Chemical composition, antibacterial and antioxidant activities of lemongrass (*Cymbopogon citratus*) essential oil and its fractions obtained by vacuum distillation

D N Do¹*, H T T Nguyen¹, T H Huynh², N P Nguyen² and X C Luu³

¹ Faculty of Environmental and Food Engineering, Nguyen Tat Thanh University, Ho Chi Minh City, Viet Nam.
² Institute of Environmental Technology, Vietnam Academy of Science and Technology, Cau Giay Dist., Hanoi, Vietnam.
³ Faculty of Drug, Cosmetic and Food, Dong An Polytechnic, Di an City, Binh Duong Province, Vietnam.

*Corresponding author: ddnhat@ntt.edu.vn

Abstract. Lemongrass (*Cymbopogon citratus*) essential oil (raw oil) is a natural product with many biological activities and is commonly used in the food and cosmetic industries. In this study, fractions of lemongrass essential oil were segregated from raw oil by vacuum fractionation. These fractions were then examined for their constitution, antioxidant and antimicrobial activities. Gas chromatography-mass spectrometry results indicated a difference in constitution and content of compounds among the fractions and raw oil. β-myrcene was the main ingredient in two fractions, F1 and F2, with content 2-4 times higher than the original content in raw oil. At the same time, F4 was the primary fraction for citral recovery, with the highest citral content of 83.53%. Fractions F3, F4, and undistilled oil (Unoil) showed better antioxidation than raw essential oil. On the other hand, the antimicrobial experiments indicated that F1, F2, and F4 had the highest activity in both cases against Staphylococcus aureus and Escherichia coli bacteria. These outcomes could increase the applicability of lemongrass essential oil in many fields, especially in medical and food applications.

1. Introduction

In recent years, consumers have become increasingly interested in natural and eco-friendly products, and they are increasingly looking for healthier and safer products [1]. Pharmaceutical companies are increasingly interested in natural sources of materials to replace synthetic compounds gradually [2]. In the food industry, synthetic additives and preservatives are increasingly limited, so there is a tendency to use additives and antibacterial substances derived from nature that is safe for health growing healthy [3]. In natural alternatives, essential oil is a potential alternative source.

Lemongrass (*Cymbopogon citratus*) is a herb mainly planted in subtropical and tropical areas. The lemongrass essential oil is commonly applied to produce flavors, detergents, cosmetics, and pharmaceuticals. The investigation has indicated that diverse chemical composites of lemongrass oil present antioxidant, antimicrobial, antifungal, anti-inflammatory, antiparasitic, and insect-repellent activities. In the food sector, lemongrass oil is normally acknowledged as safe and can be applied as a replacement food additive to synthetic types [4]. Its significant antibacterial and antioxidant features can be used in food preservation as a substitute for synthetic components, meeting today's consumer needs.
The efficiency of the biological ability of essential oils is based mainly on the chemical constitution, which depends on many different factors such as the growing conditions, harvest time, and extraction method [5-6]. In addition to the attendance of components of essential oils, the content of each ingredient also plays a critical role in their biological activity. Investigators have shown that even minor components participate in the biological function of essential oil owing to the synergistic activity. Studying the bioactive ability of different essential oil fractions can create new applications and enhance the value of essential oils.

There was much attention to the bioactive ability of lemongrass essential oil for commercial applications in various fields. The purpose of the study was to fractionate lemongrass essential oil by vacuum fractionation. Essential oil fractions, which are obtained after distillation, were determined by their physical and chemical properties. The effects of the different chemical compounds in each fraction on their antimicrobial and antioxidant activity were determined.

2. Materials and Methods

2.1 Source of lemongrass essential oil
The crude lemongrass oil was produced by steam distillation in Phong Dien district, Thua Thien Hue province, Vietnam, in October 2020. It was supplied by Suwina Company, Vietnam. Chemicals used in the study were ethanol (Merck), sodium hydroxide, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich) analytical grade.

2.2 Essential oil fractionation
The feed section was a 250-mL flask containing 100mL of raw essential oil heated by an electrical heater and fractionated on a Vigreux column with heights of 300 mm. The vapor left the distillation column was then condensed by a Liebig condenser using the 5 °C water as a cooling stream through a chiller. Finally, the condensed liquid was collected in a multi-limb vacuum receiver and remaining in the flasks until the process finished. Fractions F1-F4 were collected, respectively, based on the difference in vapor temperature at the top of the column. The internal vacuum pressure of the whole system could be reduced up to 15 mmHg and was maintained owing to a vacuum pump connecting to the receiver. The fractionation was terminated when no vapor left the distillation column.

2.3 Essential oil component analysis
The chromatography was performed on an Agilent G1530A Gas Chromatography system. The system coupled with an HP-5MS column used He as a carrier gas with a 1 mL/min flow rate. An Agilent Mass Spectrum, Model 5973N MSD, was used as the system detector. The ionization energy was 70 eV, while the ionization source temperature of 220 °C and the mass scan ranged between 40 and 400 amu. The column temperature was initially set at 60 °C and increased at a rate of 10 °C/min up to 280 °C. The injection volume was 1,0 μL with a split ratio of 1:50. The scan regime in the GC/MS was 1 second per turn.

2.4 Antioxidant activity testing
The lemongrass essential oil's antioxidant capacity was estimated as described by Brand-Williams et al. [7] with some modifications. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical solution was performed with 3.9 mg of radical and 100 mL of ethanol, then stored at 4 °C for 24 hours. This solution was diluted with ethanol to get an absorbance of 1.000 ± 0.02 at 517 nm in a Shimadzu UV-1800 Spectrophotometer (Shimadzu Corp., Japan). Aliquots of 600 μL of each oil solution with ethanol were put into a cuvette, and 2.4 mL DPPH solution was added. The mix was shaken and left in the dark for 30 min at room temperature. After that, the absorbance was determined at 517 nm in the Shimadzu UV-1800.

% Inhibition = [(Ab-As)/Ab] x 100%

Ab refers to the absorbance of the control, and As refers to the absorbance of the test oil.
2.5 Antimicrobial activity testing

Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 25923 TM) were the bacterial strains used in this investigation. Fresh cultures of microorganisms were cultivated on MHB and were incubated at 37 °C for 24 h. Lemongrass essential oil and its fractions were tested for antibacterial activity by the disk diffusion technique [8]. A 100 µL bacterial culture was spread homogeneously onto the surface of the MHA plates. Filter paper disks with a diameter of 6 mm were loaded with 10 µL of each lemongrass essential oil fraction. After 18-24 h of incubation at 37 °C, the antibacterial activity was recognized by measuring the diameter (mm) of the inhibition zones.

3. Results and discussion

3.1 Composition of the raw lemongrass essential oil and its fractions

Fractions F1, F2, F3, F4, and Unoil were recovered during fractional vacuum distillation. Table 1 showed the composition of compounds presented in the crude lemongrass essential oil and the fractions produced after the separation process. The earliest components recovered at the top of the column were 5-hepten-2-one and β-myrcene. Most of the β-myrcene was recovered in the F1 and F2 fractions during distillation. The content of β-myrcene presented in these fractions is much higher than in the raw oil (from 12.15% in crude oil to 45.21, 26.53% at the F1, F2 fractions, respectively). With this high β-myrcene content, the F1, F2 fractions have potential applications in cosmetics and food. The most abundant compound in raw lemongrass oil was citral. After the separation process, citral concentrated mainly on the F3 and F4 fractions. In which F4 was the fraction with the highest citral content (83.53%). The rest of the essential oil in the flask after the fractional distillation (undistilled essential oil) contained less volatile constituents such as geranyl acetate (5.61%), trans-α-bergamotene (95.46%), m-camphorene (9.69%), p-camphorene (4.70%). In general, the differences in the composition of fractions (F1, F2, F3, F4, Unoil) compared with raw lemongrass essential oil could lead to differences in their bioavailability.

| Component                | Raw oil (wt%) | F1 (wt%) 50-57°C | F2 (wt%) 58-60°C | F3 (wt%) 61-98°C | F4 (wt%) 100-110°C | Unoil (wt%) |
|--------------------------|--------------|------------------|------------------|------------------|-------------------|-------------|
| 2-Pentanone              | 0.30         | 0.15             | -                | -                | -                 | -           |
| 5-Hepten-2-one           | 3.73         | 14.55            | 11.13            | 4.04             | 0.29              | -           |
| β-Myrcene                | 12.15        | 45.21            | 26.53            | 8.11             | 0.60              | -           |
| 2,3-Dehydro-1,8-cineole | 0.26         | 0.16             | -                | -                | -                 | -           |
| trans-β-Ocimene          | 1.23         | 5.83             | 3.58             | 1.01             | -                 | -           |
| β-cis-Ocimene            | 0.78         | 3.78             | 2.54             | -                | -                 | -           |
| Linalool                 | 1.36         | 2.32             | 3.84             | 3.48             | 0.93              | -           |
| Perillen                 | 0.54         | 1.85             | 1.89             | 0.65             | -                 | -           |
| Citronellal              | 0.69         | 1.40             | 2.92             | 2.36             | -                 | -           |
| Isoneral                 | 1.75         | 1.16             | 2.72             | 3.18             | 2.22              | -           |
| 3,7-Dimethyl-3,6-octadienal | 2.92     | 1.69             | 4.39             | 5.78             | 4.25              | 2.99        |
| 2-Cyclohexen-1-ol        | 0.28         | 0.13             | 0.17             | 0.43             | 0.31              | -           |
| β-Citral                 | 29.4         | 7.43             | 19.55            | 34.77            | 37.85             | 11.08       |
Geraniol & 3.49 & 0.52 & 0.95 & 2.61 & 4.17 & 3.56 \\
α-Citral & 33.85 & 6.36 & 15.41 & 29.91 & 45.68 & 33.79 \\
Geranyl acetate & 1.37 & 0.18 & 0.21 & 0.88 & 0.16 & 5.61 \\
Caryophyllene & 0.47 & 0.12 & 0.06 & 0.46 & 0.56 & 1.13 \\
trans-α-Bergamotene & 0.38 & - & - & - & - & 5.46 \\
Octadecane & 0.35 & - & - & - & 2.00 & 1.05 \\
Naphthalene & 0.149 & - & - & - & - & 1.29 \\
Columbin & 0.22 & - & - & - & - & 0.76 \\
Trichloroacetic acid & 0.22 & - & - & - & - & 0.62 \\
m-Camphorene & 0.35 & - & - & - & - & 9.69 \\
p-Camphorene & 0.18 & - & - & - & - & 4.70 \\
\*: Not detected.

3.2 Antioxidant activity

The results of antioxidant ability of raw lemongrass oils and their fractions with DPPH free radical scavenging experiment were indicated in Figure 1. There was a difference in percentage inhibition between raw lemongrass and fractions (F1, F2, F3, F4, Unoil) achieved after distillation. The results displayed that F3, F4, and Unoil had better antioxidant ability than the raw lemongrass essential oil. Meanwhile, F1, F2 showed much lower antioxidant activity than raw essential oil. Previous investigations have demonstrated that citral, the prime terpenoid component of lemongrass essential oil, has excellent antioxidant activity [12]. According to the chemical profile in Table 1, citral was present in a large percentage of the composition of fractions F3 (64.68%), F4 (83.53%), and Unoil (44.87%) fractions. In comparison, citral was present in small proportions in the F1 (13.79%) and F2 (34.96%) fractions. The F3 fraction showed the highest antioxidant activity (57.6%) even though the citral content was lower than the F4 fraction, which may be owing to the synergistic effect of all its constituents, according to some previous studies [13]. In the case of Unoil, although the citral content was not high (44.87%), its antioxidant activity was higher than that of raw essential oil; this may be due to the presence of phenolics compounds with high antioxidant activity such as m-camphorene, p-camphorene, geranyl acetate. In some other studies, this trend was also observed that the residues with higher phenolic content indicated higher antioxidant ability [14].
3.3 Antibacterial activity

The antibacterial ability of the lemongrass essential oil and fractions (F1, F2, F3, F4, Unoil) was investigated through preliminary assays of the disk diffusion experiment. The antimicrobial ability was examined against gram-positive bacteria (Staphylococcus aureus) and gram-negative bacteria (Escherichia coli). The results showed that raw lemongrass essential oil and F1, F2, F3, F4, and Unoil fractions all have antibacterial effects against both Staphylococcus aureus and Escherichia coli. Gram-positive organism (Staphylococcus aureus) with inhibition zones ranging from 10–38 mm was found more sensitive to lemongrass essential oil and its fractions as compared to the gram-negative organism (Escherichia coli) with inhibition zones ranging from 2–26 mm. The diameter of the inhibitory zone (Table 2) for Staphylococcus aureus is shown in the order F2> F1> F4> Raw oil> F3> Unoil. In case of Escherichia coli, diameter of inhibitory zone (Table 2) in order F2> F4> Raw oil F1> F3> Unoil. In general, F1 and F2 fractions had good antibacterial activity; this may be due to the presence of β-myrcene in high content in these fractions; some studies have recognized that β-Myrcene enhances the antibacterial effect when combined with citral [15]. The F4 fraction with the highest citral content showed better antibacterial activity when compared to the raw lemongrass essential oil, which was consistent with the good antimicrobial role of citral in several previous studies [16]. For the residue after distillation, the least volatile fraction showed a lower antibacterial effect than the raw essential oil with both Staphylococcus aureus and Escherichia coli organisms. In summary, lemongrass essential oil and its fractions after the distillation had shown different antibacterial against both gram-negative and positive-gram bacteria. The positive-gram effect is better than compared to gram-negative organisms. The antibacterial ability of lemongrass essential oil and its fractions depended on three prime compounds: α-citral, β-citral, and β-myrcene. This was consistent with some previous reports [17].

| Oil Fraction | Diameter of Inhibition Zone (mm) |
|--------------|----------------------------------|
|              | Staphylococcus aureus | Escherichia coli |
| Raw oil      | 24.33 ± 0.36             | 12.05 ± 0.41     |

**Figure 1.** Antioxidant activity (% inhibition of lemongrass essential oil and its fractions)
4. Conclusions

Lemongrass essential oil (raw oil), its fractions (F1, F2, F3, F4), and the undistilled oil (Unoil) obtained by the fractional distillation showed differences in composition content as well as in their biological activities. This result showed that the vacuum distillation method was appropriate for producing different essential oil fractions, having better biological activities than the raw essential oil. Fractions F3, F4, and Unoil indicated better antioxidation than raw essential oil. On the other hand, fractions F1, F2, and F4 indicated better antibacterial ability than the original raw essential oil. These properties were related to the presence of various compounds in the essential oil composition. The presence of three main components α-citral, β-citral, and β-myrcene played an important role. These results contributed to the increased applicability of lemongrass essential oil. Fractions with high antimicrobial ability have the potential for medical applications as natural antimicrobial agents. The fractions can be used for their antioxidant ability in food applications, such as natural preservatives.

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References

[1] Barbarossa C and De Pelsmacker P 2016 J. Bus. Ethics 134(2) 229-247
[2] Atanasov A G, Waltenberger B, Pferschy-Wenzig E-M, Linder T, Wawrosch C, Uhrin P, Temml V, Wang L, Schwaiger S and Heiss E H 2015 Biotechnol. Adv. 33(8) 1582-1614
[3] Bondi M, Lauková A, de Niederhausern S, Messi P and Papadopoulou C 2017 Natural preservatives to improve food quality and safety Hindawi
[4] Hylgaard M, Mygind T and Meyer R L 2012 Front. microbiol. 3 12
[5] Turek C, Stintzing F C 2013 Compr. Rev. Food Sci. Food Saf. 12(1) 40-53
[6] Bakkali F, Averbeck S, Averbeck D, Idaomar M 2008 Food Chem. Toxicol. 46(2) 446-475
[7] Brand-Williams W, Cuvelier M-E, Berset C 1995 LWT - Food Sci Technol 28(1) 25-30
[8] Ha C T, Thai T H, Hien N T, Anh H T, Diep L N, Thuy D T, Nhat D D and Setzer W N 2019 Nat. Prod. Commun. 14(6) 1934578X19860370
[9] Falcao M A, Fianco A L, Lucas A M, Pereira M A, Torres F C, Vargas R M and Cassel E 2012 Phytochem Rev 11(4) 405-412
[10] Kpoviessi S, Ber J, Agban i P, Gbaguidi F, Kepadonou-Kpoviessi B, Sinsin B, Accrombessi G, Frédéric M, Moudachirou M and Quetin-Leclercq J 2014 J. Ethnopharmacol. 151(1) 652-659
[11] Verma R K, Verma R S, Chauhan A and Bisht A 2015 J. Essent. Oil Res. 27(3) 197-203
[12] Baschieri A, Ajvazi M D, Tonfack J L F, Valgimigli L and Amorati R 2017 Food Chem. 232 656-663
[13] Naik M I, Fonday A B, Jaykumar E and Bhat J A 2010 Asian Pac J Trop Dis 3(7) 535-538
[14] Olmedo R, Nepote V and Grosso N R 2014 Food Chem 156 212-219
[15] Premathilake U, Wathugala D and Dharmadasa R 2018 *Int. J. Minor Fruits, Med. Aromatic Plants* **4** 13-19
[16] Tyagi A K, Malik A 2012 *Innov Food Sci Emerg Technol* **13** 169-177
[17] Onawunmi G O, Yisak W-A and Ogunlana E 1984 *J. Ethnopharmacol.* **12**(3) 279-286