Optimization of Essential Oil Production from *Cymbopogon citratus* in Vietnam by Hydro-Distillation

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Abstract: *Citral-rich lemongrass (*Cymbopogon citratus*) essential oils have been reported to exhibit anticancer, antimicrobial, antifungal, and antioxidant properties, leading to wide applications in the food industry, pharmaceuticals, cosmetics, perfumery markets, and pest inhibition. Vietnam has abundant lemongrass, which can be centralized to develop a lemongrass-based essential oil industry. In this work, essential oils were extracted from lemongrass harvested from the Bac Giang and Phu Yen provinces of Vietnam. The study aimed to investigate the influential factors, including distillation time, raw material size, water quality, and water-to-material ratio, to optimize oil production from* C. citratus *in a 10 L hydro-distillation boiler. The maximum oil yield was obtained under the optimal conditions: distillation time of 180 min, the plant material size of 2–3 mm, reverse osmosis (RO) and/or distilled water, and water-to-material ratio of 5/1 (mL/g). The maximum yields of lemongrass oil from Bac Giang and Phu Yen provinces were determined as 2.55 and 3.96 mL/kg, with corresponding citral contents analyzed of 45.5 and 68.1%, respectively. From the experimental data, a protocol for essential oil production from Vietnam's* C. citratus *by the hydro-distillation technique was proposed.*

Keywords: *affecting factors;* Cymbopogon citratus*; essential oil production; hydro-distillation; lemongrass; total citral content*

■ INTRODUCTION

Lemon grass or lemongrass has the scientific name *Cymbopogon citratus* (DC.) Stapf, which is a tender perennial grass in the Cymbopogon genus, is native to the tropics of Maritime Southeast Asia, and it is known to many tropical countries, including India, Brazil, Mexico, and Vietnam [1]. Lemongrass belongs to the Poaceae family, growing in a clumping habit with multiple stalks. Each lemongrass bush consists of 50–100 cloves with 0.8–1.5 m high. The leaf sheaths of lemongrass are usually purple or greenish-white. It has a lemony characteristic aroma due to its main bioactive chemical compositions in leaves, stems, and roots. Lemongrass biomass generally contains 1–2% essential oil contains [2] or in the range of 2.1–2.9 mL/kg of raw materials [1]. In the literature, lemongrass essential oil contains some main ingredients including neral (19–39%), geranial (30–51%), citral (15–86%), limonene (0.76–6.00%), geranic acid (3.30%), myrcene (4–11%), geranyl acetate (0.57–4.20%) and other small amounts of linalool and citronellol [2-8]. Citral and geraniol, unsaturated aldehyde and alcohol compounds, are the most important components of typical lemongrass essential oil, which has a strong citrus scent and a flavor reminiscent of lemon. *In vitro* studies, there are numerous reports that certified valuable bioactivities of lemongrass essential oil, such as anticancer, antimicrobial, antifungal, and antioxidant, which are attributed to high citral content [3,7,9-12]. Therefore, the extracted essential oil of *C. citratus* has been applied widely in the food industry (food preservation, flavor additives in food and drink), pharmaceuticals (aromatherapy), cosmetics (bath and cleaning products), perfumery markets, and pest inhibition [2,5,13].

The technical extraction method is the most important factor affecting the yield, quality, and bioactive composition of obtained essential oil. The extraction methods have been known, such as solvent extraction [14], steam distillation [4-5,15], mechanical expression, hydro-distillation [5,8,16], and ultrasound or microwave assisted-hydrodistillation [2,8,17-18]. Among these mentioned methods, steam and/or hydro-distillation have been the most popular, simplest, and cheapest extraction methods used for plant essential oil production. The reason is that hydro-distillation-based techniques have been widely applied for plant essential oil production on an industrial scale [4-5,16,19]. In particular, the method only uses water and heat to produce purified oils from diverse plant matrices under moderate temperature and pressure conditions without using chemicals or organic solvents (e.g., ethanol, hexane, methanol), which is superior to supercritical fluid technologies [5]. Moreover, the hydro-distillation process involves completely immersing plant materials in boiling water under low pressure or vacuum to generate a steam temperature below 100 °C. This method is superior to steam distillation because of its shorter distillation time and higher oil yield with less steam used, and it preserves the original qualities of the plant. Principally, the vapor pressure matches the ambient pressure, allowing volatile constituents in plants with boiling points ranging from 150 to 300 °C to be evaporated at temperatures close to those of water. When the steam is distilled at a cooling temperature below 100 °C, the volatile compounds in the steam are also condensed into the liquid but insoluble in the water, thus, the obtained fluid is separated into two layers [20]. The lemongrass essential oil is yellow, hydrophobic in nature and lighter than water; therefore, it floats on the surface of the liquid [21]. Moreover, to obtain the maximum oil yield, it is necessary to determine the suitable extraction time, steam and energy requirement, ratio of raw material mass and water volume, origin and varieties of material, drying material method, and quality of supply water. These influencing factors have been investigated in some studies for different lemongrasses originating from some countries [1-2,5,8]. However, there has been a lack of reports of building a hydro-distillation procedure for the optimization of lemongrass essential oil yield.

Therefore, this study was aimed at investigating of main operational parameters (e.g., distillation time, material size, water source, and water-to-material ratio) affecting essential oil yield from lemongrass harvested in Bac Giang and Phu Yen provinces of Vietnam. A protocol for essential oil production from lemongrass on an industrial scale was proposed based on the obtained experimental data.

■ EXPERIMENTAL SECTION

Materials

Fresh lemongrass plants in this study were collected from two locations, one at a household in Quynh Do village, Bac Lung commune, Luc Nam district, Bac Giang province and another at BB farm, Van Hoa highland, Son Hoa district, Phu Yen province, Vietnam. They were both harvested in the summer (June to July) when the weather was hot and dry. They were dried naturally in the open air under shade at 30–35 °C for about 2–3 d. The used parts of the plant were stems, leaves, and the whole plant air dried, washed and then cut into 1–2 cm or 10–20 mm pieces with clean scissors/stainless steel knife, then ground into smaller pieces of 1–2 mm size by a blender.

Instrumentation

The experimental setup is shown in Fig. 1. The equipment is a hydro-distillation unit (Model TX05-02, China) with a maximum capacity of 10 L. The unit

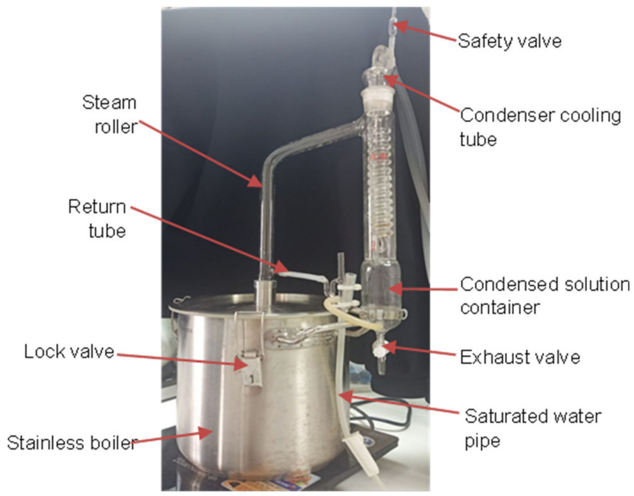

Fig 1. A 10 L hydro-distillation unit (Model TX05-02, China)

consists of a 304 stainless steel boiler with lock valves to create steam pressure during hydro-distillation.

The raw materials and water are loaded into the boiler so that the liquid and material should not be above the maximum line of 10 L. The boiler is attached to a glass steam roller, which is then connected to a glass condenser welding tube. A glass condensed container is linked at the end of the tube with an exhaust valve to remove the liquid. A separating glass funnel is used for the separation of essential oil and water layers.

Procedure

Protocol of essential oil production from C. citratus *(DC.) Stapf by hydro-distillation*

The procedure of lemongrass oil production by hydro-distillation on a lab scale is depicted in Fig. 2. Firstly, the fresh plant material was prepared by shade drying for 2–3 d after harvesting and balancing the mass. The leaves and/or stems of lemongrass were then washed, ground, or chopped into small pieces as possible. Next, 1 kg of lemongrass was loaded into the boiler with 5 L of clean water (the optimal ratio of water to raw material of 5/1, mL/g). Then, the induction or electric heater was turned on to increase the water temperature, and to create heat and pressure in the pot. When the water was boiled at 100 °C after 15 min, the medium heat mode was set and continued so that the steam evaporated and condensed by circulating cooling water continuously for 180 min. In each hour, the condensed water and essential oil extract were taken from the glass condenser and added in a 500 mL funnel for oil separation. After the layer of yellow essential oil and water were completely separated, the lemongrass essential oil was lighter than water and floated on the surface, opening the valve of the funnel to drain the water volume in the lower layer. The above yellow extracted essential oil was then collected, weighed, and the volume of the crude oil was measured. The crude oil was mixed with anhydrous Na_2SO_4 as a drying agent and then filtered to obtain the pure oil for extra analysis. Finally, the lemongrass essential oil was stored in a dark glass with a tight lid and kept in the refrigerator at 4 °C.

Effect of distillation time on oil yield. In this experiment, 1 kg of lemongrass separated into leaves and stems originating from Bac Giang province were ground as possible to about 2–3 mm pieces and added in 5 L volume of distilled water. During 4 h, the water inlet for the condenser is kept at a temperature of 30–35 °C. The volume of essential oil and the condensed water were measured after each hour. As a result, the optimized extraction time for lemongrass essential oil in the 10 L hydro-distillation unit was determined and selected to be set up in the later experiments.

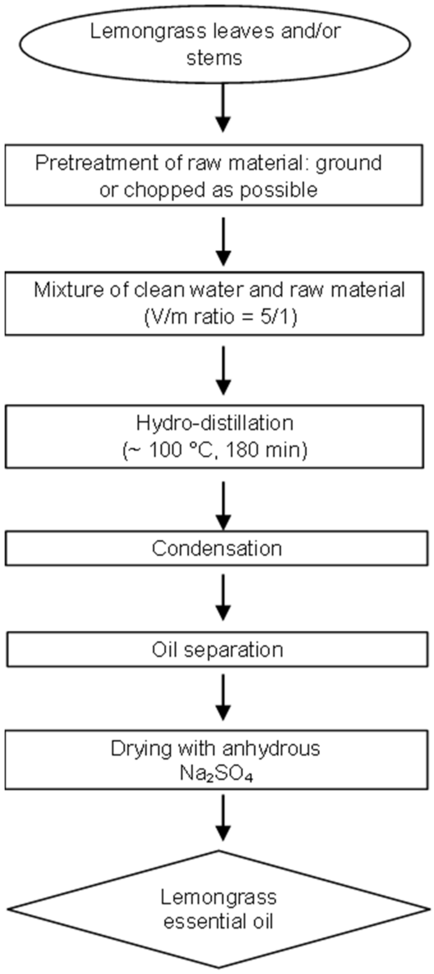

Fig 2. The procedure of lemongrass essential oil production by hydro-distillation

Effect of material size on oil yield. The target of this experiment was to choose a suitable size of raw material for optimal essential oil extraction. The lemongrass stem and leaves in Bac Giang province were divided, and each part of the plant was chopped into 10–20 mm in size and the others 2–3 mm in size. The weight of input material per batch was 1 kg, and the volume of distilled water was 4 L. The distillation time lasted for 3 h.

Effect of water quality on oil yield. Three supply water sources in this experiment were investigated, including reverse osmosis (RO) water as clean water, distilled water, and groundwater with a certain turbidity and yellow color as unclean water. The whole plant of lemongrass in Bac Giang province was 1 kg with a size of 2–3 mm, the distilled water was 4 L, and the distillation time was 3 h.

Effect of water-to-material ratio on oil yield. Similarly, 1 kg of lemongrass plant with the size of 2–3 mm in Bac Giang province was prepared. The investigated ratios of distilled water and raw material per batch were 3/1, 4/1, 5/1, and 7/1 (V/m, mL/g). The distillation time was 3 h.

Effect of plant origin on oil yield. The whole plants (stems and leaves) with an input mass of 1 kg per batch collected from two different locations, including a household in Bac Giang province and BB farm in Phu Yen province of Vietnam, were prepared in this experiment. The V/m ratio of distilled water and plant material was chosen to be 5/1 (mL/g), and the distillation process in 180 min. In all experiments, the yellow essential oil was lighter than water and floated on the surface of the fluid, thus, crude oil was easily separated, filtered, and dried by anhydrous Na_2SO_4 to obtain pure lemongrass essential oil.

Analysis

Determination of oil yield. Essential oils were directly measured in the burette. The oil yield ratio was determined by the volumetric method and expressed as mL of oil harvested per kg of plant materials used for each experiment (mL/kg).

GC-MS conditions for chemical composition analysis of essential oil. A GC-MS instrument (Nexis GC-2030, MS 2020 - Shimadzu, Japan) equipped with a SH-Rxi-5Sil MS model column (30 m long, 0.25 mm id and 0.25 µm film thickness) with helium carrier gas with a flow rate of 1.0 mL/min was used to determine the chemical composition of the essential oil. Extract samples were prepared for analysis on GC/MS by taking a volume of 10 µL, mixing in 1 mL of methanol and vortexed for 15 min. Mass spectra were taken at 70 eV. The temperature programming of the oven included an initial hold at 50 °C for 2 min at 20 °C min^{-1} and a rise to 100 °C for 2 min at 4 °C min^{-1} followed by the additional rise to 240 °C for 1 min at 15 °C min^{-1}. A final rise to 280 °C held for 5 min was allowed for a complete column clean-up. The injector was set at 250 °C with a sample volume of 2.0 µL. The prepared samples were injected into the GC with the injector in the split mode ratio of 1/25. MS quadrupole temperature was set at 150 °C and MS source at 230 °C. The analysis mode was a full scan that identified the compounds of the essential oils by both their Kovats indices and NIST/EPA/NIH mass spectral database (NIST14).

Statistical analysis. Experiments were carried out in duplicate, and results were expressed as average ± standard deviation (SD). Statistical analysis using paired t-test (two samples for means) yielding p-values less than 0.05 was declared as significant. The analysis data was collected using the software package Excel 2016 (Microsoft, Washington, USA).

■ RESULTS AND DISCUSSION

Effect of Distillation Time

The purpose of this experiment was to study the yield of essential oil from lemongrass stems and leaves obtained with the extraction time each hour or after 60 min. The volume of the extracted oil was measured, as shown in Fig. 3.

The highest yield of essential oil recorded at the first hour or 60 min was 2.15 and 2.30 mL/kg of lemongrass stems and leaves, respectively, which accounted for over 92% of the total obtained essential oil yield. After 60 min, the yield was sharply reduced with time. In particular,

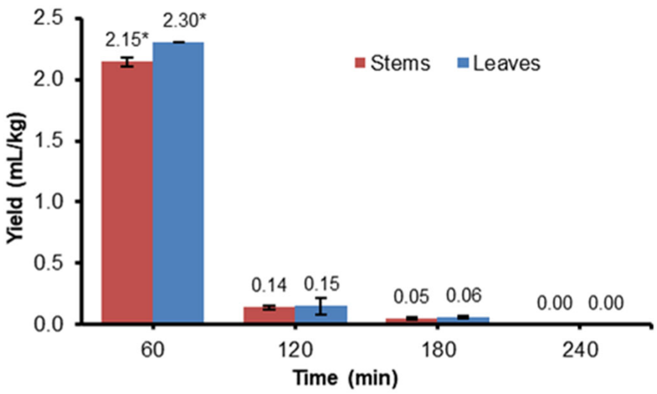

Fig 3. Effect of distillation time on lemongrass essential oil yield obtained in each hour

the oil yields extracted from both stem and leaf parts were insignificantly obtained, about 0.15 and 0.05 mL/kg, corresponding to 120 and 180 min, respectively. Notably, in the 4th hour or in the period of 180–240 min the condensed essential oil on the surface of the liquid container has not been seen, thus, no essential oil yield was obtained. The results revealed that the lemongrass essential oil was almost extracted in 60 min and finished the extraction process in a total of 180 min. It is unnecessary to prolong the distillation time to save energy and time. The distillation time of 180 min in this study agreed with the results achieved by others in a lab-scale production [1,22]. Distillation time may be prolonged to 240–300 min due to the production in a pilot or plant scale with hundreds kg of raw material [4,6]. In all, the optimal time for maximum essential oil yield obtained by hydro-distillation in a lab scale was 180 min which was selected to conduct the next experiments.

Effect of Material Size

In this experiment, both leaf and stem parts of lemongrass were studied with both 10–20 mm and 2–3 mm sizes, which were supposed to influence the essential oil yield as expressed in Fig. 4. It was shown in Fig. 4 that the smaller size of lemongrass for both leaves and stems produced more yield of essential oil. The yields of essential oil from leaves and stems at 2–3 mm size were 2.47 and 1.95 mL/kg, respectively, which were statistically significantly higher than those of 2.1 and 1.63 mL/kg at the size of 10–20 mm (p-values of 0.034 and 0.040 < 0.05). Additionally, the yields obtained from lemongrass leaves were higher than that of the plant stem. In agreement with the study of Akhihiero et al. [23], it was reported that the smallest particle size of 4 mm produced the largest amount of essential oil while the 15 mm size produced the smallest yield. When the size of the material is reduced, the more surface area is in contact with water, the more accessible it is for the oil molecules to escape from plant tissues into the steam, getting a higher yield of essential oil. As a result, the lemongrass plant used in the next experiments should be ground into small pieces of 2–3 mm to obtain the highest amount of essential oil.

Effect of Water Quality

It was found that the quality of water sources affected the essential oil yield on both sides, negatively or positively. Therefore, three supply water sources with different qualities were chosen to study the essential oil recovery efficiency. As shown in Fig. 5, the clean water, such as distilled water and treated water by RO system resulted in insignificantly different yields of lemongrass essential oil as 1.95 and 2.10 mL/kg, respectively (with p-value of $0.066 > 0.05$). However, by distilling in groundwater with high turbidity, and light-yellow color, it was about 1.63 mL/kg, which was remarkably lower than that of the distilled water or RO water with the p-values of 0.040 or 0.048, respectively (p-values < 0.05).

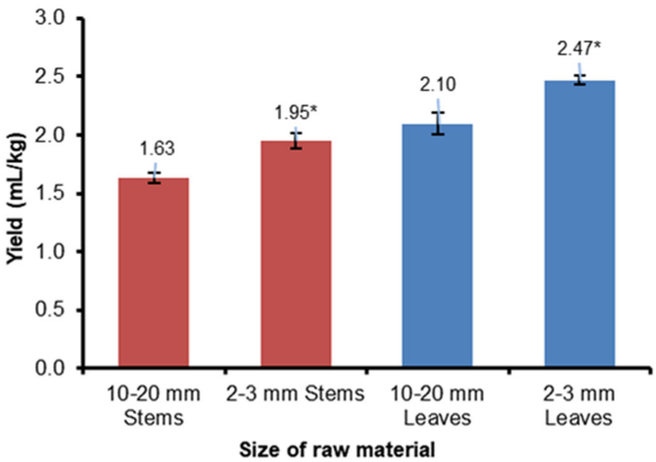

Fig 4. Effect of size of raw material parts on lemongrass essential oil yield

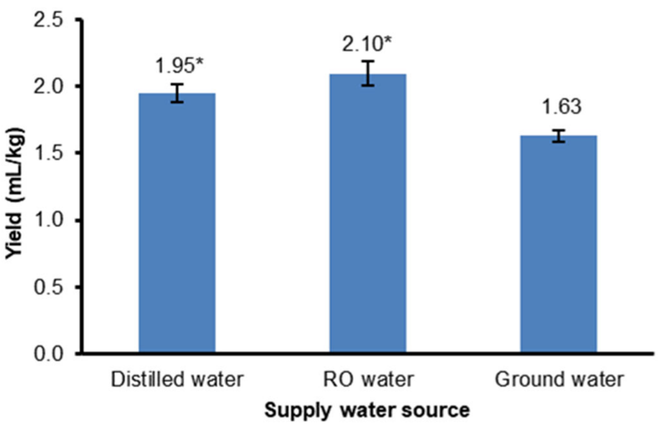

Fig 5. Effect of supply water quality on lemongrass essential oil yield

It was explained that the groundwater with high turbidity and yellow might contain ions, impurities, and residues interfering with the release of essential oil molecules during distillation. This affecting factor has not been reported in the literature before. Therefore, it is necessary to use clean water for the hydro-distillation of lemongrass in order to achieve high essential oil extraction efficiency.

Effect of Material-to-Water Ratio

In this experiment, the tested V/m ratios of water volume to material mass were 3/1, 4/1, 5/1 and 7/1. The result is shown in Fig. 6. It was revealed that the V/m ratio of water volume to raw material mass strongly affected the lemongrass oil yields. After 180 min of hydro-distillation, the highest yield of lemongrass whole plant (2.55 mL/kg) was obtained with the V/m ratio of 5/1 where the equivalent distilled water volume was 5 L and the raw material mass was 1 kg per batch. When the distilled water volume was smaller than 5 L, such as 4 and 3 L, the essential oil yield was reduced to 1.95 and 1.63 mL/kg, respectively. On the other hand, when the water volume increased to 7 L, the oil yield was also not increased and even reduced to 2.1 mL/kg. Illustratively, the condensed water volume measured after each hour was about 750–800 mL/h, after 180 min of hydro-distillation, the remaining water volume in the boiler with the input water of 3 and 4 L was almost exhausted to 700 mL led to the limitation of steam volume. While the input water volume was too much in this study 7 L, the remaining water volume in the boiler after 180 min was rather high, about 2 L. The significantly higher water volume in the boiler wasted of more time and energy to create vapor, and the condensed oil amount obtained from the steam was slightly smaller and required more time to obtain the maximum oil yield. In this study, the optimal V/m ratio of water and plant material was chosen to be 5/1 (L/kg or mL/g) to get the maximum yield of lemongrass essential oil.

Effect of Origin of Raw Material

To evaluate the effect of raw material origin on the essential oil yield, the whole plants were taken from two different provinces of Vietnam, Bac Giang and Phu Yen, the data is shown in Fig. 7.

It was obtained about 3.96 mL/kg of essential oil for the plant from Phu Yen province, which was statistically significantly higher than that of Bac Giang province with 2.55 mL/kg (p-value of $0.034 < 0.05$). The lemongrass was grown naturally by a household in Bac Giang province, while it was grown with a careful cultivation regime and breed selection by BB farm in a highland of Phu Yen province. This means that the origin and cultivation of plant materials were strongly impacted by the essential oil yields.

Data shown in Fig. 8 are chromatographic peaks of bioactive compounds detected in lemongrass essential oils from Bac Giang and Phu Yen provinces of Vietnam by GC-MS. It is presented in Table 1 about the mass compositions of bioactive compounds in the plant

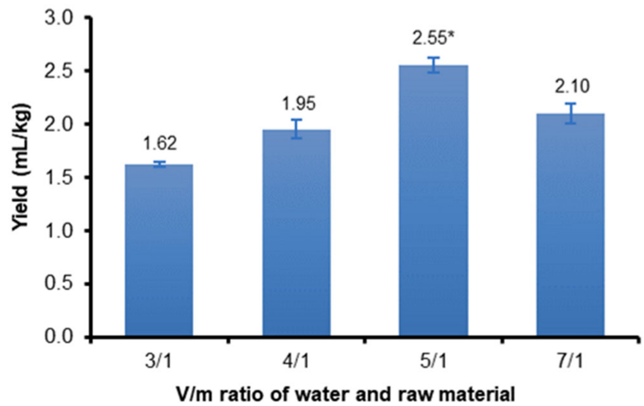

Fig 6. Effect of V/m ratio of water volume to raw material mass on lemongrass essential oil yield

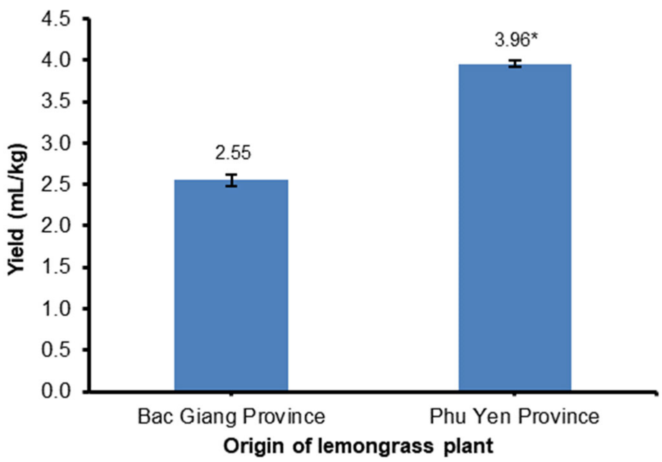

Fig 7. Effect of origin of raw material on lemongrass essential oil yield

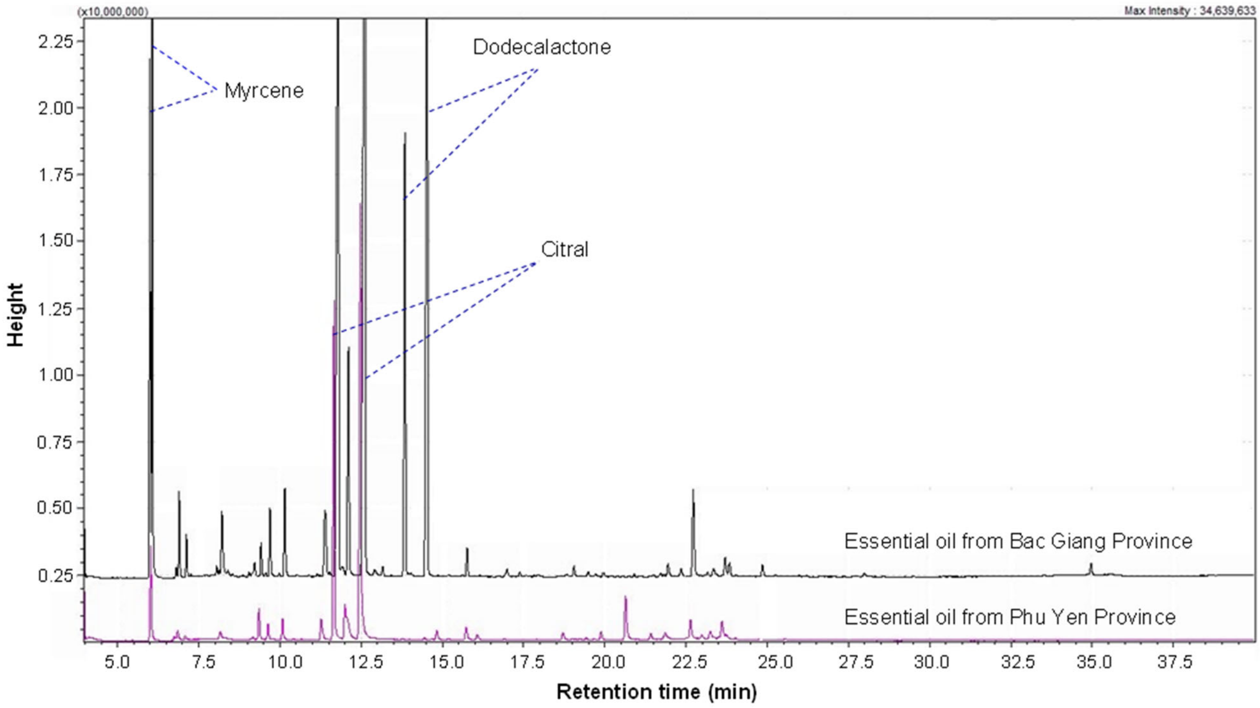

Fig 8. Chromatographic peaks of lemongrass essential oils originated from Bac Giang and Phu Yen provinces of Vietnam

Table 1. Bioactive compounds of lemongrass essential oils analyzed by GC-MS

No.	Real-time	Bioactive compound	Bac Giang Province	Phu Yen Province
			Composition (%)	Composition (%)
1	5.20	Pinene	0.00	0.07
2	5.50	Camphene	0.00	0.08
3	5.90	Sulcatone	6.14	0.00
4	6.00	Myrcene	11.44	6.33
5	6.80	Limonen	0.14	0.27
6	6.90	Ocimene (Pinene)	1.12	0.91
7	7.20	Ocimene (Pinene)	0.59	0.37
8	8.20	Linalood	1.24	0.71
9	9.40	Citronellal	0.55	2.32
10	9.70	Isoneral	1.10	1.05
11	10.10	Isogeranial	1.50	1.50
12	11.30	Citronellol	1.80	2.05
13	11.70	Citral	19.76	26.32
14	12.10	Geraniol	4.21	6.43
15	12.60	Citral	23.16	39.23
16	13.80	Dodecalactone	7.86	0.00
17	14.50	Dodecalactone	15.56	0.00
18	14.80	Citronellyl acetate	0.00	0.84
19	15.70	Geranyl acetate	0.50	1.25
20	16.00	Farnesol	0.00	0.46
21	17.00	Caryophyllene	0.21	0.00

No.	Real-time	Bioactive compound	Bac Giang Province	Phu Yen Province
			Composition (%)	Composition (%)
22	17.40	α-Bergamotene	0.08	0.00
23	18.70	Germacren	0.00	0.76
24	19.40	Himachalene	0.00	0.00
25	19.70	γ-Muurolene	0.00	0.81
26	19.90	Cadinene	0.09	0.00
27	20.60	Elemol	0.00	4.55
28	22.70	Selin-6-en-4α-ol	1.94	2.12
29	23.20	Guaiol	0.00	1.00
30	23.70	Cadinol	0.48	0.57
31	23.90	Intermedeol	0.29	0.00
32	24.80	Juniper camphor	0.26	0.00
		Total	100.00	100.00

Table 2. Lemongrass oil essential yield and total citral content by steam and/or hydro distillation method with different conditions and origins

Origin of the lemongrass plant	Distillation method and equipment	Distillation conditions	Essential oil yield (mL/kg)	Total citral (%)	Ref.
Jammu, India	Steam distillation, plant scale, capacity: 1000 kg	Raw material mass: 100 kg, whole plant size: chopped, 300 min	4.7–5.5	80–85	[4]
Zaria, Nigeria	Steam distillation, pilot plant, capacity: 100 kg/day	Ratio of water and raw material: 25 L/20 kg, leaf size: 2–3 mm, 90 min	NA	40.5	[5]
Burji Fatih, Khartoum city, Sudan	Hydro-distillation using a Clevenger apparatus	Water volume: 200 mL, leaf size: crushed as possible, 240 min	NA	65.5	[6]
Quy Hop district, Nghe An province, Vietnam	Steam distillation, unit volume 180 L, capacity: 25–30 kg of material per batch	Raw material mass: 25–30 kg, ratio of water and raw material: 5.66 L/kg, whole plant size: 10 mm, 180 min	2.98	61.6	[22]
Thanh Oai district, Hanoi city, Vietnam	Steam distillation, unit capacity: 15 kg of material per batch	Raw material mass: 8.5–9 kg, stem size: 20 mm, 180 min	2.1–2.9	70.5	[1]
Mekong Delta provinces of Vietnam	Hydro-distillation using a Clevenger apparatus	Raw material: 100 g, water volume: 400 mL, leaf size: 20 mm, 360 min	0.2% (v/w)	83.9	[8]
Bac Giang province, Vietnam	Hydro-distillation, unit volume: 10 L	Ratio of water and raw material: 5 L/kg, whole plant size: 2–3 mm, 180 min	2.55	45.5	This study
Phu Yen province, Vietnam	Hydro-distillation, volume: 10 L	Ratio of water and raw material: 5 L/kg, whole plant size: 2-3 mm, 180 min	3.96	68.1	This study

essential oils from both locations. In general, the biochemical compositions of essential oils from both provinces were almost similar, with 24–29 substances found, in which total citral consisting of the isogeranial, isoneral, and citral, the most typical bioactive substance with the highest mass percentage of 45.5 and 68.1% from

Bac Giang and Phu Yen provinces, respectively. Despite the total citral content in the plant from Phu Yen province being significantly higher than that in Bac Giang province, there were more bioactive substances such as dodecalactone and sulcatone found in the plant from Bac Giang province. According to the citral content requirements of TCVN 11425:2016 (ISO 3217:2016) in the range of 60–85% [24], the essential oil from the farm in Phu Yen met the Vietnam national standard of lemongrass oil. In Table 2, it was shown that the oil yields of Vietnam lemongrass distilled in small-scale equipment were in the same range of up to 4 mL/kg, which was smaller than that of India with a significantly higher plant scale [4]. However, the total citral content in this study, especially originated in Phu Yen province of Vietnam, was abundant, meeting the requirement [24]. The lemongrass essential oil with the high content of citral, its cis-isomer (isoneral), myrcene and other aldehydic compounds were confirmed to be a potentially valuable antifungal and anti-inflammatory agent for the prevention and treatment of acute inflammatory skin conditions, meat broiler preservation [6-7,25]. In all, the lemongrass in Việtnam with a good cultivar and cultivation regime, and optimal hydro-distillation conditions will provide high quality and yield of essential oil.

■ CONCLUSION

In this study, the raw material of lemongrass should be ground into small pieces as possible to obtain a better yield of essential oil. The optimal distillation time for lemongrass oil extraction in a 10 L hydro-distillation unit was 180 min, ratio of clean water volume to raw material mass was 5 L/kg. For the first time, the supply water quality was reportedly evaluated to have effects on the essential oil yield. It was also confirmed that plants with good cultivation and breed selection resulted in better oil yield and quality. The yields of lemongrass essential oil from two locations, Bac Giang and Phu Yen provinces of Vietnam, were 2.55 and 3.96 mL/kg, respectively. The total citral content with the highest bioactive compounds in the lemongrass oil of Vietnam analyzed by GC-MS was 45.5 and 68.1%, equivalent to Bac Giang and Phu Yen provinces, meeting the requirement of citral according to TCVN 11425:2016 (ISO 3217:2016) in the range of 60–85%. As a result of the influential factors, the protocol of lemongrass essential oil production on a lab scale by hydro-distillation was concluded to optimize the oil yield from the material originating from Vietnam. This is also confirmed that lemongrasses in Vietnam with good cultivation and breed selection, give high quality and yield of essential oil, providing an abundant citral source for many applications in the food industry, pharmaceuticals, cosmetics, and perfumery markets.

■ ACKNOWLEDGMENTS

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■ CONFLICT OF INTEREST

Nothing declared.

■ AUTHOR CONTRIBUTIONS

Thi Cuong Vu conducted the experiments, Thi Cam Van Do processed data, wrote and revised the manuscript. All authors agreed to the final version of this manuscript.

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