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Analysis of Volatile Organic Compounds, Antioxidant, Tyrosinase Inhibitory, and Antimicrobial Activities of Essential Oils from Citronella Grass and Kaffir Lime

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Abstract

This research investigates the volatile organic compounds (VOCs), antioxidant properties, and antimicrobial effects of essential oils from citronella grass and kaffir lime, aiming to assess their potential as natural alternatives to synthetic chemicals in community products. Essential oils were extracted using a prototype distiller, yielding 1.67 % (v/w) from citronella grass and 2.36 % (v/w) from kaffir lime. The chemical compositions were analyzed using gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR). Antioxidant activities were assessed through assays for 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH$^{\bullet}$), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid radicals ($ABTS^{\bullet+}$), and ferric reducing antioxidant power (FRAP). Tyrosinase inhibition and antimicrobial activity were evaluated, with molecular docking studies supporting the findings. Major components in citronella grass oil included geraniol (35.99 % w/w), geranyl acetate (10.73 % w/w), α-citral (7.34 % w/w), and β-citral (5.72 % w/w), while kaffir lime oil contained D-limonene (20.72 % w/w) and (-)-β-pinene (20.19 % w/w). Antioxidant assays revealed lower free radical scavenging activities for both oils compared to Trolox, attributed to their non-polar nature. Citronella grass oil exhibited superior free radical scavenging, due to its molecular structure and electron-donating groups. Tyrosinase inhibition assays showed kaffir lime oil was more effective (IC_{50} = 195.68 µg/mL) than citronella grass oil (IC_{50} = 380.45 µg/mL), likely due to smaller, ring-structured molecules. These observations are supported by molecular docking studies. Antibacterial tests demonstrated higher potency of citronella grass oil over kaffir lime oil and clindamycin, with its smaller molecular size enhancing bacterial cell wall interactions. Stability tests revealed that citronella grass oil was initially more effective against *Staphylococcus aureus* but its activity declined by 45 % on the 2nd day, while still outperforming kaffir lime oil. Essential oils with smaller, simpler molecules provide rapid action but may lose effectiveness over time, whereas complex molecules offer stable, long-term inhibition. Both citronella grass and kaffir lime oils show potential as natural alternatives to synthetic chemicals and can be utilized in products such as soaps, creams, lotions, and disinfectants.

Keywords: Essential oils, Citronella grass, Kaffir lime, Antioxidants, Tyrosinase inhibition, Antimicrobial activities

Introduction

Thailand is one of the most biodiverse countries due to its tropical location just above the equator and its proximity to the sea. These topographic conditions are ideal for the growth of a wide variety of plants, each containing important compounds with numerous benefits [1,2]. One of the important compounds in plants that is widely used is essential oils. These oils have beneficial properties and are utilized in various ways, such as in medicine, food ingredients or additives, and cosmeceuticals, among others. Essential oils are composed of various complex volatile compounds derived from different parts of plants, containing both major and minor constituents. They are used as natural plant protectants due to their pesticide properties, low toxicity to mammals, and easy biodegradability. Essential oils exhibit a range of biological activities, including fragrance, antiseptic, antioxidant, anticancer, antiprotozoal, anti-inflammatory, immunomodulatory, and antimicrobial properties. They are also utilized in the treatment of neurodegenerative diseases, diabetes, and hyperpigmentation [3-7].

Sakon Nakhon is a province located in the upper northeastern region of Thailand. It is rich in biodiversity, surrounded by mountain ranges to the west and south. The topography consists of uneven, undulating plains with natural water sources and abundant forest resources. Most of the area is dedicated to agriculture. Based on this information, Sakon Nakhon province is a significant source of herbs, encompassing both naturally occurring varieties and those cultivated as economic crops. Citronella grass and kaffir lime are considered medicinal plants commonly found in local communities in Sakon Nakhon province. These plants are easy to grow, grow quickly, and require minimal maintenance. Both contain important compounds, namely essential oils, which help deter pests. Citronella grass (*Cymbopogon nardus*) belongs to the same family as lemongrass but has a stronger pungent aroma. The benefits of citronella grass are numerous: It is commonly used to extract essential oils, boil for

drinking water, make incense, and create mosquito repellent spray. It is also effective in preventing and eliminating insect pests. Additionally, it has properties that help expel wind, relieve bloating and flatulence, aid digestion and appetite, and prevent and treat oral diseases. Moreover, it inhibits bacteria, fungi, and some viruses [3,8-10].

Kaffir lime (*Citrus hystrix* DC.) is a small perennial plant with hard wood. Its trunk and branches have slightly long thorns. The dark green leaves have a smooth surface and are quite thick, emitting a pleasant aroma due to the presence of oil glands. The fruit is dark green, resembling a lemon with a rough outer surface dotted with oil glands (hesperidium). When young, the fruit is dark green; it turns bright yellow when ripe and contains many seeds. Kaffir lime can be used for consumption and as an external medicine. Its properties include: Kaffir lime skin acts as a carminative in the intestines, relieves congestion, aids menstruation, expels gas, and serves as a heart tonic. The fruit has a sour taste that helps expel phlegm, fix sticky saliva, neutralize gas in the intestines, counteract mismanifested poison, cure bloating, stimulate appetite, relieve headaches, relieve colic, treat scurvy, and the oil from the skin helps prevent dandruff and makes hair black and shiny [4,6,7,11-13]. In addition, essential oil from bergamot has been found to exhibit antibacterial, antioxidant, anticancer, and anti-inflammatory activities [4,11,12].

Essential oils from medicinal plants have garnered significant attention due to their diverse applications in pharmaceuticals, cosmetics, and traditional medicine. Among these, citronella grass and kaffir lime are known for their bioactive properties, including antioxidant, antimicrobial, and enzyme inhibitory activities. VOCs play a crucial role in defining the biological activities of these oils, with their chemical composition directly influencing their efficacy. Similarly, antioxidants are essential for neutralizing free radicals, which can prevent oxidative stress-related damage, while tyrosinase inhibitors are vital for controlling hyperpigmentation disorders. Antimicrobial properties further expand their potential for applications in disinfectants and preservation. Previous studies have analyzed the VOC profiles of various essential oils and their biological activities. However, differences in extraction methods, plant varieties, and environmental factors often lead to variability in results. This research utilizes a prototype essential oil distiller, an advanced steam distillation apparatus capable of processing over 50 kg of plant material per batch, to ensure sufficient

yields for comprehensive analysis and comparison. Such scalable technology bridges the gap between laboratory studies and commercial production, providing a foundation for local economic development.

Therefore, this research focuses on analyzing the VOCs, antioxidant properties, tyrosinase inhibitory activities, and antimicrobial effects of essential oils from citronella grass and kaffir lime. Advanced techniques, including *in vitro* assays and molecular docking simulations, will be employed to investigate their bioactivities and chemical compositions. The findings will be compared with existing literature to highlight the unique properties of these oils. The aim of this research is to evaluate the bioactive properties of essential oils from citronella grass and kaffir lime, extracted using a prototype essential oil distiller. The goal is to add value to local medicinal plants, with the resulting information contributing to the development of traditional medicines and cosmeceutical products by community enterprise groups in Sakon Nakhon province.

Materials and methods

Materials and reagents

All chemicals used in this work, namely (±)-6-Hydroxy-2,5,7,8-tetramethyl-chromane-2-carboxylic acid (Trolox), ascorbic acid, tyrosinase enzyme from mushrooms, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), potassium persulfate, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), hydrochloric acid, iron (III) chloride hexahydrate, sodium acetate, acetic acid, disodium hydrogen phosphate, sodium hydrogen phosphate, dimethyl sulfoxide (DMSO), and l-dihydroxyphenylalanine, ethanol, were of analytical grade (AR) and were purchased from Sigma-Aldrich, Fluka, QRëC, Merck, Acros Chemical Co. Ltd., and Carlo Erba. The citronella grass and kaffir lime were obtained from the Bueng-Thawai Subdistrict area, Tao Ngoi District, Sakon Nakhon province, in the northeastern region of Thailand. These samples were washed several times with water to ensure cleanliness. The cleaned citronella grass samples were cut into small pieces, using all parts except the root. For the kaffir lime, the whole fruit was cut into small pieces to prepare for essential oil extraction. The extraction of essential oils from citronella grass and kaffir lime samples was performed using fresh, non-dried plant material.

Extraction of essential oil from citronella grass and kaffir lime using the prototype essential oil distiller

This research involved the distillation of essential oil from citronella grass and kaffir lime using a prototype essential oil distiller. The main components of the prototype distiller include a stainless-steel tank for storing raw materials. Inside the tank, a stainless-steel grid holds the raw materials above the water. When heated, the water vaporizes, and the steam extracts the essential oils from the samples on the grid. The steam carrying the essential oils is then transferred through a joint pipe connecting the sample tank to a stainless-steel cooling tank. The distillation process for extracting essential oils from these plant samples is continuous and takes approximately 2 - 3 h per cycle, depending on the type of raw material used. **Figure 1** shows a schematic diagram of the prototype essential oil distiller, including both the model and the working machine, as well as a flowchart illustrating the distillation process for citronella grass and kaffir lime.

The samples of citronella grass and kaffir lime, cut into small pieces weighing 20 kg, were loaded onto a stainless-steel grate and then placed into a holding tank containing approximately 30 L of water. The water in the sample tank was heated until it turned into vapor, initiating the process of extracting essential oil from the citronella grass and kaffir lime samples, which took about 3 h. The next step was to remove some impurities from the essential oil samples using a separatory funnel. Finally, the essential oil was stored in a glass bottle with a tight lid and refrigerated at 4 °C for use in the next step of the experiment. In addition, this prototype essential oil distillation machine is designed for distilling essential oils from both fresh and dry herbal plant samples, with a production capacity of 50 kg. It is suitable for the needs and potential of community enterprise groups. Moreover, this prototype features easy operation steps, convenient maintenance, low cost, and portability, allowing it to be easily transported to various locations.

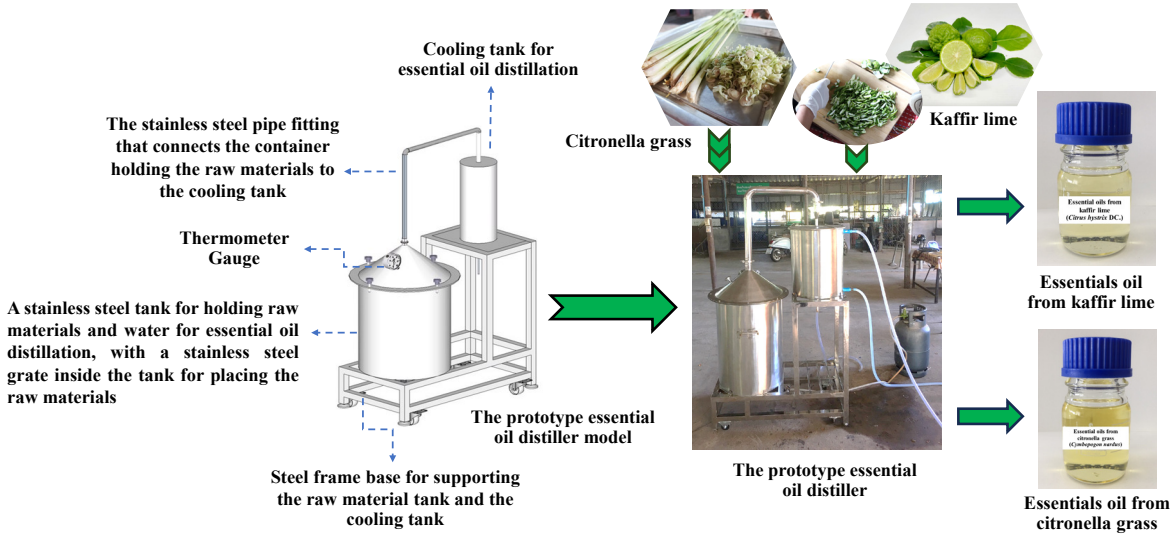

Figure 1 A schematic diagram showing the extraction of essential oils from citronella grass and kaffir lime using a prototype distiller.

Analysis of chemical composition of essential oil from citronella grass and kaffir lime using gas chromatography-mass spectrometry (GC-MS)

The obtained essential oil from citronella grass and kaffir lime was then analyzed for its chemical composition using gas chromatography (Agilent 7890A)-mass spectrometry (Agilent 7000B) (GC-MS). The type of column used was an HP-5 capillary column with dimensions of 30 m × 0.25 mm and a film thickness of 0.25 µm. Sample preparation involved pipetting 50 mg of the sample, dissolving it in 1 mL of ethanol, filtering it through a 0.45 µm filter, and injecting 1 µL of the solution. The conditions of the gas chromatography apparatus were as follows: An injection temperature of 250 °C, a column flow rate of 1.5 mL/min using helium gas, and a transfer line temperature of 250 °C. The conditions of the mass spectrometry instrument were as follows: The ion source was electron ionization (EI), the scan mode ranged from mass 35 to mass 550, the ion source temperature was 230 °C, and the electron energy was 70 eV, respectively. The GC-MS chromatogram obtained from the analysis was compared with the chemical composition data from the database of each standard compound.

Characterization of essential oil from citronella grass and kaffir lime using ^{1}H and ^{13}C-NMR

The obtained essential oils from citronella grass and kaffir lime were characterized using ^{1}H and ^{13}C-NMR techniques on a Brüker AscendTM 600 MHz spectrometer at the Vidyasirimedhi Institute of Science and Technology, Thailand. Approximately 5 mg of the essential oil sample was diluted with 5 mL of deuterated chloroform ($CDCl_3$) solvent in a clean and dry NMR tube. The sample tube was then placed in an NMR instrument and the spectrum was measured using the ^{1}H-NMR technique to provide information about the protons in the molecule. Additionally, the ^{13}C-NMR measurement was performed to provide information about the carbon atoms in the sample molecule. Since the essential oil sample obtained was a crude extract, the data identified from both the ^{1}H-NMR and ^{13}C-NMR analyses were used to confirm the functional groups in the compound structure. This information will be very useful in supporting the chemical composition analysis results of the essential oil samples obtained by the GC-MS technique.

Antioxidant capacity assay

Many antioxidant assays rely on the single electron transfer reaction, which causes a color change when the antioxidant is reduced. To evaluate the antioxidant capacity of the obtained essential oils from

citronella grass and kaffir lime, assays based on the consumption of stable free radicals (ABTS and DPPH) and the capacity of antioxidants to reduce ions (FRAP) were conducted.

DPPH scavenging assay for antioxidant activity

The DPPH• assay was conducted following the method described by Phewphong *et al*. [1] and Preecharram *et al*. [13,14]. A 1000 µg/mL Trolox standard solution was prepared and subsequently diluted to obtain solutions with concentrations of 0.625, 1.25, 2.50, 5.00, 7.50, 10.00, 12.50, 15.00, 17.50, 20.00, 22.50, 25.00, 50.00, 100.00, 250.00, 500.00, and 1000.00 µg/mL. To prepare the essential oil sample solution, approximately 10 mg of essential oil was weighed and dissolved in 5 mL of 50 % v/v DMSO. The volume was then adjusted to 10 mL to obtain an extract solution with a concentration of 1000 µg/mL. The analysis of DPPH• antioxidant activity of samples was conducted by pipetting 100 µL of samples at various concentrations into a 96-well plate. To each well, 100 µL of 0.06 mM DPPH• solution was added. The plate was then incubated in the dark at room temperature for 30 min. Absorbance was measured using a microplate reader at 515 nm, with at least 3 replicates performed for each sample. The percentage of DPPH scavenging activity was calculated using a specified equation.

$$\text{DPPH}^{\bullet}\text{ scavenging activity (\%)} = [(A_{control} - A_{sample})/A_{control})]\times 100 \quad (1)$$

$A_{control}$ is the absorbance of the DPPH radical solution in the respective extraction solvent. A_{sample} is the absorbance of each essential oil in the DPPH radical solution.

ABTS scavenging assay for antioxidant activity

The antioxidant capacity of each essential oil was analyzed using the $ABTS^{\bullet+}$ radical scavenging assay. The reaction of the protonated sample with $ABTS^{\bullet+}$ radicals resulted in a fading of the blue-green color, leading to a decrease in absorbance at 734 nm. The test was performed on samples of the extracts, using Trolox as a reference standard, as reported by Raspo *et al*. [7] and Moller *et al*. [15]. The preparation of the Trolox standard solution was performed in the same manner as for the DPPH• scavenging assay for antioxidant activity. Similarly, the preparation of essential oil sample solutions to a concentration of 100 µg/mL was carried out using the same procedure as for the DPPH• scavenging assay. The antioxidant efficiency of essential oil samples was analyzed by pipetting 100 µL of sample solutions at various concentrations into a 96-well plate. To each well, 100 µL of $ABTS^{\bullet+}$ reagent was added, and the plate was incubated in the dark at room temperature for 30 min. Absorbance was measured using a microplate reader at 734 nm, with at least 3 replicates performed for each sample. The percentage of $ABTS^{\bullet+}$ scavenging activity can be calculated using the following equation:

$$ABTS^{\bullet+}\text{ scavenging activity (\%)} = [(A_{control} - A_{sample})/A_{control})]\times 100 \quad (2)$$

$A_{control}$ is the absorbance of the $ABTS^{\bullet+}$ solution without the sample (control). A_{sample} is the absorbance of the $ABTS^{\bullet+}$ solution with the essential oil sample. This equation provides the percentage of $ABTS^{\bullet+}$ radicals that were scavenged by the sample, indicating its antioxidant activity.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay measures the ability of antioxidants to reduce iron in an acidic medium. This assay was conducted following the methods described by Raspo *et al.* [7] and Moller *et al.* [16]. The preparation of the Trolox standard solution was conducted in the same way as for the DPPH• scavenging assay for antioxidant activity. Likewise, the essential oil sample solutions were prepared to a concentration of 1000 µg/mL using the same procedure as that used for the DPPH• scavenging assay. The antioxidant efficiency of the essential oil samples was assessed by pipetting 100 µL of sample solutions at various concentrations into a 96-well plate. To each well, 100 µL of FRAP reagent was added, and the plate was incubated in the dark at room temperature for 30 min. Absorbance was measured using a microplate reader at 593 nm, with a minimum of 3 replicates performed for each sample. To calculate the results of the FRAP assay as milligrams of Trolox equivalent (mg TE) per gram of crude essential oil sample using a specified equation.

$$\text{FRAP (mg TE/g crude sample)} = \frac{(A_{sample} - A_{blank}) \times \text{slope}}{\text{dilution factor}} \times \frac{100}{\text{weight of crude sample}} \quad (3)$$

where A_{sample} is the absorbance of the sample, A_{blank} is the absorbance of the blank control, slope is the slope value from the Trolox standard curve, dilution factor is the dilution ratio of the sample, and weight of crude essential oil sample is the weight of the crude essential oil sample used (in grams), respectively.

Tyrosinase inhibitory activity test by dopachrome method

The tyrosinase inhibitory activity test using the dopachrome method, with ascorbic acid as a reference standard, was modified from the research reports of Masuda *et al.* [17], Özer *et al*. [18], and Srisuksomwong *et al*. [19]. The process begins with the preparation of a 0.067 M sodium phosphate buffer solution at pH 6.8. Next, a tyrosinase enzyme solution is prepared by weighing 0.5 mg of tyrosinase enzyme and dissolving it in the 0.067 M sodium phosphate buffer (pH 6.8) in a 10 mL volumetric flask. The volume is then adjusted to 10 mL to obtain the tyrosinase enzyme solution. The L-DOPA solution was prepared by weighing 0.6 mg of L-DOPA and dissolving it in 0.067 M sodium phosphate buffer (pH 6.8) in a 10 mL volumetric flask. The volume was then adjusted to 10 mL. The L-DOPA solution was prepared by weighing 0.6 mg of L-DOPA and dissolving it in 0.067 M sodium phosphate buffer (pH 6.8) in a 10 mL volumetric flask. The volume was then adjusted to 10 mL. The preparation of ascorbic acid standard solutions at various concentrations involved weighing 50 mg of ascorbic acid and dissolving it in 50 % v/v DMSO-in a 50 mL volumetric flask. The volume was then adjusted to 50 mL, resulting in an ascorbic acid standard solution with a concentration of 1000 µg/mL. From this 1000 µg/mL solution, ascorbic acid standard solutions with concentrations of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 250, and 500 µg/mL were prepared. The preparation of sample solutions at various concentrations involved weighing 10 mg of the essential oil sample and dissolving it in 50 % v/v DMSO in a 10 mL volumetric flask. The volume was then adjusted to 10 mL to obtain a sample solution with a concentration of 1000 µg/mL.

The tyrosinase inhibitory activity test was conducted by evaluating the inhibitory activity of the standard ascorbic acid samples prepared at different concentrations. Test group A (control) consisted of 80 µL of phosphate buffer (pH 6.8), 40 µL of tyrosinase solution, and 40 µL of 50 % v/v DMSO. Test group B (blank control) included 120 µL of phosphate buffer (pH 6.8) and 40 µL of 50 % v/v DMSO. Test group C (test sample) included 80 µL of phosphate buffer (pH 6.8), 40 µL of tyrosinase solution, and 40 µL of the sample solution. Test group D (blank sample) consisted of 120 µL of phosphate buffer (pH 6.8) and 40 µL of the sample solution. The mixed solution was then shaken and incubated at 25 °C for 10 min. Following this, 40 µL of L-DOPA solution was added to each test tube, and the mixture was shaken and incubated again at 25 °C for 10 min. The absorbance was measured using a microplate reader at 475 nm, and the percentage of tyrosinase inhibition was calculated using the following equation.

$$\% \text{ Tyrosinase inhibition} = \frac{(A_{control} - B_{blank\ control}) - (C_{test\ sample} - D_{blank\ sample})}{(A_{control} - B_{blank\ control})} \times 100 \quad (4)$$

where $A_{control}$ is the absorbance of the control group (without the test substance but with the tyrosinase enzyme), $B_{blank\ control}$ is the absorbance of the blank control (without the test substance and the tyrosinase enzyme), $C_{test\ sample}$ is the absorbance of the test sample (with the test substance and the tyrosinase enzyme), and $D_{blank\ sample}$ is the absorbance of the blank sample (with the test substance but without the tyrosinase enzyme), respectively.

Molecular docking of Tyrosinase inhibitory activity test

All 3-dimension ligand structures were downloaded from The National Institutes of Health (NIH) database (https://pubchem.ncbi.nlm.nih.gov). The conformation of each ligand was then fully optimized at the B3LYP/6-31G(d) level using Gaussian 09 prior to docking simulations [20]. Tyrosinase from the mushroom *Agaricus bisporus* was used as the protein target, and its structure was downloaded from the Protein Data Bank (http://www.pdb.org), PDB ID: 2Y9X [21]. All water molecules, tropolone inhibitor, and non-interacting ions were removed. Missing hydrogen atoms and side chains were added using

AutoDockTools 1.5.6 (ADT) [22]. Gasteiger charges were assigned, and all non-polar hydrogens were merged with their respective carbon atoms [23]. Some amino acids in the active site, such as His263, PHE264, MET280, VAL283, and ASN260, were partially designated as flexible. The grid box was set to 40×40×40 grid points in the x, y, and z dimensions, centered on the reference tropolone ligand, with a grid spacing of 0.375 Å. Energy grid maps were generated using the AutoGrid 4.2.6 program (http://autodock.scripps.edu). All docking calculations were performed using AutoDock 4.2.6 [22], employiFng the Lamarckian genetic algorithm (LGA) with 200 runs, 2.5×10^6 energy evaluations, and default values for other parameters [24]. Protein-ligand interactions were visualized using Discovery Studio Visualizer 2024 [25].

Antibacterial activity test of essential oil from citronella grass and kaffir lime

The antibacterial activity of the essential oils obtained from citronella grass and kaffir lime was measured using the paper disk diffusion method. This method is widely used in clinical microbiology laboratories for routine antibiotic susceptibility testing and to help guide appropriate antibiotic therapy [3,7]. This study tested the inhibitory activity against Gram-positive bacteria, namely *Staphylococcus aureus* TISTR2329, *Staphylococcus epidermidis*, *Bacillus subtilis* TISTR1248, and *Bacillus cereus*, as well as against Gram-negative bacteria, namely *Escherichia coli* TISTR 527. The essential oils obtained from citronella grass and kaffir lime samples were used as crude extracts without dilution to test their antibacterial activity. The experimental procedure was based on the research reports of Raspo *et al*. [7], Bilal and Hossain [26] and Rehab and Hossain [27]. The experimental procedure began with the preparation of a bacterial suspension to match the turbidity standard of 0.5 McFarland. The bacteria were then cultivated on agar plates by spreading the bacterial suspension evenly over the surface to create a bacterial lawn. Meanwhile, place the 6 mm filter paper disks on the agar plate, then pipette the sample, positive control, and negative control onto each disk. This study used clindamycin as a positive control and DMSO as a negative control. All the disk plates were incubated at a temperature of 37 °C for 24 h. After incubation, the diameter of the inhibition zones was measured to assess antibacterial activity. The inhibition was recorded by calculating the diameter of the growth inhibition zones in millimeters (mm), and the antibacterial activities were determined.

Results and discussion

Extraction and chemical composition analysis of the essential oil from citronella grass and kaffir lime

Figure 1 displays a schematic diagram of the essential oil extraction process from citronella grass and kaffir lime using a prototype distiller. In this process, fresh, non-dried plant material from both citronella grass and kaffir lime was used for the extraction of essential oils. The calculated yields of essential oils from citronella grass and kaffir lime were found to be 1.67 and 2.36 % (v/w), respectively, relative to the weight of the fresh samples. These percentage yields of essential oils were obtained by extracting the samples 3 times to ensure the maximum amount of essential oil was extracted. The essential oil extracted from both raw materials has a clear yellow color, as shown in **Figure 1**. It was observed that the essential oil from citronella grass has a darker yellow tone compared to that from kaffir lime. In comparison with the research report by Kakaraparthi *et al*. [28], the oil content of citronella grass was found to range from 0.9 to 1.7 % of the dry sample. The variation in essential oil content and composition of citronella grass can be attributed to the time of harvest and weather conditions. Wu *et al*. [29] investigated the extraction of essential oil from citronella grass leaves using supercritical carbon dioxide (SFE), with a focus on its antioxidant and antimicrobial activities. They determined that the optimal conditions for the highest essential oil yield were an extraction time of 120 min, an extraction pressure of 25 MPa, an extraction temperature of 35 °C, and a CO_2 flow rate of 18 L/h. Under these conditions, the mean essential oil yield was 4.40 %. Similarly, Husni *et al.* [4] reported that the essential oil content from kaffir lime peel was 1.74 % based on fresh weight. In contrast, Weng *et al.* [30] found that hydro-distillation of kaffir lime peel collected in Vietnam produced a pale yellow, almost transparent liquid with a yield of 4.6 % based on fresh weight. Therefore, the experimental results indicate that the percentage of essential oils extracted from both

citronella grass and kaffir lime depends on factors such as the extraction method, harvesting time, and cultivation conditions.

The chemical compositions of essential oils from citronella grass and kaffir lime were analyzed using GC-MS. The analysis revealed 14 major chemical compounds in the essential oil from citronella grass and 16 major compounds in the essential oil from kaffir lime. The GC-MS results for the essential oil of citronella grass are presented in **Figure 2** and **Table 1**. The 5 main volatile organic constituents of citronella grass found in the highest concentrations were geraniol (35.99 % w/w), geranyl acetate (10.73 % w/w), α-citral (7.34 % w/w), β-citral (5.72 % w/w), and a combination of (R)-(+)-citronellal and citral diethyl acetal (5.44 % w/w). The results of this study are consistent with the findings of Devi *et al*. [3], Kakaraparthi *et al.* [28], Wu *et al.* [29], and Weng *et al.* [30], who reported that geraniol, citral, geranyl acetate, and citronellal are the major constituents of citronella grass essential oil. It is important to note that the percentage of each active compound in essential oil extracted from citronella grass can vary. Factors such as different cultivation areas, harvesting times, and extraction methods directly influence the concentration of these phytochemicals, even within the same plant species.

Table 1 The chemical composition of aroma essential oils from citronella grass.

Peak number	Retention time (min)	Compound Name	Compound formula	Molecular weight	Composition (% w/w)
1	18.49	4-Nonanone	C_9H1_8O	142.24	0.89
2	19.93	β-Linalool	$C_{10}H_{18}O$	154.25	3.38
3	22.51	(R)-(+)-Citronellal	$C_{10}H_{18}O$	154.25	5.44
4	26.25	Citronellol	$C_{10}H_{20}O$	156.27	4.48
5	26.68	β-Citral	$C_{10}H_{16}O$	152.24	5.72
6	27.68	Geraniol	$C_{10}H_{18}O$	154.25	35.99
7	28.14	α-Citral	$C_{10}H_{16}O$	152.24	7.34
8	31.76	Citronellol acetate	$C_{12}H_{22}O_2$	198.30	1.18
9	33.14	Geranyl acetate	$C_{12}H_{20}O_2$	96.29	10.73
10	34.36	β-Caryophyllene	$C_{15}H_{24}$	204.357	2.27
11	35.48	Citral diethyl acetal	$C_{12}H_{22}O_2$	198.30	5.44
12	38.27	γ-Cadinene	$C_{15}H_{24}$	204.35	1.27
13	39.72	Hedycaryol	$C_{15}H_{26}O$	222.37	4.06
14	61.40	Bis-(2-ethylhexyl)-phthalate	$C_{24}H_{38}O_4$	390.56	1.87

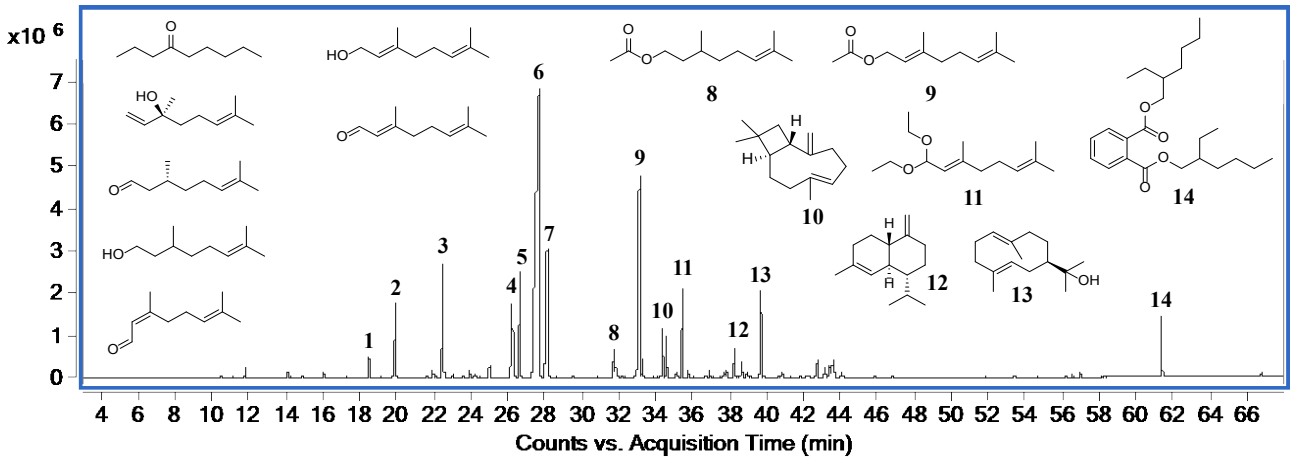

Figure 2 GC-MS chromatogram of the chemical composition of aroma essential oils from citronella grass.

Table 2 The chemical composition of aroma essential oils from kaffir lime.

Peak number	Retention time (min)	Compound name	Compound formula	Molecular weight	Composition (% w/w)
1	11.14	(-)-α-Pinene	$C_{10}H_{16}$	136.24	1.76
2	13.35	(-)-β-Pinene	$C_{10}H_{16}$	136.24	20.19
3	14.29	β-Myrcene	$C_{10}H_{16}$	136.23	1.03
4	14.79	α-Phellandrene	$C_{10}H_{16}$	136.23	0.89
5	15.46	4-Terpinenyl acetate	$C_{12}H_{20}O_2$	196.28	4.33
6	15.89	m-Cymene	$C_{10}H_{14}$	134.22	2.29
7	16.17	D-Limonene	$C_{10}H_{16}$	136.23	20.72
8	17.69	γ-Terpinene	$C_{10}H_{16}$	136.23	5.22
9	18.40	Ethyl 2-(5-methyl-5-vinyltetrahydrofuran-2-yl)-propan-2-yl carbonate	$C_{13}H_{22}O_4$	242.31	2.08
10	19.16	Terpinolene	$C_{10}H_{16}$	136.23	4.29
11	19.91	β-Linalool	$C_{10}H_{18}O$	154.25	1.44
12	22.48	(R)-(+)-Citronellal	C10H18O	154.25	4.48
13	22.97	endo-Borneol	C10H18O	154.25	4.52
14	23.63	4-Terpineol	$C_{10}H_{18}O$	154.25	12.30
15	24.33	α-Terpineol	$C_{10}H_{18}O$	154.25	9.14
16	26.18	Citronellol	$C_{10}H_{20}O$	156.27	1.57

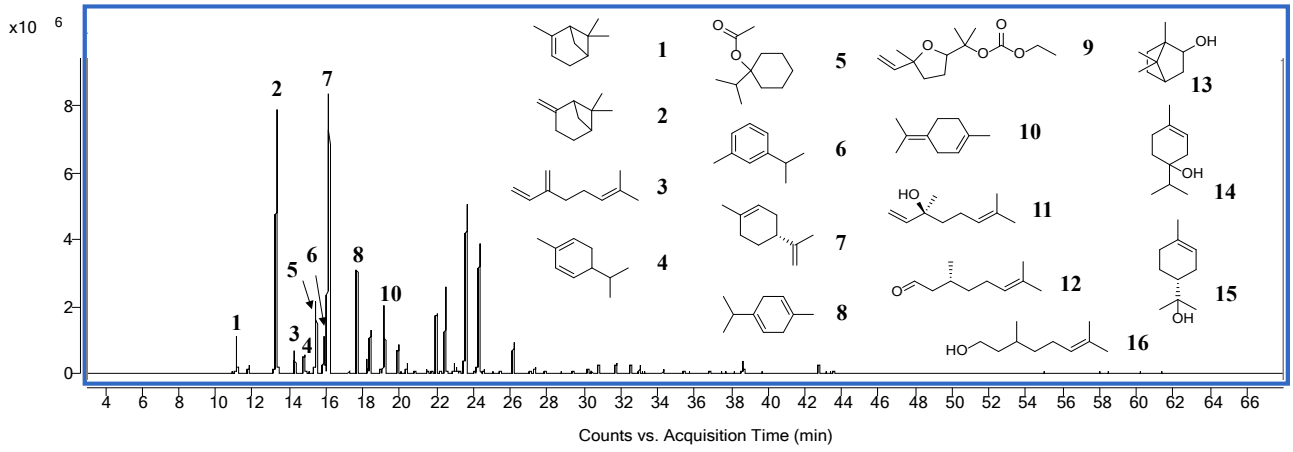

Figure 3 GC-MS chromatogram of the chemical composition of aroma essential oils from kaffir lime.

The results of the chemical composition analysis of the essential oil extracted from kaffir lime are presented in the GC-MS chromatogram in **Figure 3**, with detailed chemical composition provided in **Table 2**. The experimental results revealed that the major compounds in the essential oil extracted from kaffir lime were D-limonene (20.72 % w/w), (-)-β-pinene (20.19 % w/w), 4-terpineol (12.30 % w/w), α-terpineol (9.14 % w/w), and γ-terpinene (5.22 % w/w). The results of this study align with the findings of Husni *et al.* [4], Aripin *et al.* [11], and Siti *et al.* [31], who reported that D-limonene and β-pinene are the main constituents of the essential oil extracted from kaffir lime. The concentrations of these phytochemicals may vary for the same reasons discussed for the essential oil extracted from citronella grass; specifically, different cultivation

areas, harvesting times, and extraction methods directly influence their concentrations, even within the same plant species.

NMR-based chemical structure analysis of citronella grass and kaffir lime essential oils

This research investigated the chemical structures of essential oils from citronella grass and kaffir lime using ^{1}H and ^{13}C-NMR, aiming to provide more accurate and detailed insights. **Figures 4** and **5** present the ^{1}H-NMR and ^{13}C-NMR spectra of the aroma essential oils from citronella grass. The analysis showed that the essential oil from citronella grass contains organic structures with functional groups such as ether, alcohol, ester, and aldehyde, with almost no aromatic compounds detected. The primary hydrocarbon functional groups identified were alkene, allylic, methylene, and benzylic. These findings are consistent with the GC-MS analysis, which revealed that the citronella grass essential oil is composed mainly of straight and branched-chain organic compounds, with fewer ring structures and aromatic groups.

Similarly, **Figures 6** and **7** show the ^{1}H-NMR and ^{13}C-NMR spectra of the aroma essential oils from kaffir lime, respectively. The analysis revealed that the essential oil extracted from kaffir lime contains organic structures with functional groups including ether, alcohol, ester, aromatic, ketone, and aldehyde. Additionally, hydrocarbon functional groups such as alkene, allylic, methylene, and benzylic were identified. These findings align with the results from GC-MS analysis, which also indicated that the kaffir lime essential oil comprises a mixture of various compounds, featuring both aromatic and aliphatic functional groups in cyclic, straight-chain, and branched-chain forms. The NMR analysis of essential oils from both plant samples identified not only the functional groups within the organic compounds but also provided insights into the chemical structures, such as straight chains, branched chains, rings, and aromatic rings. These findings highlight the differences in the composition and chemical structures of the essential oils extracted from each plant, which likely influence their chemical properties and phytochemical activities.

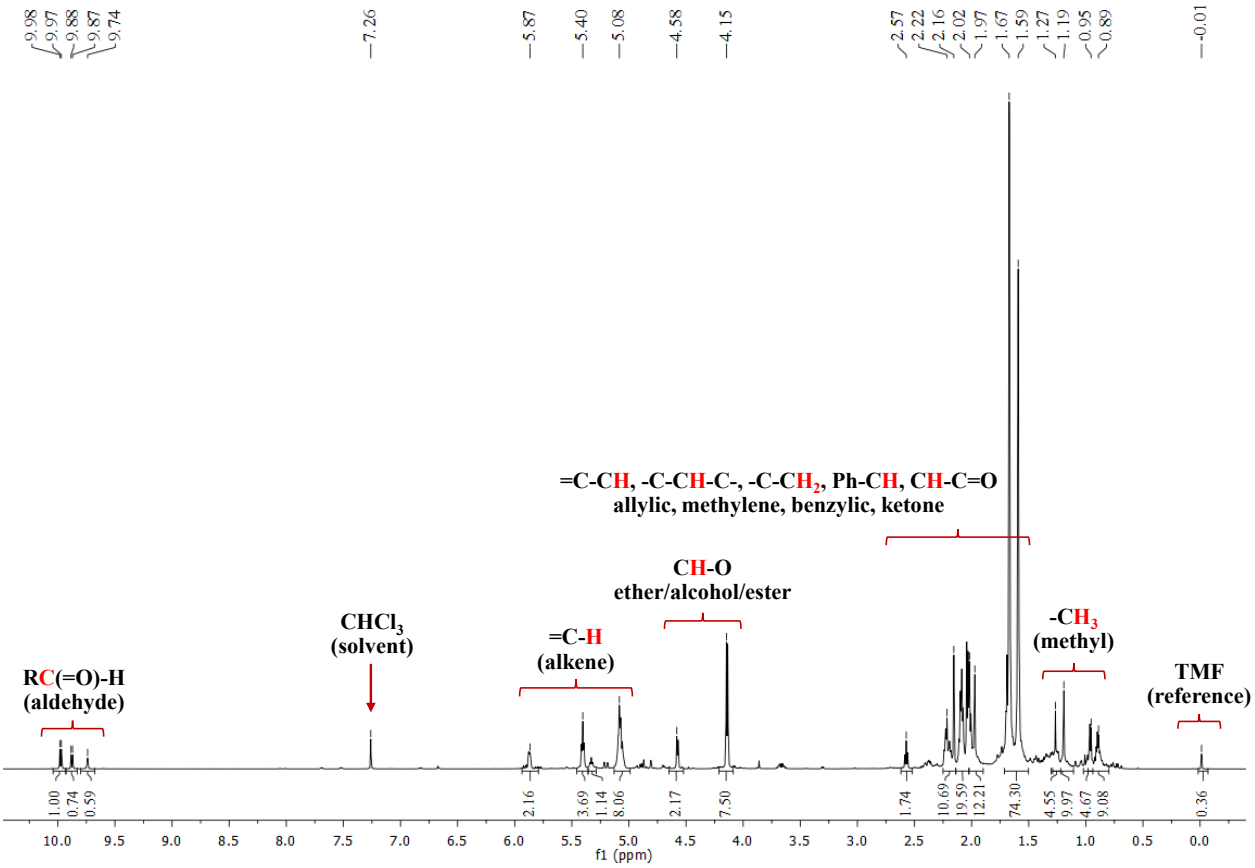

Figure 4 ^{1}H-NMR spectra of aroma essential oils from citronella grass.

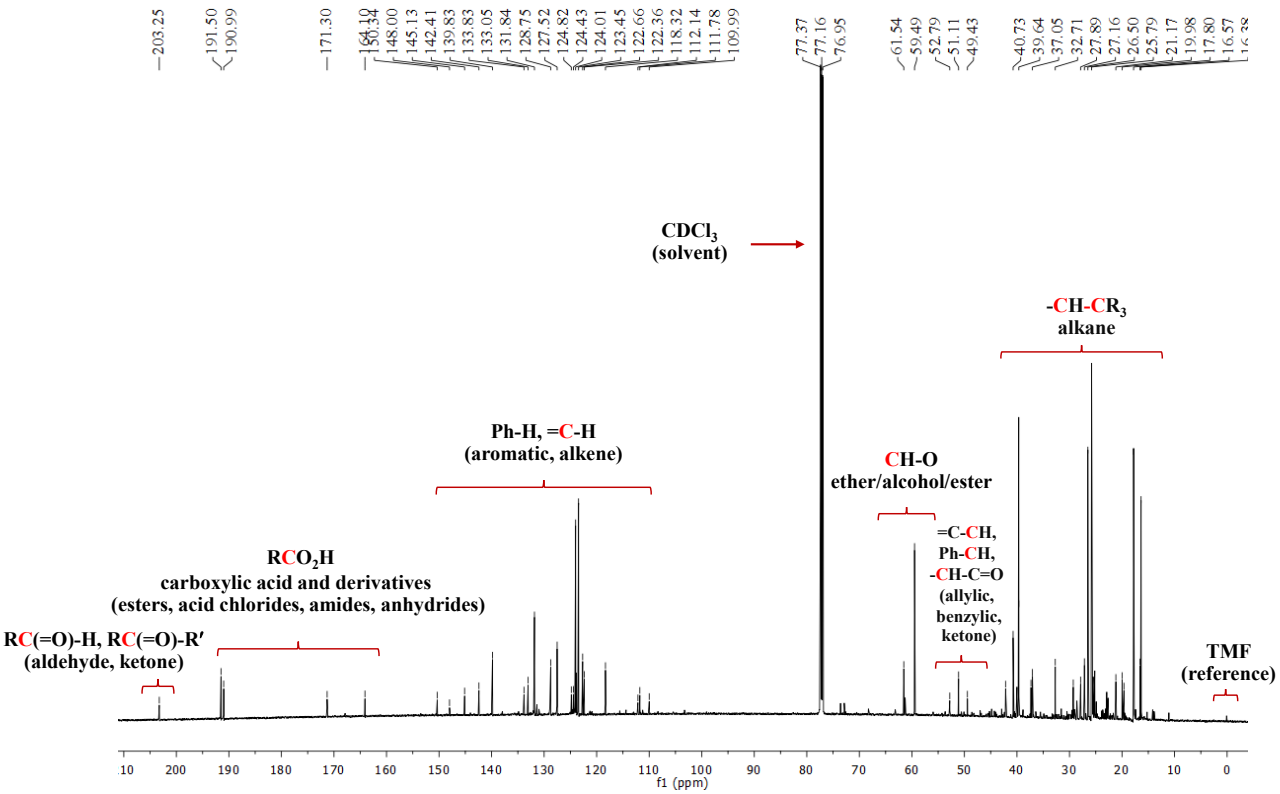

Figure 5 ^{13}C-NMR spectra of aroma essential oils from citronella grass.

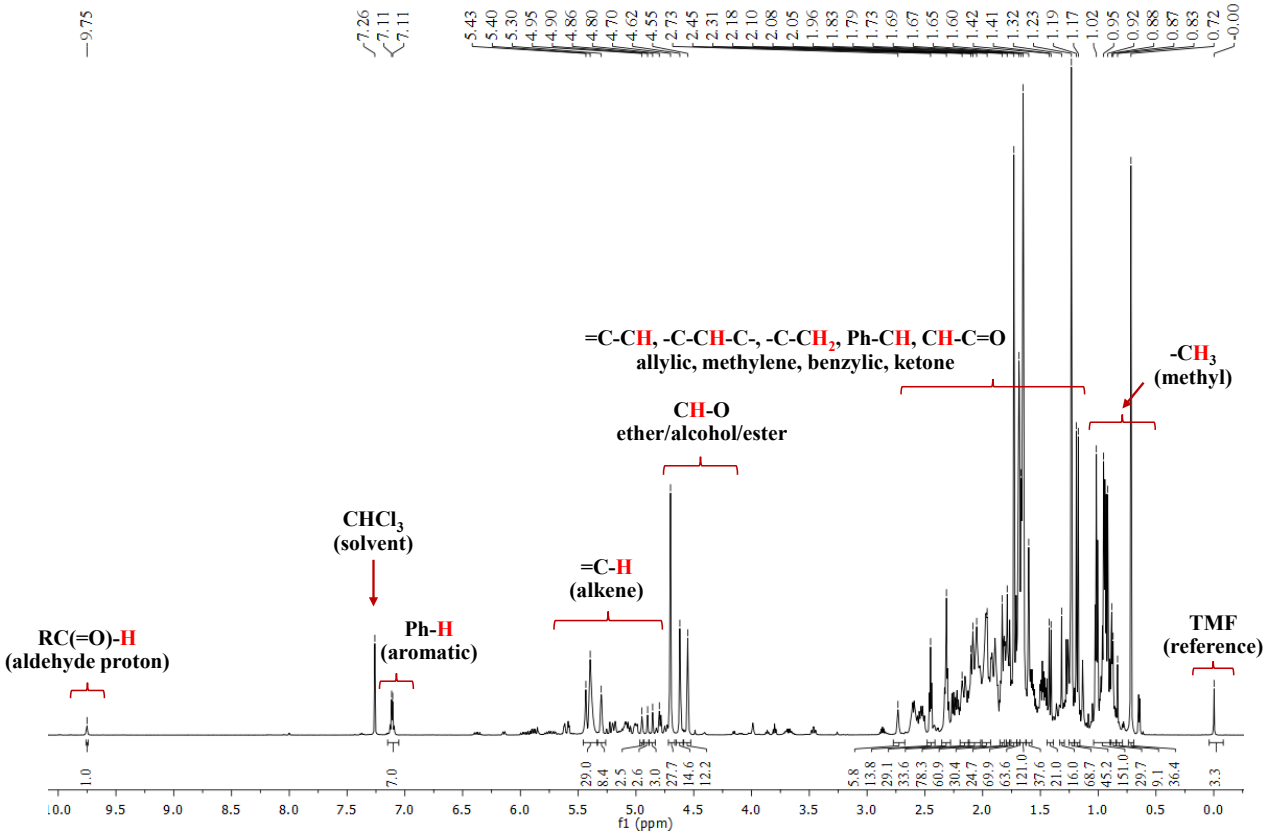

Figure 6 ^{1}H-NMR spectra of aroma essential oils from kaffir lime.

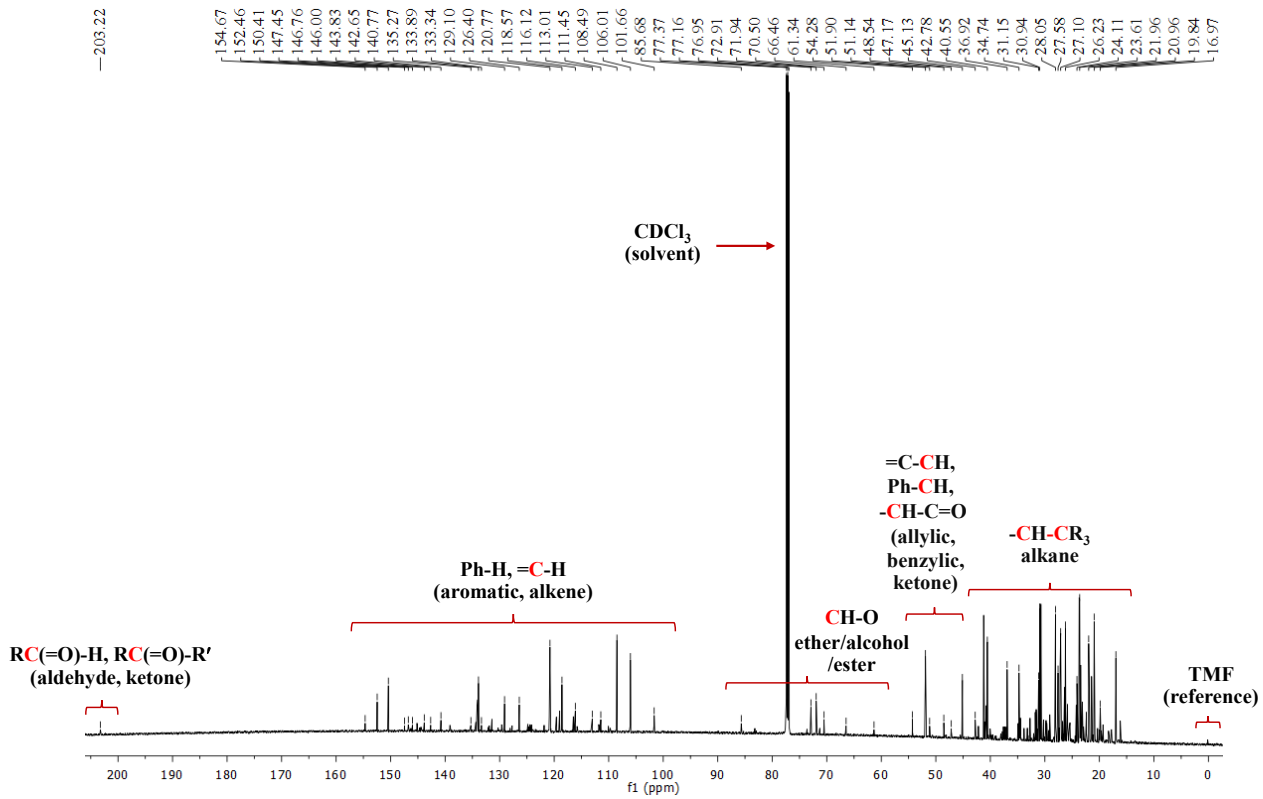

Figure 7 ^{13}C-NMR spectra of aroma essential oils from kaffir lime.

Antioxidant activity of citronella grass and kaffir lime essential oils

This research investigated the antioxidant activity of essential oils extracted from citronella grass and kaffir lime using 3 methods: DPPH$^{\bullet}$ and ABTS$^{\bullet+}$, reported as % inhibition, and FRAP, reported as mg TE/g crude extract. The antioxidant activity results of citronella grass and kaffir lime essential oils are presented in **Table 3**. The results of the antioxidant activity analysis using the DPPH$^{\bullet}$ scavenging assay indicated that the standard compound Trolox exhibited an inhibition value of 33.38 ± 0.61 % at a concentration of 5 µg/mL. In comparison, the essential oil from kaffir lime showed an inhibition value of 13.74 % ± 0.46, while the essential oil from citronella grass exhibited an inhibition value of 16.86 % ± 1.69 at a concentration of 1000 µg/mL. For the ABTS$^{\bullet+}$ antioxidant activity test, the essential oils were tested at a concentration of 100 µg/mL, with Trolox as the standard at 25 µg/mL. The test results showed that the standard compound Trolox exhibited an inhibition value of 53.35 ± 2.86 %, while the essential oil samples extracted from kaffir lime and citronella grass exhibited inhibition values of 27.06 ± 2.14 and 44.61 ± 1.94 %, respectively.

These experimental results indicated that the essential oils extracted from both kaffir lime and citronella grass had lower efficacy in inhibiting free radicals compared to the standard Trolox. This may be due to the essential oil samples being non-polar or low-polar compounds, while the antioxidants DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ are relatively polar. Consequently, during the antioxidant activity tests, the samples and free radicals did not dissolve effectively together, even with the use of 50 % v/v DMSO as the solvent, which is moderately polar and can dissolve both polar and non-polar substances to some extent. This rationale and explanation are consistent with the research reports by Moller *et al.* [15], Moller *et al.* [16], Siti *et al.* [31], Anggraeny *et al.* [32], and Budiarto *et al.* [33], who studied the antioxidant activity of essential oils extracted from different plants. They found that these essential oils had low radical scavenging activity compared to reference compounds, likely because essential oils are non-polar substances. In some experiments, polar solvents were used to extract

essential oils. For example, the ethanol or ethyl acetate fractions showed the highest antioxidant activity, followed by the water residue and hexane fractions. Both the ethanol or ethyl acetate fractions and the water residue contained alkaloids, saponins, tannins, phenolics, and flavonoids, while the hexane fraction contained some alkaloids, phenolics, and flavonoids, which are low-polarity compounds. Non-polar or low-polar extracts generally exhibit lower antioxidant activity compared to polar extracts.

In the study of antioxidant effects using the FRAP assay, the method measures the antioxidant capacity of a substance by assessing its ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), which produce a blue color. The intensity of this blue color is directly proportional to the antioxidant power of the sample [16,19,34]. The results showed that the essential oil from citronella grass has a higher FRAP value (113.44 ± 4.16 mg TE/g crude extract) compared to the essential oil from kaffir lime (6.54 ± 0.18 mg TE/g crude extract), indicating greater antioxidant activity. The FRAP value reflects a substance's ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), with higher values signifying stronger antioxidant power. Therefore, citronella grass essential oil, with its higher FRAP value, is more effective at neutralizing free radicals than kaffir lime essential oil. The results of this experiment align with studies on antioxidant activity using $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ techniques, which found that essential oils extracted from citronella grass generally exhibit higher antioxidant efficiency than those from kaffir lime. The structure and chemical composition of the aroma essential oils from citronella grass are primarily composed of straight and branched-chain organic compounds, with fewer ring structures and aromatic groups. The main compounds include geraniol, geranyl acetate, α-citral, β-citral, citronellal, and citral diethyl acetal, all of which belong to functional groups such as ethers, alcohols, esters, and aldehydes. These compounds, characterized by straight and branched-chain structures, as well as carbonyl (C=O), hydroxyl (O–H), and alkene (C=C) functional groups, can easily donate electrons to free radicals. While the main chemical constituents and structure of kaffir lime essential oil include cycloalkene rings and alcohol (hydroxyl; O–H) functional groups. As a result, its ability to donate electrons to free radicals is lower than that of citronella grass essential oil.

Table 3 Antioxidant activity of the essential oil from citronella grass and kaffir lime.

Samples	$DPPH^{\bullet}$ (% inhibition)	$ABTS^{\bullet+}$ (% inhibition)	FRAP (mg TE/g crude extract)
Trolox	33.38 ± 0.61	53.35 ± 2.86	-
The essential oil from citronella grass	16.86 ± 1.69	44.61 ± 1.94	113.44 ± 4.16
The essential oil from kaffir lime	13.74 ± 0.46	27.06 ± 2.14	6.54 ± 0.18

Tyrosinase inhibitory effect of the essential oil from citronella grass and kaffir lime

Tyrosinase is an enzyme essential for melanin production which gives color to skin, hair, and eyes. It catalyzes 2 main reactions: The hydroxylation of tyrosine to DOPA and the oxidation of DOPA to dopaquinone, which eventually leads to melanin formation. Tyrosinase inhibition plays a crucial role in various fields, particularly in beauty and medicine. By reducing melanin production, tyrosinase inhibitors can lighten skin and diminish the appearance of dark spots, such as freckles, melasma, and acne scars. Additionally, in the food industry, tyrosinase inhibition is important for preventing the browning of fruits and vegetables when they are cut or bruised, helping to maintain the quality and appeal of these foods [18,35-39]. Therefore, this research aims to investigate the inhibitory effect of essential oils extracted from citronella grass and kaffir lime on the tyrosinase enzyme. These essential oils are derived from a prototype essential oil distiller. The findings from this study will provide a valuable database for the application of these essential oil extracts in various fields, including their incorporation into cosmetic products (e.g., soap, cream, body lotion, facial

cleansing gel, etc.) and their use in preserving the freshness and quality of fruits and vegetables by preventing browning when cut or bruised.

The experimental results for the inhibitory activity of essential oils from citronella grass and kaffir lime on the tyrosinase enzyme are presented in **Table 4**. The table reports the tyrosinase inhibition results as IC_{50} (µg/mL) values, indicating the concentration at which the compound inhibits 50 % of the original tyrosinase activity [17,35,36]. The study results showed that ascorbic acid (standard solution) had an IC_{50} value for tyrosinase inhibition of 58.38 ± 1.97 µg/mL. In comparison, the essential oil from kaffir lime exhibited an IC_{50} value of 195.68 ± 6.23 µg/mL, and the essential oil from citronella grass had an IC_{50} value of 380.45 ± 3.10 µg/mL. The experimental results indicate that the ascorbic acid standard solution has the strongest inhibitory effect on the tyrosinase enzyme, as it requires the smallest amount of substance to inhibit 50 % of the original tyrosinase activity. The essential oils from kaffir lime and citronella grass exhibited tyrosinase inhibitory activity approximately 3 times and 6.6 times lower, respectively, than that of the ascorbic acid standard solution. Furthermore, when comparing the inhibitory activities of the 2 essential oils, it was found that the essential oil extracted from kaffir lime demonstrated more than twice the inhibitory activity of the essential oil extracted from citronella grass. The higher inhibitory effect of the essential oil extracted from kaffir lime on tyrosinase, compared to that of the essential oil from citronella grass, can be attributed to the chemical composition of the oils. The main components of kaffir lime essential oil, D-limonene and (-)-β-pinene, have ring chemical structures and are small molecules that can interact more effectively with the tyrosinase enzyme, thereby inhibiting its activity. In contrast, the primary components of citronella grass essential oil, geraniol and geranyl acetate, possess branched-chain chemical structures, which hinder their ability to interact with and inhibit the tyrosinase enzyme as effectively.

Table 4 Inhibitory activity on tyrosinase enzyme of the essential oil from citronella grass and kaffir lime.

Samples	Tyrosinase inhibition IC_{50} ± SD (µg/mL)
Ascorbic acid (standard solutions)	58.38 ± 1.97
The essential oil from kaffir lime	195.68 ± 6.23
The essential oil from citronella grass	380.45 ± 3.10

Molecular docking of tyrosinase inhibitory activity test

In addition to *in vitro* methods, *in silico* approaches, such as molecular docking simulations, can be employed to study the binding mechanisms of active compounds against their target proteins. Molecular docking is a powerful computational tool that allows researchers to predict and analyze the binding conformation, mode, and residues involved in protein-ligand interactions. It enables the identification of ligands that geometrically fit into the protein's binding site, thereby facilitating the design of molecules with strong binding affinity and inhibitory potential [40-43]. In this study, the binding energy and interactions of selected ligands with Agaricus bisporus tyrosinase were predicted using *in silico* molecular docking simulations conducted with AutoDock 4.2. The docking study involved 5 interesting compounds, such as ascorbic acid, geraniol, geranyl acetate, (-)-β-pinene, and D-limonene. These were evaluated for their binding affinity and interaction patterns with the tyrosinase enzyme. The post-docking structures shown in **Figure 8** and **Table 5** illustrate the binding interactions between the ligand and the receptor. Ascorbic acid was used as the native ligand/comparator due to its well-documented antioxidant and tyrosinase inhibitory properties. This provided a valuable reference for assessing the effectiveness of other docking compounds in inhibiting tyrosinase.

The docking structures of ascorbic acid revealed 2 possible arrangements within the active site of the enzyme. The 1st conformation, represented by the green structure, showed that all hydroxyl groups of ascorbic acid formed hydrogen bonds with the carbonyl group of Asn260. Additionally, the ligand exhibited van der Waals interactions with other amino groups around the active site, as shown in **Figure 8(b)**. The observed binding energy of ascorbic acid, –5.23 kcal/mol, indicated a moderate interaction with tyrosinase. This value serves as a reference to evaluate whether other compounds bind more strongly or weakly to the enzyme. The post-docking analysis of geraniol and geranyl acetate, compounds found in high concentrations in the essential oil from citronella grass, revealed distinct binding modes. The hydroxyl group of geraniols formed a hydrogen bond with Asn260, while the long-chain hydrocarbons interacted with the active site surface via hydrophobic interactions (**Figure 8(c)**). This resulted in a binding energy of –5.06 kcal/mol. For geranyl acetate, the acetoxy group introduced slightly more steric hindrance compared to geraniol. Post-docking analysis revealed 3 different conformations (**Figure 8(d)**). For the most likely structure of geranyl acetate, despite having higher energy compared to other molecules, it exhibited only a 27 % occupation at this site, suggesting lower binding affinity and specificity for the enzyme's active site. The docking of (-)-β-pinene and D-limonene, which are found in essential oils from kaffir lime, highlighted their unique nonpolar hydrocarbon structures. Although these ligands lacked hydrogen-bonding sites with the amino groups in the active site, they formed strong bindings through hydrophobic interactions. They exhibited nearly 100 % binding site identity, with docking scores of 98 and 81 % for (-)-β-pinene (**Figure 8(e)**) and D-limonene (**Figure 8(f)**), respectively. This high binding site identity highlights the significant specificity of these ligands toward the tyrosinase enzyme, consistent with the lock-and-key model.

Table 5 Molecular docking results of the bioactive compounds with tyrosinase enzyme.

Compounds	Conformation	% Occupation	Binding energy (ΔG; kcal/mol)	Inhibition constant (K_i)
Ascorbic acid	1	37	–5.23	147.77 uM
	2	14	–5.08	190.21 uM
Geraniol	1	28	–5.06	194.43 uM
	2	27	–5.18	159.44 uM
	3	17	–4.91	250.68 uM
Geranyl acetate	1	27	–6.08	35.09 uM
	2	18	–5.54	86.33 uM
	3	15	–5.50	93.35 uM
(-)-β-Pinene	1	98	–3.97	1.23 mM
D-Limonene	1	81	–4.61	417.76 uM

Notes: 100 % occupation corresponds to a docking score of 200, Inhibition constant calculated from binding free energy, $Ki = \exp(\Delta G/RT)$ where R is the universal gas constant (1.985×10^{-3} kcal mol^{-1} K^{-1}) and T is the temperature (298.15 K).

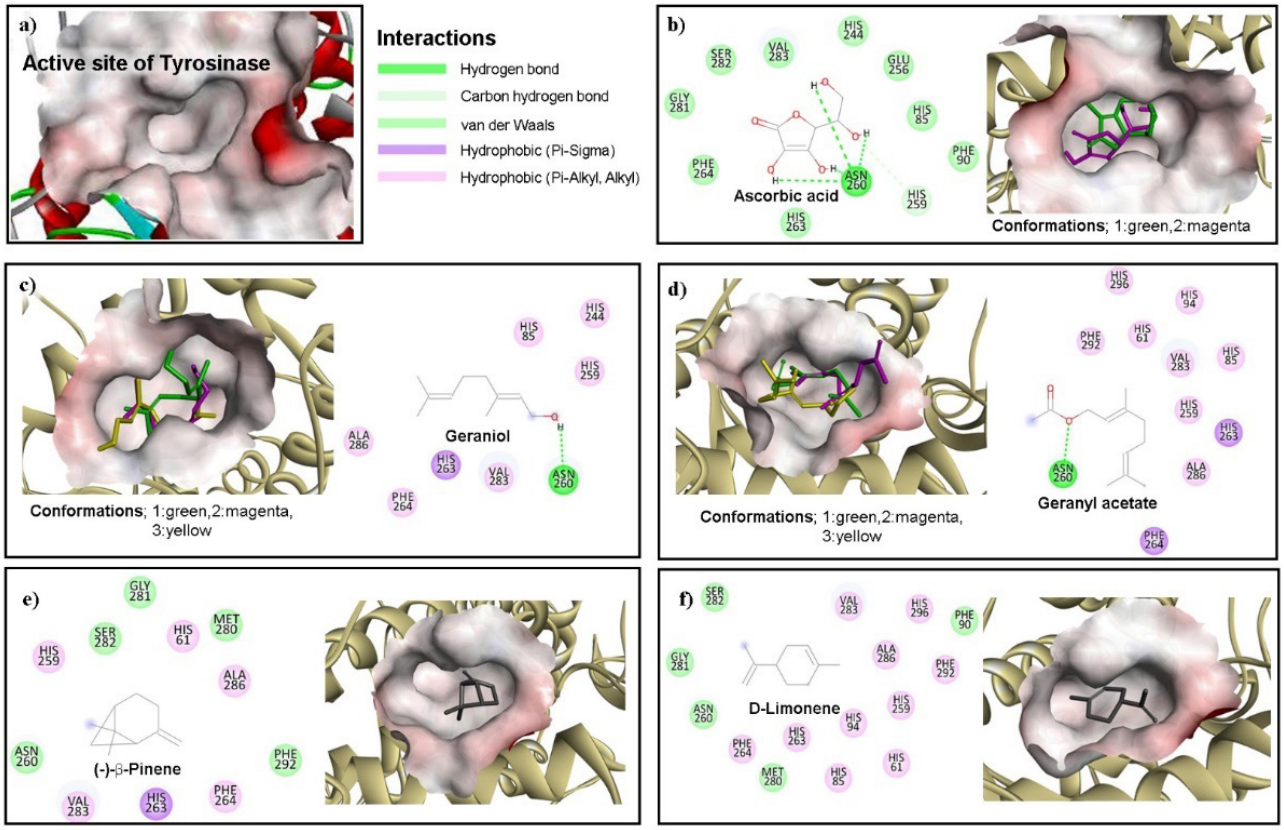

Figure 8 Post docking structures of bioactive compound with tyrosinase, active site of tyrosinase (a), 3D structures and binding interaction of ascorbic acid (b), Geraniol (c), Geranyl acetate (d), (-)-β-pinene (e), and D-Limonene (f).

Antibacterial activity test of essential oil from citronella grass and kaffir lime

Antimicrobial agents are widely used worldwide to treat various diseases. Many of these agents are derived from plants and other natural sources. Essential oils extracted from local herbs present an interesting option for studying antibacterial properties due to their availability, affordability, and relative safety [3,20,44]. This research aims to investigate the antibacterial activity of essential oils extracted from citronella grass and kaffir lime, local medicinal plants commonly found in households in Sakon Nakhon province, Thailand. The study evaluated the inhibitory effects of these essential oils against Gram-positive bacteria, including *Staphylococcus aureus* TISTR 2329, *Staphylococcus epidermidis*, *Bacillus subtilis* TISTR 1248, and *Bacillus cereus*, as well as against the Gram-negative bacterium *Escherichia coli* TISTR 527. The antibacterial efficacy was tested by measuring the inhibition zone of each extract against Gram-positive and Gram-negative bacterial strains, as presented in **Table 6** and **Figure 9**. The study results indicated that the essential oil extracted from citronella grass exhibited superior inhibitory efficacy against all tested bacteria compared to the essential oil extracted from kaffir lime.

The experimental result can be explained by the chemical composition and structure of the essential oil extracted from citronella grass. This oil primarily contains compounds such as geraniol, geranyl acetate, α-citral, β-citral, (R)-(+)-citronellal, and citral diethyl acetal, which feature functional groups like ether, alcohol, ester, and aldehyde, with almost no aromatic compounds. Additionally, citronella grass essential oil is mainly composed of straight and branched-chain organic compounds, with few ring structures and aromatic groups. The chemical structure of these compounds can be divided into 2 main parts: The hydrophilic portion, which consists of various functional groups such as R–(C=O)–O–R, R–(C=O)–H, –OH, R–O–R, and R–(C=O)–R, and the hydrophobic portion, which is composed of both saturated and unsaturated hydrocarbon chains as shown

in the chemical structure in **Figure 10**. From the experimental results in **Table 6**, it was found that the essential oil from citronella grass has a strong inhibitory effect on both Gram-positive and Gram-negative bacteria, as indicated by the relatively large size of the inhibition zones. This phenomenon can be explained by the antibacterial mechanism of citronella grass essential oil, particularly its effect on the cell wall and cell permeability. According to various research reports, Gram-positive bacteria are more sensitive to hydrophobic compounds because the hydrophobic layer surrounding their cells may facilitate the entry of these molecules into the cell. Meanwhile, Gram-negative bacteria are more reactive to hydrophilic molecules, which allows these molecules to easily penetrate the outer membrane and enter the cell [44-47]. From the experimental results, it can be observed that the essential oil extracted from citronella grass contains main compounds with both hydrophilic and hydrophobic properties. This dual nature likely facilitates the penetration of the bacterial cell wall, contributing to the high efficiency observed in the antibacterial activity test.

Table 6 Antibacterial activity test of essential oil from citronella grass and kaffir lime.

Bacteria strains	**Diameter of inhibition zones (mm)**		
	Essential oil from citronella grass	**Essential oil from kaffir lime**	**Clindamycin (positive control)**
Gram-positive bacteria			
-*Staphylococcus aureus* TISTR2329	42.24	20.55	34.77
-*Staphylococcus epidermidis*	13.02	13.18	10.08
-*Bacillus subtilis* TISTR1248	33.79	16.27	20.75
-*Bacillus cereus*	32.44	15.74	25.10
Gram-negative bacteria			
-*Escherichia coli* TISTR 527	33.28	30.15	36.87

Notes: The negative control (DMSO) does not inhibition zones.

Additionally, various research reports support the experimental results regarding the antibacterial mechanism. Possible mechanisms by which essential oils interfere with bacterial proliferation include: 1) Disintegration of the bacterial outer membrane or phospholipid bilayer, 2) Alteration of the fatty acid composition, 3) Increased membrane fluidity leading to leakage of potassium ions and protons, 4) Interference with glucose uptake, and 5) Inhibition of enzyme activity or cell lysis [45,49,50]. Among all the possible mechanisms of bacterial inhibition, it is evident that the primary process by which citronella grass essential oil exhibits its excellent properties is through the penetration of the essential oil or disruption of the bacterial cell wall. These factors help explain the antibacterial activity observed in the essential oil extracted from kaffir lime. According to chemical composition and structure analysis by GC-MS and NMR techniques, the essential oil predominantly contains D-limonene, (-)-β-pinene, and γ-terpinene, which are non-polar (or low-polar) alkene functional groups with hydrophobic ring structures, making up about 70 % of the total compounds. While the oil also contains compounds with polar and hydrophilic R–(C=O)–O–R and –OH functional groups, these are still part of ring hydrocarbon structures and account for about 30 % of the total compounds as shown in the chemical structure in **Figure 10**. This directly impacts the efficiency of permeation and cell wall destruction of the bacteria used in this experiment, which is likely to be lower than that of the essential oil extracted from citronella grass.

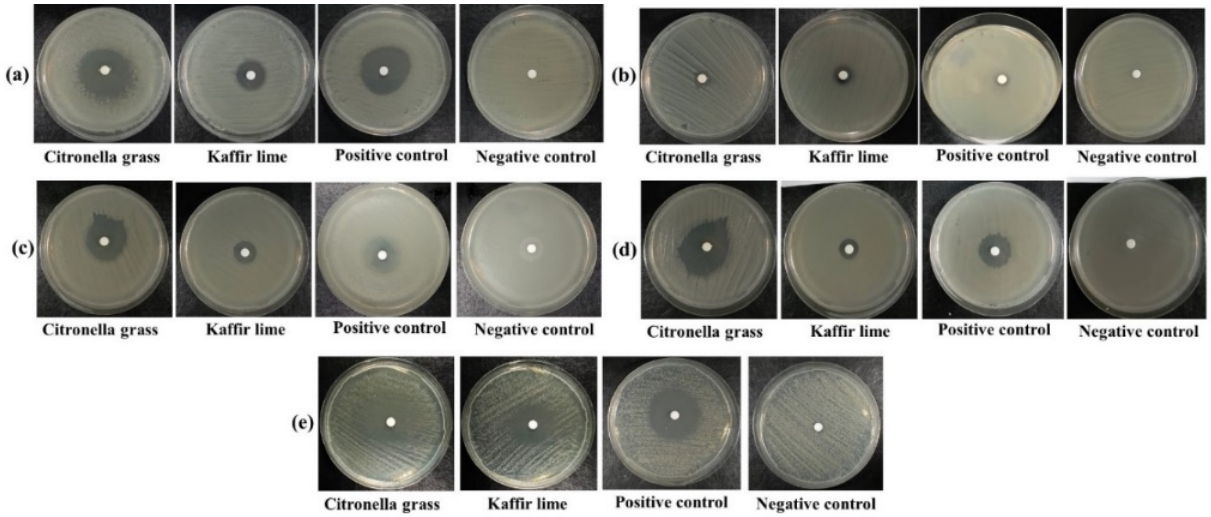

Figure 9 Inhibition zone of bacterial strains: (a) *Staphylococcus aureus* TISTR2329, (b) *Staphylococcus epidermidis*, (c) *Bacillus subtilis* TISTR1248, (d) *Bacillus cereus*, and (e) *Escherichia coli* TISTR 527 using various samples of essential oil from citronella grass and kaffir lime, clindamycin (positive control), and DMSO (negative control).

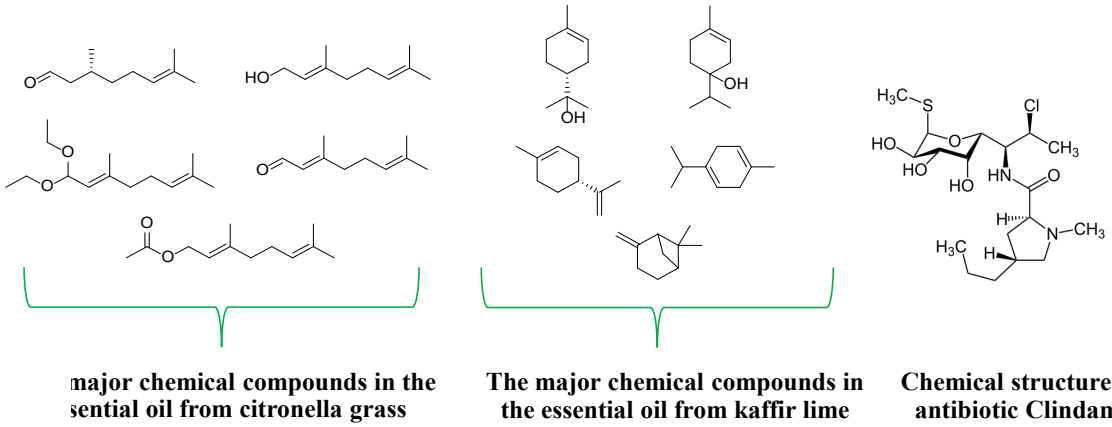

Figure 10 The chemical structures of the major compounds in the essential oils from citronella grass and kaffir lime, and the chemical structure of the antibiotic clindamycin.

Furthermore, this research compared the antibacterial activity of essential oils from citronella grass and kaffir lime with clindamycin, which was used as a positive control. Clindamycin is an antibiotic commonly used to treat bacterial infections by inhibiting bacterial growth in the body. It is often prescribed for conditions such as acne, skin infections, respiratory tract infections, oral infections, intestinal infections, and bacterial vaginosis, among others. Clindamycin is a lincosamide antibiotic with a complex chemical structure that includes an amino sugar (methylthio-lincosamide) featuring a thioether group (–S–) and an amino group ($–NH_2$), both essential for its activity. It also contains a pyrrolidine ring, a 5-membered nitrogen-containing structure that adds rigidity and enhances binding. A chlorinated phenyl group increases the molecule's hydrophobicity, aiding its penetration into bacterial cells. The core structure of clindamycin is derived from lincomycin, with a modification at the 7-position—replacing a hydroxyl group with a chlorine atom—to enhance its antibacterial potency as displayed in the chemical structure in **Figure 10**. This balanced hydrophilic and hydrophobic structure allows clindamycin to effectively bind to bacterial ribosomes, inhibiting protein synthesis and preventing bacterial growth [41-54].

The experimental results showed that essential oil from citronella grass exhibits better antibacterial activity against Gram-positive than clindamycin, while Gram-negative bacteria are inhibited similarly to clindamycin.

The explanation for this experimental result is that clindamycin has a larger molecular structure compared to the compounds found in citronella grass essential oil, making it less accessible for interaction with the bacterial cell wall, despite having more functional groups. Consequently, when comparing the number of molecules per unit surface area of the bacterial cell wall, smaller molecules are more likely to contact the bacterial cell wall than larger molecules. In other words, larger molecules experience a steric hindrance that limits their ability to contact and penetrate the bacterial cell wall.

Antibacterial stability test of essential oil from citronella grass and kaffir lime

In this experiment, *Staphylococcus aureus* was chosen as the test bacteria because it naturally resides on human skin and is a common cause of skin inflammation. If hands are not properly cleaned before handling food, this bacterium can contaminate the food, leading to ingestion. Once inside the body, *Staphylococcus aureus* produces a heat-resistant toxin called enterotoxin, which can cause food poisoning within 1 - 6 h of consumption. Symptoms include sudden onset of nausea, vomiting, diarrhea, and stomach pain, typically without fever. In severe cases, shock may occur [55-58]. Another important reason is the formation of the inhibition zone for *Staphylococcus aureus* when using essential oils from citronella grass and kaffir lime in the test. The inhibition zone is clearer and more distinct than that observed with other bacteria in the test. Therefore, the efficacy against *Staphylococcus aureus* was tested after 6 days of incubation by measuring the diameter of the inhibition zone around each disk to assess antibacterial activity every day. The experimental results are shown in **Figure 11(a)**. On the 1st day of the test, it was observed that the inhibition of *Staphylococcus aureus* by essential oil from citronella grass was greater than that achieved with both clindamycin and essential oil from kaffir lime. However, on the 2nd day of the test, it was found that essential oil from citronella grass had reduced antibacterial activity by approximately 45 %, which was lower than that of clindamycin but still higher than essential oil from kaffir lime. The decreasing trend in antibacterial efficacy of kaffir lime essential oil was similar, whereas clindamycin exhibited a consistent efficacy. From the 3rd to the 6th day of the experiment, the 3 antibacterial agents displayed a consistent trend, with only slight decreases in efficacy. **Figures 11(b) - 11(e)** shows the inhibition zones of essential oils from citronella grass and kaffir lime after 1 day and 6 days of incubation.

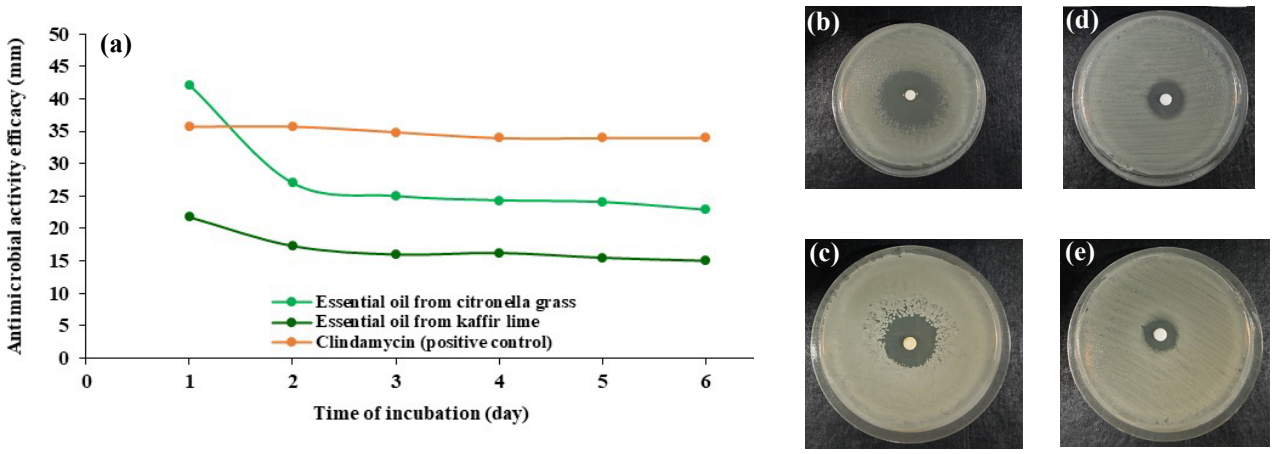

Figure 11 (a) antimicrobial efficacy test against *Staphylococcus aureus* TISTR2329 after 6 days of incubation. Inhibition zone of essential oil from citronella grass (b) after 1 day and (c) after 6 days of incubation. Inhibition zone of essential oil from kaffir lime (d) after 1 day and (e) after 6 days of incubation.

These study results suggest that the chemical structures of the major compounds in the essential oils from citronella grass contain both hydrophilic and hydrophobic components. Their small molecular size allows them to effectively penetrate and destroy bacterial cell walls, particularly during the 1st day of incubation. However, it is predicted that after the 1st day, the concentration of antibacterial active molecules decreases, leading to a reduction in inhibition on the 2nd day of incubation, with a slight decline that stabilizes from the 3rd to 6th days. This phenomenon was also observed in the essential oil samples from kaffir lime. On the other hand, although the antibiotic clindamycin exhibits less inhibitory effect against *Staphylococcus aureus* than the essential oils from citronella grass on the 1st day, its inhibitory effect remains stable from the 2nd to the 6th day of incubation. This stability is likely due to the more diverse functional group composition in the chemical structure of clindamycin compared to the essential oils from citronella grass and kaffir lime, as shown in **Figure 10**. Therefore, when comparing individual molecules, it can be observed that a single molecule of clindamycin may be capable of binding to more than 1 bacterial cell wall. This ability is a key factor contributing to the high stability of clindamycin's antibacterial efficacy. From all these experimental data, it can be concluded that the key findings of this work are as follows: Antibacterial compounds with simple chemical functional groups and small molecular sizes can act quickly due to their rapid permeability and ability to cleave cell walls, but their inhibition stability decreases over time. Conversely, antibacterial compounds with complex chemical functional groups and larger molecular sizes may exhibit lower initial inhibition activity, but their inhibition stability remains consistent over time.

Examples of the application of essential oils from citronella grass and kaffir lime as ingredients in various products

The final topic of this research focuses on the application of essential oils from citronella grass and kaffir lime in various products, making it both appropriate and relevant. The data were collected from the Bueng-Thawai Subdistrict area in Tao Ngoi District, Sakon Nakhon Province, where the raw materials of citronella grass and kaffir lime were initially studied. This topic highlights the practical applications and potential benefits of these essential oils, offering insights into their commercial and everyday uses. It also aligns with the growing trend toward natural and sustainable products. Examples of their applications include the production of soaps, creams, and lotions, where they are generally used in amounts of approximately 1 - 3 %. In mosquito repellent sprays, they are typically added at 5 - 10 % by weight, depending on individual preference. They are also used in disinfectant sprays, herbal massage oils, and multipurpose cleaners, as shown in **Figure 12**. The essential oils from citronella grass and kaffir lime can be incorporated into soap products to enhance both fragrance and therapeutic properties. Citronella grass oil is valued for its mosquito-repellent and antibacterial qualities, while kaffir lime oil adds a refreshing scent. In skincare, citronella grass oil's antibacterial properties make it effective for acne treatments. It is also widely used in mosquito repellent products due to its insect-repelling capabilities and high antibacterial activity. On the other hand, kaffir lime oil provides antioxidant and tyrosinase-inhibiting properties that can improve overall skin health. Although its antibacterial properties are less pronounced compared to citronella grass oil, it's refreshing scent makes it suitable for inclusion in disinfectant sprays. Both essential oils can be blended into massage oils, with citronella grass oil assisting in muscle relaxation and kaffir lime oil offering a refreshing, invigorating experience. Additionally, citronella grass oil is effective in cleaning products due to its antibacterial properties and pleasant scent. Exploring the use of these natural essential oils highlights their advantages over synthetic alternatives and emphasizes the value of local herbs in creating effective and eco-friendly products.

Figure 12 Examples of their applications include the production of soaps, creams, lotions, mosquito repellent sprays, disinfectant sprays, herbal massage oils, and multipurpose cleaners produced in the Bueng-Thawai Subdistrict area, Tao Ngoi District, Sakon Nakhon province.

Conclusions

The study on essential oils from citronella grass and kaffir lime, extracted using a prototype distiller, revealed yields of 1.67 and 2.36 %, respectively. GC-MS and NMR analysis identified the main components of citronella grass oil as geraniol (35.99 %), geranyl acetate (10.73 %), and citrals, while kaffir lime oil primarily contained D-limonene (20.72 %) and (-)-β-pinene (20.19 %). Antioxidant tests (DPPH$^{\bullet}$, ABTS$^{\bullet+}$, FRAP) showed that both oils had lower efficacy than Trolox, likely due to their non-polar nature. However, citronella grass oil was more effective than kaffir lime oil due to its straight and branched-chain structures and functional groups that readily donate electrons. In tyrosinase inhibition, kaffir lime oil (IC_{50} of 195.68 µg/mL) was over twice as effective as citronella grass oil (IC_{50} of 380.45 µg/mL). This greater efficacy is attributed to the small, ring-structured molecules in kaffir lime oil, which interact more effectively with the tyrosinase enzyme, in contrast to the branched-chain structures in citronella grass oil. These findings are supported by molecular docking studies. Molecular docking studies of the most abundant bioactive compounds in both essential oils revealed that, although the compounds from kaffir lime exhibited lower binding energy compared to those from citronella grass, they demonstrated significantly higher binding site specificity (lock and key model). This increased specificity is primarily attributed to major hydrophobic interactions between the kaffir lime compounds and the enzyme's active site. These interactions suggest that the essential oils from kaffir lime are likely more effective at binding to the tyrosinase enzyme, indicating a greater potential for inhibiting its activity compared to the essential oils from citronella grass.

The antibacterial activity test revealed that citronella grass essential oil was more effective than kaffir lime oil and clindamycin. This enhanced efficacy is due to the smaller molecular size of citronella grass oil's compounds, which better interact with bacterial cell walls compared to the larger clindamycin molecules. In the stability test, citronella grass oil initially showed greater effectiveness against *Staphylococcus aureus* than both kaffir lime oil and clindamycin. However, its efficacy decreased by about 45 % on the 2nd day, falling below clindamycin but still above kaffir lime oil. All 3 agents maintained consistent activity from the 3rd to 6th day, with only minor declines. In summary, antibacterial compounds with simple, small molecules act quickly but lose efficacy over time, while those with complex, larger molecules have more stable long-term inhibition despite lower initial activity.

Additionally, the essential oils from citronella grass and kaffir lime exhibit properties comparable to or exceeding those of standard synthetic chemicals. This makes them valuable for further development and application in a variety of community products. These essential oils, extracted using a prototype distiller, can be utilized in the production of items such as soaps, creams, lotions, mosquito repellent sprays, disinfectant sprays, herbal massage oils, and multipurpose cleaners. Incorporating these safe local herbs not only promotes the use of natural ingredients but also enhances the value of these indigenous plants.

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