, for efficiency of the process, it is important for the essential oil to contact more quickly. Several factors determine the quality and quantity of essential oil, for instance, the raw material will determine the quality of the essential oil produced. Optimal material conditions also affect the quality of essential oils which related to the appropriate method of picking and the age of sample. The post-harvest handling of essential oils is not the same for leaves, flowers, stems, skins, rhizomes, or seeds. Pre-treatment
applied to sample prior extraction process includes size reduction to increase the surface area and drying or heating in order to ease the extraction of the oil. Method that is used in production of essential oil also determine the quality and quantity of essential oil produced. Inappropriate or errors of production process can reduce not only yield but also quality of the essential oil produced. This study was aimed to investigate how pre-treatment prior to distillation affect the bioactive content, antioxidant, and microbial activity of essential oil from lemongrass leaf and stem.

2. Materials and methods

2.1. Pre-treatment and essential oil extraction
Lemongrass (Cymbopogon citratus) plant used in the study was collected from Sumbang village, Banyumas, Central Java. Lemongrass plant was harvested at 4 months of age. Dried lemongrass leaf and stem were prepared using fresh lemongrass stalk that were washed, sorted, chopped into smaller size, and dried in cabinet dryer at 60°C until dry-broken for millet.

Prior to distillation of dried leaf and dried stem, the samples subjected to pre-treatment were divided into two groups, heated using microwave of 225 watt for 5 min (M1) and non-heated (M2). Lemongrass essential oil was extracted by hydro-distillation (direct distillation) at 3, 4 and 5 h. The lemongrass essential oil (LE) was kept at a room temperature in glass bottle away from the sunlight.

2.2. Characterization

2.2.1. Yield. The LE obtained was then dried with anhydrous sodium sulphate and the volume was measured. The yield was expressed as the volume of LE obtained from 100 g of dried samples (% v/w) [16].

2.2.2. Refractive index. The LE were subjected to tests of index of refraction according to Colombian Technical Standard 289 (2002a) (Normas Técnicas Colombianas, NTC) using an Abbe refractometer (Atago DR-A1, Tokyo, Japan) [16].

2.2.3. Antioxidant activity analysis, DPPH methods. DPPH scavenging activity of lemongrass oil was determined according the procedure of [17]. For assay, 1 gram sample was taken to a test tube and 9 ml ethanol (96 %) was added to the sample tube, then massed for 24 hours. Then 4 ml filtrate was transferred to a test tube and 1 ml of the fresh prepared solution of DPPH was added and stored for 30 min. The absorbance of the sample was measured at 517 nm using spectrophotometer UV-VIS double bin (Shimadzu, Japan). A mixture of 1 ml of DPPH solution plus 4 ml of ethanol was used as a control. All analyses were run in duplicates. The DPPH radical scavenging effect was calculated as inhibition of percentage according to the following formula:

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\text{Inhibition of percentage (\%) = \left[ \frac{A_{c(o)} - A_{a(t)}}{A_{c(o)}} \right] \times 100}
\]

where: Ac(o) is an absorbance of control DPPH solution at 0 min and Aa(t) is absorbance of test sample after 20 min.

2.2.4. Antimicrobial activity. Antibacterial activity testing was conducted using well diffusion method. The test culture bacteria (S. aureus and E. coli) were incubated at 37°C for 24 hours in NB medium. A total of 200 µL of tested bacterial culture suspension was added to 200 mL Nutrient Agar (NA) medium with a bacterial density of 106 CFU/mL. The agar medium was poured into a petri dish and allowed to solidify. Furthermore, on the media, wells were prepared in 6 mm diameter. A total of 60 µL LE of leaf and stem were put into each well and incubated at 37°C for 24 h. Observation and measurement of the inhibition zone (mm) was carried out by measuring the clear area around the well.
2.3. Statistical analysis
Data obtained were analyzed using the F test (variance test) at the 95% confidence level and further test (DMRT) to get the best treatment.

3. Results and discussion

3.1. Yield
This study showed that there was a significant difference in yield of lemongrass essential oil (LE), in variation of pre-treatment method (microwave heating, M1 and without heating, M2), sample type (dried leaf and dried stem), and distillation time (3, 4, and 5h) (Fig.1). The highest yield of 2.623 % v/w obtained from stem with microwave heating (M1) before distillation for 5 h. While the lowest yield of 0.2 % v/w obtained from leaf without heating (M2) before distillation for 3 h.

![Figure 1. The mean value of yield of lemongrass essential oil (LE of lemongrass essential oil (LE)) in various pre-treatment methods (microwave heating M1 and without heating, M2), sample types (dried leaf and dried stem), and distillation times (3, 4, and 5h)](image)

Overall, this study indicated that microwave heating pre-treatment (M1) before distillation produces higher lemongrass essential oil (LE) yields than that of without heating (M2) in both samples, leaf and stem. Microwave heating works by heating the moisture inside the cells and evaporate it, producing a high pressure on the cell wall. The pressure builds up inside the biomaterial which modifies the physical properties of the biological tissues (cell wall and organelles disrupter) improving the porosity of the biological matrix. This would allow better penetration of water through the matrix and improved yield of the essential oil [6]. This result is in align with Kawiji [7] who reported that distillation of fresh lemongrass-stalk which crushed before distillation gave more essential oil than that without crushed. Therefore, pre-treatment using microwave heating potential in increasing the yield of LE.

The stem gave a higher yield than the leaf in all pre-treatment method and distillation time. The stem produces a higher amount of essential oil than the leaf because during the distillation process, in the same treatment, the essential oil content in the stem is higher than the leaf. This result in accordance with Ewansiha et al, [8] which reported that the leaf of lemongrass contain lower essential oil than the root. Based on distillation time, longer distillation time produced higher yield. It is because the longer contact time between the oil source material and the solvent make the oil more easily
extracted, so that the longer penetration process of boiling water to the material, the more oil produced.

3.2. Antioxidant activity of the lemongrass essential oil

This study showed that there was a significant difference in antioxidant activity of LE in various pre-treatment methods (microwave heating, M1 and without heating, M2), sample types (dried leaf and dried stem), and distillation times (3, 4, and 5h) (Fig.2). This study showed that microwave heating prior to distillation of both lemongrass and stem produced LE with higher antioxidant activity compared to that without heating. An ingredient can be said to be active as a free anti-radical or antioxidant if the percentage of reduction (% inhibition) is more than or equal to 50% [9].

The highest antioxidant activity of 86.33% resulted from lemongrass stem, with were heated by microwave prior to distillation at 4 h. While the lowest of 45% resulted from lemongrass leaf without heating, distilled for 3 h (Fig.2). Considering the yield (Fig 1), the lowest yield also exhibit the lowest antioxidant activity (Fig.2) which is from the leaf without heating distilled for 3 h. However, the highest antioxidant activity was not from the highest yield (Fig.1), it was from stem with microwave heating prior to distillation, but at different distillation time, namely 4 and 5 h. It indicates that distillation for 4 h gave LE the optimum bioactive content while longer distillation time (5h) caused destroying the bioactive content. This is in accordance with Masrifah [10] which states that the decrease in antioxidant activity is due to the interaction between the solvent and the material, resulting in the formation of prooxidant compounds and decrease the antioxidant activity.

The stem has a higher antioxidant activity value than the leaf which can inhibit free radical compounds. The different results were due to differences in the content of bioactive components in the lemongrass, the stems had higher geranial, mineral, and mirsen compounds than the leaf of the citronella. The presence of α-citral (geraniol) and β-citral (neral) components contained in lemongrass essential oil likely acts as an antioxidant and scavenger of free radicals [11]. Based on variations in distillation time, it can be concluded that the longer the distillation time, the higher the antioxidant value. However, at 5 hours the value of antioxidant activity decreased, therefore, 4 hours distillation time is the optimum point to produce antioxidant activity. It is because the longer the distillation time, the more heat received by the material to evaporate the oil cells from the material so that the total geraniol and mineral will be higher. At the 5 hour distillation time, the geraniol and mineral content

![Figure 2. The mean value of antioxidant activity of lemongrass essential oil (LE) in various pre-treatment methods (microwave heating M1 and without heating, M2), sample types (dried leaf and dried stem), and distillation times (3, 4, and 5h)](image-url)
are decrease is due to the material being distilled too long, causing geraniol and mineral to decompose into isoprene compounds. While the heating treatment with microwave (M1) resulted in a higher antioxidant value of essential oils compared to without heating (M2). This effect occurs because the conventional heating process occurs through a heat gradient, whereas in microwave heating, heating occurs through direct interaction between the material and microwaves. The difference in temperature profiles in conventional heating and heating using microwaves results in faster energy transfer, and has the potential to improve the quality of the oil produced [12].

3.3. Refractive index of the lemongrass essential oil

The result of this study showed that there was a significant difference in refractive index of LE in variation of pre-treatment method (microwave heating, M1 and without heating, M2), sample type (dried leaf and dried stem), distillation time (3, 4, and 5h) (Fig.3). Microwave heating pre-treatment produced LE with higher refractive index compare to that of without heating. Furthermore, it has also showed that LE from leaf had lower refractive index than that of stem. Both sample, leaf and stem, showed that the longer the distillation time resulted in a smaller refractive index of LE. According to Yulianti and Suyanti [13], the refractive index of lemongrass essential oil is 1.483 - 1.489.

![Figure 3. The mean value of refractive index of lemongrass essential oil (LE) in various pre-treatment methods (microwave heating M1 and without heating, M2), sample types (dried leaf and dried stem), and distillation times (3, 4, and 5h)](image)

3.4. Antimicrobial activity of the lemongrass essential oil

This study results showed that there was a significant difference in antimicrobial activity toward S. aureus and E. coli of LE in variation of pre-treatment method (microwave heating, M1 and without heating, M2), sample type (dried leaf and dried stem), and distillation time (3, 4, and 5h) (Fig.4). Microwave heating prior to distillation on both lemongrass and stem produced LE with higher antimicrobial activity compared to that without heating.

The highest antimicrobial activity of 29.67 mm inhibition toward S. aureus and 14.67 mm inhibition zone toward E. coli were resulted from lemongrass stem with microwave heating prior to distillation at 4 h. Meanwhile, the lowest of 7.33 mm inhibition toward S. aureus was resulted from lemongrass leaf without heating, distilled for 3 h and 4.33 mm inhibition zone toward E. coli was resulted from lemongrass leaf without heating, distilled for 5 h (Fig.4). Considering the highest antioxidant activity obtained from stem with microwave heating prior to distillation at 4 h (Fig.2), this result is in align with antioxidant activity of LE because distillation for 4 h gave LE the optimum bioactive content [10].
Figure 4. The mean value of antimicrobial activity as inhibition zone toward *S. aureus* and *E. coli* of lemongrass essential oil (LE) in various pre-treatment methods (microwave heating M1 and without heating, M2), sample types (dried leaf and dried stem), and distillation times (3, 4, and 5h).

Lemongrass extract contains tannins, saponins, alkaloids, flavonoids, and essential oils. Those various ingredients in lemongrass have a considerable antibacterial activity [14]. Those compounds can damage the bacterial cell wall and change the composition of the bacterial cell. The mechanism of phenolic and terpenoid compounds as antibacterial through damaging the cell wall structure and disrupt the work of active transport and proton strength in the bacterial cytoplasmic membrane [15]. According to Bota et al., [15], the main chemical composition of lemongrass oil are citral, citronellal, geraniol, and citroneol which are formed by the elements carbon (C), Hydrogen (H), and oxygen (O) with the elemental formulas C10, H16, 18, 20 and O that are terpenoid compounds of the monoterpen group (C10). The essential oil content can be used as an anti-bacterial agent.

4. Conclusion
Overall, this study resulted that microwave heating before distillation of dried lemongrass both leaf and stem increase its antioxidant and antimicrobial activity.

Acknowledgements
Thank you to the Rector of the University of Sudirman and the Head of the General Soedirman University Research and Community Service Institute (LPPM) for the funds provided through the PDUPT Funded Research Fund 2018 with the Decree of the Head of LPPM: No Kep. 1633/UN23.14/PN.01.00/2018

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