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# Extraction of Essential Oil from Ultrasound Pre-treated Citronella Grass (*Cymbopogon nardus*) Leaves by Hydro-distillation Method

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The global demand on essential oil derived from citronella grass (*Cymbopogon nardus*) leaves as fragrances, aroma and remedies ingredients has been steadily increasing towards commercial scale for decades. Therefore, a study to enhance citronella grass essential oil production capacity through ultrasound pre-treatment is performed. This study investigated the effect of various operating parameters, such as leaf particle size (ground, 5 mm and 10 mm), ultrasound pre-treatment time (10, 20 and 30 min) and the leaf particle mass to extracting water volume (SW) ratio (1:4.0 to 1:12.0) on the essential oil yield. The chemical and physical properties of the citronella oil, namely bioactive constituents, specific gravity, refractive index and colour, were also determined. The experimental results indicate that increased ultrasound pre-treatment time and S/W ratio promoted to the enhancement of oil yields by up to 56.94 % and 19.59 %. The GC-MS analysis proved that citronella oil contains mainly citronellal, geraniol, neral and geranial. Physical properties evaluation also revealed that the specific gravity, refractive index and colour of the extracted citronella oil were of acceptable ranges. The results indicated that the increase of S/W ratio and ultrasound pre-treatment time potentially increase the extraction yield. This study suggests that the combined ultrasound pre-treatment and hydro-distillation is a promising method for extracting high quality citronella oil and could be implemented to scale-up the citronella oil production capacity.

## 1. Introduction

*Cymbopogon nardus* L. or citronella grass, is a member of the genus of *Cymbopogon* (*Poaceae* family) originated from South India and Sri Lanka, which is popularly recognized as serai wangi in Malaysian peninsular and Indonesia. Recently, it can also be easily encountered in other tropical regions of the Asian, American, and African continents. Citronella grass has narrow-long leaves that are rich in silica thorns aligned along the leaf edges. Usually, these leaves bear a lot of glandular hairs, which each has a wider basal cell than the distal one. Although citronella grass is a non-woody plant, its cell wall is also composed of cellulose, hemicellulose, and lignin (Aizan et al., 2008). However, the lignin content in the lignocellulosic structure is lower than that in wood (Kaur and Dutt, 2013). The leaves contain roughly 1–2 % of essential oils, which is mainly composed of geraniol, citronellal, limonene, eugenol and citronellol (Katiyar et al., 2011). However, the chemical composition of the citronella oil varies broadly, which depends on the genetic diversity, geographical origin, climate, cultivation practice, maturity, extraction techniques and parts of the plant (Yen and Lin, 2017). Presently, India, Sri Lanka, Malaysia, Madagascar, West Africa, Taiwan, Argentina, Brazil, Mexico, Guatemala, Ecuador, and the West Indies are regarded as the world's main producers of citronella oil, which contribute a total annual production of about 5000 tonnes (Skaria et al., 2007). For centuries, citronella oil has served its remarkable roles in the beverages, foodstuffs and traditional medication. Because citronella oil is enjoying an increasing global demand due to its wide utilisations in the perfume, soap, cosmetics, flavouring, and healthcare industries, citronella grass is now considered as a highly promising plant commodity by agricultural entrepreneurs (Silva et al., 2011).

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Although hydro-distillation and steam distillation are the most common techniques for extracting essential oil from citronella grass, its lengthy operation can lead to undesirable thermal degradation and hydrolysis of some key components (Reverchon and De Marco, 2006). Hence, a proper pre-treatment should be carried out to break down the lignocellulosic constituents of its raw materials for efficient citronella oil extraction (Bhutto et al., 2017). Milling is generally combined with other pre-treatments to enhance the surface area of the raw material, which provides larger accessibility for further processes (Ravindran and Jaiswal, 2016). Ultrasonication produces a cavitational destabilisation of the lignocellulose matrices through generation of oxidising radicals and alteration of surface structure (Bhutto et al., 2017). During ultrasound propagation in the liquid solvent, the microbubble collapses reduce the thickness of the transfer boundary, and thus increase the solid/liquid actual heat and mass transfer area, and significantly speed up the transfer phenomena. Therefore, application of ultrasound in the extraction processes of bioactive compounds from plants can reduce the extraction time and enhance the yield of the active ingredients in the extraction product (Xu et al., 2019). In this work, ultrasonication is selected as the pre-treatment method for citronella grass prior to hydro-distillation where sonication time, hydro-distillation time, SW ratio, and leaf particle size were further studied to find the best operating condition to obtain the highest oil yield extracted from *C. nardus*. Then, the chemical and physical characteristics of the citronella oil and microstructure of the citronella grass leaf particles were also analysed.

## 2. Methodology

### 2.1 Plant sample and chemicals

*C. nardus* leaves were purchased from a traditional market in Semarang, Central Java, Indonesia. They were rinsed with flowing water to get rid of undesirable particles and dirt. Then, the cleaned leaves were dehydrated in an electric oven (Memmert, Germany) at 100 °C overnight to prevent any microbial degradation during storage. Prior to use, the dried leaves were cut into pieces of approximately 5 mm and 10 mm length using a sharp kitchen knife and further sonicated using (BUC 65L, B-One Ultrasonic Cleaner, China) at 30 °C and various exposure times. The chemicals used in this study were the products of Sigma-Aldich, Singapore.

### 2.2 Hydro-distillation experiments and oil yield calculation

Approximately 75 g of oven-dried *C. nardus* leaves was extracted by the hydro-distillation using a Clevenger-type apparatus at various SW ratios for 5 h. The preheating was measured from the moment the heating mantle was turned on until that of the extracting water began to boil. The steam leaving the boiling flask was condensed in a Liebig condenser and the condensate was collected in the connected separation tube, where the oil is separated from the water. The extracted oil was dehydrated by flowing it over anhydrous sodium sulphate bed and was then stored at 4 °C in hermetically sealed dark glass flasks with rubber lids until yield and compositional analysis without any further purification. The oil yields were calculated using Eq(1):

$$\text{Yield (\% m/m)} = \frac{\text{mass of extracted essential oil (g)}}{\text{mass of leaf particles (g)}} \times 100\% \qquad (1)$$

### 2.3 Chemical and physical characterisations of citronella oil

Citronella oil composition was examined by gas chromatography coupled with mass spectrometry (GC–MS) on Agilent 6890N Network GC System, Agilent J&W Capillary. The GC analysis was performed on a Varian 3300 type gas chromatograph by employing an FID detector, an apolar capillary column DB-5 (30 m × 0.25 mm i.d.; film thickness 0.25 µm) and a polar column supelcowax 10 with the same specifications. The operating conditions of the DB-5 and Supelcowax columns were as follows: from 50 °C (5 min), 50 °C to 250 °C at the rate of 2 °C/min. The temperature of the injector and detector were set at 250 and 300 °C, respectively. Helium was used as the carrier gas and continuously flown at 1.50 mL/min. Undiluted essential oil samples (0.2 µL) were injected manually using a glass syringe. The components of oil samples were identified based on their retention time, computer matching by comparing with the peaks of the Wiley and Adams library search data (Hamzah et al., 2014). The mass percentage of the identified components was computed from the peak area of the chromatogram without any correction factor and was calculated relatively. The specific gravity, refractive index and colour of the citronella oil samples were measured using a Densitometer DA-130N, an Atago No. 68534, and a Lovibond Tintometer Model E, respectively.

## 3. Results and discussion

### 3.1 Comparison of pre-treatment conditions on essential oil yield

The essential oil yields obtained from hydro-distillation of raw and ultrasound pre-treated citronella grass leaves are compared. As presented in Figure 1 (a), hydro-distillation of raw citronella grass leaves experienced a sluggish oil extraction in the first 60 min at which 0.11 % m/m yield was achieved. Up to 60 min hydro-distillation process, the yield is comparable to the accumulative essential oil yield of *C. nardus* grown in Thailand reported by Nakahara et al. (2003), which was 0.12 % m/m. However, the oil yield increased sharply between 60 to 150 min hydro-distillation and reached 0.28 % m/m. Under this period of time, the essential oil bearing parenchymatous cells of the leaves surface had already opened and the citronella oil diffused easily from the inner part to the leaves surface leading to a rapid oil removal by the boiling water and water vapor. As expected, as extraction time went by, the accumulated oil yields did not increase significantly. The depletion of citronella oil in the citronella grass leaves was likely the cause of this phenomenon. Beyond 240 min, the oil yields were nearly constant and the highest oil yield (0.31 % m/m) was achieved at 300 min. The yield obtained in this study was about 3 times than that reported by Tran et al. (2019) who extracted essential oil from lemongrass (*C. citratus*) leaves by hydro-distillation, but lower than that achieved by Toledo et al. (2016), which was 0.7 % m/m. The difference in the botanical source of the raw material may be the cause of this finding (Yen and Lin, 2017).

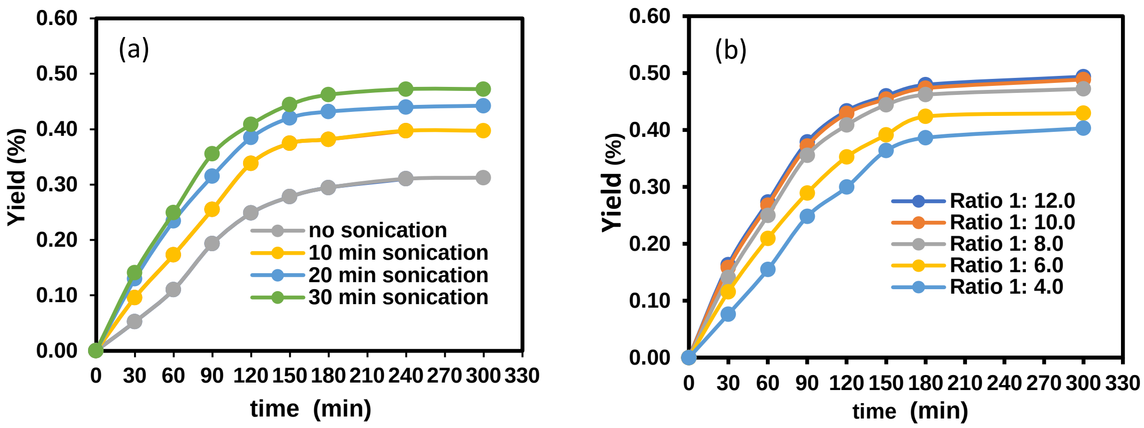


*Figure 1: Profiles of citronella oil cumulative yield at different pre-treatment condition (a) and SW ratio (b)*

Figure 1 (a) shows that higher oil yields were obtained from hydro-distillation of ultrasound pre-treated citronella grass leaves, from which a longer time used for ultrasound pre-treatment also resulted in higher oil yields. Highest oil yield obtained from hydro-distillation of ultrasound pre-treated citronella grass leaves was 0.47 % m/m, which was 50 % higher than the yield obtained from citronella grass without ultrasound pre-treatment. Lewinsohn et al. (1998) reported that citronella oil is stored inside the parenchymatous cells, which are fixed firmly and deeply in the adaxial side the citronella grass leaf mesophyll. The result confirmed that during ultrasound pre-treatment, ultrasonic waves propagation in liquid medium generates cavitation bubbles due to the pressure variation, inciting a breakdown of cell walls, eases rapid diffusion of the boiling water into the essential oil cells of citronella grass and then facilitate the washing out of the cell contents, which results in higher oil yield (Tiwari and Mason, 2012). Hence, ultrasound pre-treatment performed better yield, a shorter hydro-distillation time and less thermal degradation compared to the conventional methods.

### 3.2 Effect of hydro-distillation time on essential oil yield

The effect of the hydro-distillation time of citronella grass essential oil yield is also shown in Figure 1 (b). Obviously, the ultrasound pre-treated citronella grass leaf particles rapidly released their oil content from 0 to 90 min as indicated by almost linear increase of oil yield with hydro-distillation time. This phenomenon is more likely due to the large portion of readily accessible essential oil on the broken parenchymatous cells upon ultrasound pre-treatment. During the hydro-distillation, the increase of temperature results in an increase in pressure within the parenchymatous cells citronella grass leaves. As the pressure exceeds the maximum level, the cell walls break, and the essential oil is released (Balti et al., 2017). Simultaneously, the boiling water easily diffuses into the broken parenchymatous cells, lowering essential oil viscosity and pushing the oil outside.

However, as the hydro-distillation time was extended from 90 to 180 min, the proportion of essential oil in the parenchymatous cells of citronella grass has been depleted resulting in a slower essential oil release. The accumulated oil yield was almost constant as the hydro-distillation time was extended further (180 to 300 min), which indicated that the residual oil in the parenchymatous cells of citronella grass has been totally depleted. In addition, prolonged exposure of essential oil with high temperature and heat may induce the degradation of its key constituents, which finally reduce its quality. Based on the experimental results, the most feasible time for citronella grass oil extraction process by hydro-distillation with conventional heating is 180 min.

### 3.3 Effect of solid:water ratio on essential oil yield

Theoretically, the intensity of physical contacts between raw material and solvent will affect the oil yield. The effect of SW ratio on the oil yield is depicted in Figure 1 (b). By increasing the pre-treated SW ratio from 1:4.0 to 1:10.0, the oil yield increased from 0.40 % m/m to 0.49 % m/m. During hydro-distillation, the heat is transferred from water to the plant material leading to the break up the parenchymatous cells and the essential oil is swept away by the steam. At the same time, the boiling water easily diffuses into these broken parenchymatous cells of citronella grass, lowering essential oil viscosity and pushing the essential oil outside, which leads to increase the oil yield.

As the SW ratio was further increased to 1:12.0, the oil yield did not increase significantly. Excessive use of water will emulsify the oil and hinder the oil – water separation. Too much use of water was also proven to reduce the extraction efficiency due to increased energy consumption and longer extraction time (Jadhav et al., 2020). As shown in Figure 1 (b), the SW ratio of 1:10.0 can be considered as the most economical hydro-distillation condition from which 0.49 % m/m oil yield can be achieved in 5 h. Similar good condition was reported by Pornpunyapat et al. (2011) for the hydro-distillation of essential oil from Gaharu wood particles.

### 3.4 Effect of particle size on essential oil yield

Figure 2 depicts the effect of leaf particle size on the accumulative yield of citronella grass essential oil. There was no significant difference in essential oil yield in the first 30 min. However, a stronger effect of leaf particle size on the oil yield was observed at longer hydro-distillation time. As expected, the oil yield increased with decreasing leaf particle size from 10 mm to 5 mm. This phenomenon can be explained by the fact that smaller particle size leads to a higher surface area, making extraction more efficient. However, the oil yield decreased sharply as leaf particle size was further decrease by grinding of citronella grass leaves. The result proved that an extensive size reduction process would not be suitable for the attainment of efficient essential oil extraction because a significant portion of the volatile matters will be lost via vaporisation during size reduction process.

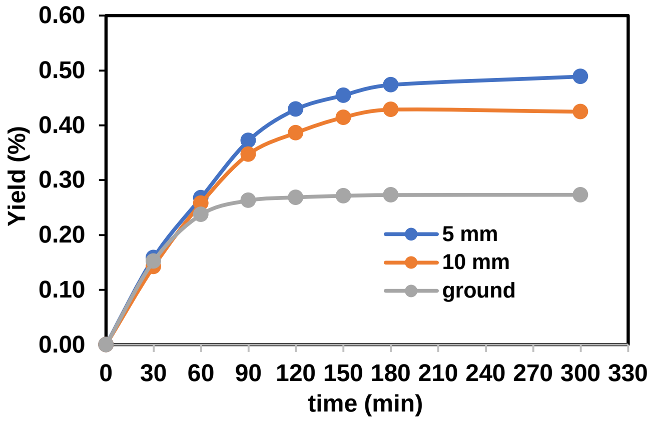


*Figure 2: Profiles of cumulative yield of essential oil as a function of particle size*

### 3.5 Chemical and physical characteristics of citronella oil

In total, 14 compounds were identified in the citronella oil by GC-MS analysis. Ten of these compounds (β-myrcene, limonene oxide, citronellal, citronellol, neral, geranial, geraniol, eugenol, β-caryophylene and δ-cadinol) were the same with citronella oil extracted from citronella grass grown in Thailand reported by Chanthai et al. (2012). The mass percentages of neral, geranial and geraniol were also almost the same, which all being higher than that reported by Hamzah et al. (2014) for citronella oil derived from citronella grass grown in Malaysia. However, it is in good agreement that neral (7.97 %), geranial (10.15 %), geraniol (54.90 %) and citronellal (23.21 %) are the major compounds of *C. nardus* essential oil, which give the unique

characteristic of aromatic grass aroma (Koba et al., 2009). The chemical composition of the essential oil may be influenced by the genetic diversity, geographical origin, climate, cultivation practice, maturity, extraction techniques and parts of the plant (Yen and Lin, 2017).
Refractive index, specific gravity and colour are the basic physical characteristics that define the quality of citronella oil and influence consumer's acceptance. The physical observation revealed that the refractive index and specific gravity of the extracted citronella oil at 20 °C were approximately 1.47 and 0.89, respectively. These values fall within the acceptable criteria of good quality citronella oil (Hamzah et al., 2014). Usually, the commercial grade citronella oil is clear yellow in colour. The citronella oil obtained by hydro-distillation with ultrasound pre-treatment exhibited a unique colour in the range of 0.7R 4Y. The characteristics of the colour red was due to the presence of trans-β-caryophyllene and γ-cadinene constituents in the citronella oil (Harjeet et al., 2011) and being in good agreement with the chemical characteristics obtained from GC-MS analysis.

### 3.6 Microstructural analysis of citronella grass leaf particles

A microscopic observation using scanning electron microscope analyser was performed to confirm that the morphology of citronella grass leaf particles altered as they underwent ultrasound pre-treatment and hydro-distillation. The results are presented in Figure 3 (a), (b) and (c).

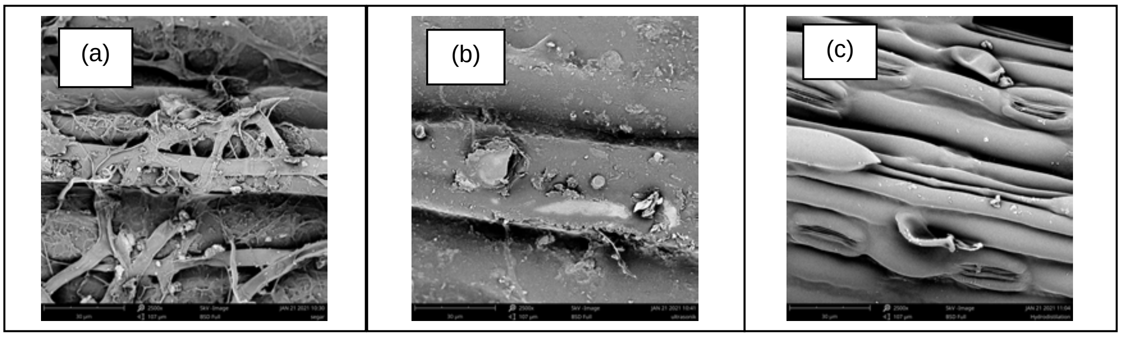


*Figure 3: The SEM images of citronella grass leaf particles (a) fresh, (b) after sonication and (c) after hydro-distillation*

The freshly cut citronella grass leaf particles (Figure 3 (a)) were covered by strong lignin and connected by hemicellulose layers. The citronella grass leaf particles that had undergone ultrasound pre-treatment (Figure 3 (b) exhibited some microfractures, but less cell wall degradation than hydro-distillation. The cell walls of the hydro-distilled leaf sample were less dense and exhibited severe swelling in addition to clear microfractures due to ultrasound pre-treatment (Hamzah et al., 2014). The secretory cavities (Figure 3 (c)), which produce and store essential oil were scattered throughout the surface of the citronella leaf after hydro-distillation. They invariably swell and burst due to the osmotic swelling of the glandular cells, which is attributed to the heat transfer that occurs only through conduction and convection. Hence, the ultrasound pre-treatment is supposed to cause rupture of the cell walls, thus producing further bursts, developing schizogenous cavities and intensify heat and mass transfer processes during hydro-distillation process (Xu et al., 2019).

## 4. Conclusions

This study successfully showed the potential of ultrasound pre-treatment to enhance the extraction performance of *C. nardus* essential oil. Hydro-distillation of 5 mm citronella grass leaf particles for 180 min with 30 min ultrasound pre-treatment using SW ratio of 1:10.0 achieved the highest oil yield (0.49 % m/m). The GC-MS analysis revealed that the ultrasound pre-treatment eases the release of citronella oil containing major fragrance constituents, namely citronellal, geraniol, neral and geranial of 23.21 %, 54.90 %, 7.97 % and 10.15 %, respectively. The specific gravity (0.89), refractive index (1.47) and colour (0.7R 4Y) of the citronella oil met the consumer acceptance ranges. The combined ultrasound pre-treatment and hydro-distillation process offer advantages compared to the conventional hydro-distillation method, mainly due to its shorter operation time and a lower solvent volume consumption, which leads to lower energy and solvent costs.

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