

ANTIBACTERIAL ACTIVITY OF VETIVER, CITRONELLA, AND EAST INDIAN LEMONGRASS ESSENTIAL OILS AGAINST *BACILLUS SUBTILIS*: A PRELIMINARY DISK-DIFFUSION STUDY

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Abstract

Antimicrobial resistance has increased interest in plant-derived antibacterial agents. This study aimed to evaluate and descriptively compare the antibacterial activities of Vetiver (*Chrysopogon zizanioides*), Citronella (*Cymbopogon nardus*), and East Indian lemongrass (*Cymbopogon flexuosus*) essential oils against *Bacillus subtilis*. Preliminary antibacterial screening was conducted using an adapted disk-diffusion method. Gentamicin and sterile distilled water served as positive and negative controls, respectively. Inhibition-zone diameters were measured after 24 h and reported as mean $\pm$ standard deviation from three independent biological replicates. All three oils produced measurable inhibition zones. Vetiver oil had the largest observed mean zone among the oils (17.33 ± 1.53 mm), followed by East Indian lemongrass oil (15.00 ± 1.00 mm) and Citronella oil (13.67 ± 1.53 mm). Gentamicin produced a mean

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zone of 24.33 ± 0.58 mm. Sterile distilled water produced no inhibition beyond the 6-mm disk. Because no inferential statistical analysis was performed, treatment differences should be interpreted descriptively rather than as statistically significant. The findings show that the three oils inhibited *B. subtilis* under the tested conditions. Further studies should chemically characterize the tested oil batches, reproduce the screening findings, and evaluate additional Gram-positive and Gram-negative bacterial species.

Keywords: Essential oil, *Bacillus subtilis*, Antibacterial activity, Vetiver, Disk diffusion

Introduction

Antimicrobial resistance reduces the effectiveness of conventional antibiotics and contributes to persistent bacterial infections (Touati et al., 2025). These challenges have increased interest in plant-derived antimicrobial agents. Essential oils are complex mixtures of volatile phytochemicals, including terpenes, phenols, aldehydes, and alcohols, and several have demonstrated antibacterial activity (Reichling, 2020; Touati et al., 2025).

Essential oils from Poaceae species are of particular interest. Citronella (*Cymbopogon nardus*) oil commonly contains citronellal, citronellol, and geraniol, whereas East Indian lemongrass (*Cymbopogon flexuosus*) oil is generally rich in citral, principally geraniol and neral (Gao et al., 2020; Elmi et al., 2025). Published analyses of Vetiver (*Chrysopogon zizanioides*) oil report sesquiterpenoid constituents such as khusimol, vetiverol, and alpha-vetivone (Thongmee et al., 2025). These literature-derived profiles are summarized in Table 1; the chemical composition of the batches used in this experiment was not measured.

Previous studies have reported antibacterial effects of Poaceae-derived essential oils against Gram-positive and Gram-negative organisms (Budzyńska et al., 2011; Gao et al., 2020; Touati et al., 2025). However, direct comparisons

of Vetiver, Citronella, and East Indian lemongrass oils against *B. subtilis* are limited. Although *B. subtilis* can form biofilms, the present experiment assessed only growth inhibition in a disk-diffusion assay and did not assess biofilm formation, inhibition, or eradication. Therefore, this study aimed solely to evaluate and descriptively compare the antibacterial activities of the three oils against *B. subtilis* and to identify patterns warranting further quantitative investigation.

Table 1. Major phytochemical constituents reported in previous literature

Essential oil	Scientific name	Major reported constituents*
Vetiver	<i>Chrysopogon zizanioides</i>	Khusimol, vetiverol, α -vetivone
Citronella	<i>Cymbopogon nardus</i>	Citronellal, citronellol, geraniol
East Indian lemongrass	<i>Cymbopogon flexuosus</i>	Citral (geranial and neral), myrcene

* Compiled from previous studies (Elmi et al., 2025; Thongmee et al., 2025). These constituents were not independently quantified in the present study.

Materials and Methods

Study variables

The independent variable was the essential-oil treatment. The dependent variable was inhibition-zone diameter. Controlled conditions included the bacterial inoculum, incubation temperature and duration, disk diameter, agar medium, and nominal treatment volume.

Essential oils

Commercial essential oils of Vetiver (*Chrysopogon zizanioides*), Citronella (*Cymbopogon nardus*), and East Indian lemongrass (*Cymbopogon flexuosus*) were obtained from Chemipan Corporation Co., Ltd. (Bangkok, Thailand). The oils were used without further dilution and stored in sealed amber-glass bottles at 4 °C until use. Available product-identification and traceability information is summarized in Table 2.

Table 2 Identification and traceability information for the essential-oil products used in the study

Essential oil	Botanical source and plant part	Product description	Supplier and product identifier	Batch/lot number
Vetiver	<i>Chrysopogon zizanioides</i> ; roots/rootlets ¹	Steam-distilled; supplier-designated Pure Essential Oil ²	Chemipan Corporation Co., Ltd., Thailand; Product ID 989; code AB2203-A ³	Not recorded; could not be recovered
Citronella	<i>Cymbopogon nardus</i> ; leaves/aerial parts ¹	Steam-distilled; supplier-designated Pure Essential Oil ²	Chemipan Corporation Co., Ltd., Thailand; AB0307; Product ID 173 ³	Not recorded; could not be recovered
East Indian lemongrass	<i>Cymbopogon flexuosus</i> ; leaves/aerial parts ¹	Steam-distilled; supplier-designated Pure Essential Oil; supplier specification: total citral <80% ²	Chemipan Corporation Co., Ltd., Thailand; AB1204; Product ID 181 ³ ; origin listed as India	Not recorded; could not be recovered

¹ Plant parts are the conventional source materials for these oils but were not independently verified for the tested bottles.

² Product description and extraction method were reported by the supplier and were not independently verified; the oils were not chemically characterized by GC-MS.

³ Product identifiers were obtained from Chemipan product documentation.

The lot numbers of the experimental bottles were not recorded; therefore, publicly available certificate batch numbers were not assigned to the tested samples.

Bacterial strain and inoculum preparation

Bacillus subtilis TISTR 008 was obtained from the Thailand Institute of Scientific and Technological Research, Pathum Thani, Thailand, and used as the test organism. The reference stock strain was maintained on nutrient-agar slants at 4 °C and subcultured before each bioassay. A single isolated colony was inoculated into 5 mL of sterile nutrient broth and incubated aerobically at 37 °C

with shaking at 150 rpm for 18–24 h. The bacterial suspension was adjusted with sterile 0.85% (w/v) physiological saline to match a 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL, and was used within 15 min of preparation.

Disk-diffusion screening assay

Preliminary antibacterial screening was performed using an agar disk-diffusion procedure adapted from Maleki & Bakhsh (2023), with modifications to the agar medium, essential-oil loading volume, control preparations, incubation duration, and number of independent biological replicates. Mueller–Hinton agar plates containing 20 mL of medium at an approximate depth of 4 mm were uniformly inoculated with the standardized bacterial suspension using sterile cotton swabs streaked in three directions to produce confluent growth. The assay was used for comparative screening only; clinical interpretive breakpoints were not applied.

Sterile Whatman No. 1 filter-paper disks (6-mm diameter) were loaded with 20 μ L of undiluted essential oil in two successive 10- μ L aliquots and allowed to absorb under aseptic conditions for 5 min to minimize direct runoff onto the agar. Gentamicin sulfate solution (100 μ g/mL) was applied at 10 μ L per disk, providing a final dose of 1 μ g/disk, as the positive control. Sterile distilled water (20 μ L/disk) served as the negative control. The loaded disks were placed on the inoculated agar with sterile forceps and pressed gently to ensure complete contact. Plates were inverted and incubated aerobically at 37 °C for 24 h.

Following incubation, clear zones of inhibition were measured across two perpendicular axes using a precision digital vernier caliper (Mitutoyo, Japan; accuracy ± 0.01 mm), including the 6 mm disk diameter. For disks with no detectable halo, the value was recorded as 6.00 mm (indicating 0 mm inhibition beyond the disk). All assays were conducted in three independent biological replicates performed on separate days.

Data analysis

Inhibition-zone diameters were summarized as mean $\pm$ standard deviation. Given $n = 3$ and the preliminary screening design, no inferential statistical tests were performed. Treatment comparisons are therefore descriptive and do not establish statistically significant differences.

Biosafety considerations

Standard aseptic technique and laboratory biosafety practices were used. Culture media, bacterial cultures, and contaminated materials were autoclaved before disposal.

Results

Antibacterial activity against *Bacillus subtilis*

All three essential oils produced visible inhibition zones against *B. subtilis* under the tested disk-diffusion conditions. The gentamicin positive control also produced a visible inhibition zone, whereas the sterile distilled-water negative control produced no inhibition beyond the paper disk.

The mean inhibition-zone diameters are presented in Table 3. Gentamicin produced a mean zone diameter of 24.33 ± 0.58 mm. Among the essential oils, Vetiver oil had the largest observed mean inhibition zone (17.33 ± 1.53 mm), followed by East Indian lemongrass oil (15.00 ± 1.00 mm) and Citronella oil (13.67 ± 1.53 mm).

The distilled-water control produced a recorded diameter of 6.00 ± 0.00 mm, which corresponded to the diameter of the paper disk itself. Therefore, this result represented 0 mm of inhibition beyond the disk and was interpreted as no detectable antibacterial activity.

Based on the observed mean values, the descriptive order of inhibition-zone diameter was:

Gentamicin > Vetiver > East Indian lemongrass > Citronella > distilled water

This ordering represents a descriptive comparison only. Because the study included three independent biological replicates and no inferential statistical analysis, the observed differences should not be interpreted as statistically significant.

Table 3 Disk-diffusion screening results against *Bacillus subtilis*

Treatment	Zone diameter (mm), mean $\pm$ SD	Interpretation
Vetiver (<i>C. zizanioides</i>)	17.33 $\pm$ 1.53	Measurable inhibition zone
East Indian lemongrass (<i>C. flexuosus</i>)	15.00 $\pm$ 1.00	Measurable inhibition zone
Citronella (<i>C. nardus</i>)	13.67 $\pm$ 1.53	Measurable inhibition zone
Gentamicin (1 μ g/disk)	24.33 $\pm$ 0.58	Measurable inhibition zone
Distilled water	6.00 $\pm$ 0.00	No inhibition beyond disk

Note: Reported zone diameters include the 6-mm paper disk. Results were obtained from three independent biological replicates and were analysed descriptively.

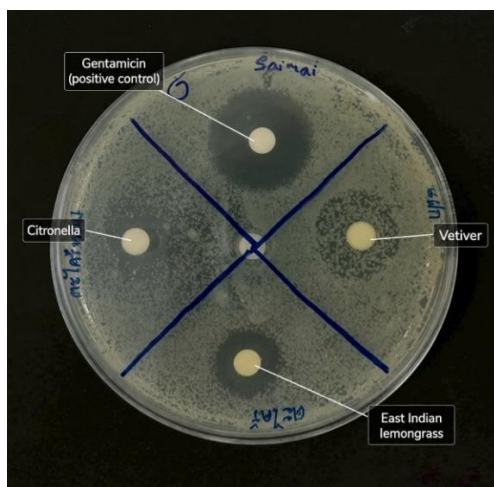


Figure 1 Representative disk-diffusion screening of essential oils against *Bacillus subtilis*. Disk positions were as follows: gentamicin positive control, top; Citronella, left; Vetiver, right; and East Indian lemongrass, bottom.

The sterile distilled-water negative control was tested separately and is not shown.

Discussion

The present study provides preliminary evidence that Vetiver (*Chrysopogon zizanioides*), Citronella (*Cymbopogon nardus*), and East Indian lemongrass (*Cymbopogon flexuosus*) essential oils inhibit *Bacillus subtilis* under the tested disk-diffusion conditions. All three oils produced measurable inhibition zones, whereas sterile distilled water produced no inhibition beyond the paper disk. Vetiver oil had the largest observed mean zone among the oils, followed by East Indian lemongrass and Citronella oils. Because the comparisons were descriptive, this ordering should not be interpreted as demonstrating statistically significant differences.

The observed activity is broadly consistent with evidence that essential oils derived from Poaceae species can inhibit bacterial growth (Budzyńska et al., 2011; Gao et al., 2020; Elmi et al., 2025; Touati et al., 2025). Essential oils contain complex mixtures of volatile constituents that may affect bacterial membranes, permeability, enzymes, and cellular metabolism (Reichling, 2020; Touati et al., 2025). These mechanisms were not investigated directly; therefore, the inhibition observed here cannot be attributed to a specific mechanism.

Published analyses indicate that Vetiver oil may contain sesquiterpenoids, whereas Citronella and East Indian lemongrass oils commonly contain citronellal-, geraniol-, and citral-related compounds (Gao et al., 2020; Elmi et al., 2025; Thongmee et al., 2025). However, the experimental oil batches were not analysed by gas chromatography–mass spectrometry. The observed effects therefore cannot be assigned to individual constituents or presumed compositional differences among the oils.

Disk-diffusion findings require cautious interpretation because inhibition-zone diameter reflects both bacterial growth inhibition and diffusion of the tested material through agar. The hydrophobicity, molecular size, polarity, and volatility of essential-oil constituents can influence diffusion; consequently, a larger zone does not necessarily establish greater intrinsic antibacterial potency

(Balouiri et al., 2016). Gentamicin and the essential oils also differ in composition, dose, solubility, and diffusion behaviour and should not be interpreted as therapeutically equivalent.

This study assessed inhibition of planktonic bacterial growth only. Although *B. subtilis* can form biofilms, no biofilm-formation, inhibition, or eradication assay was performed. Accordingly, the disk-diffusion findings provide no evidence of antibiofilm activity (Budzyńska et al., 2011; Kavanaugh & Ribbeck, 2012).

Several limitations should be considered. Only one bacterial species was tested, so the findings cannot establish an antibacterial spectrum or be generalized to other Gram-positive or Gram-negative organisms. Only three independent biological replicates were conducted, and no inferential statistical analysis was performed. Disk diffusion is also influenced by agar-diffusion behaviour and provides only preliminary comparative screening. Furthermore, the lot numbers of the tested products were not recorded, the product information was not independently authenticated, and the chemical composition of the experimental batches was not verified. Cytotoxicity, stability, formulation characteristics, and safety were not evaluated. These limitations preclude clinical, pharmaceutical, food-preservation, or industrial claims.

Future work should reproduce the disk-diffusion findings using fully traceable, chemically characterized oil batches, a larger number of independent biological replicates, and additional Gram-positive and Gram-negative bacterial species. If antibiofilm or practical applications are proposed, those properties should be evaluated directly using appropriate biofilm, stability, and safety assays.

Overall, the three essential oils produced measurable inhibition zones against *B. subtilis* under the specified conditions. Vetiver oil had the largest observed mean zone among the oils, but the descriptive findings do not establish statistically significant differences or relative antibacterial potency. The results

should therefore be interpreted as preliminary, organism-specific screening evidence.

Conclusion

Under the tested disk-diffusion conditions, Vetiver, Citronella, and East Indian lemongrass essential oils produced measurable inhibition zones against *Bacillus subtilis*. Vetiver oil had the largest observed mean zone among the three oils; however, the results were descriptive and do not establish statistically significant differences or relative antibacterial potency. The conclusions are restricted to preliminary growth-inhibition screening of *B. subtilis* under the specified conditions and should not be generalized to other bacterial species, antibiofilm activity, safety, or practical application.

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