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In Vitro Antibacterial Activity of Calamansi Peels and Lemongrass Leaves Ethanol Extracts Against *Escherichia coli*

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ABSTRACT

This study investigated the antibacterial potential of locally abundant plant materials in the Philippines, focusing on calamansi peel (*Citrus microcarpa*) agro-waste and lemongrass (*Cymbopogon citratus*) leaves as sustainable alternatives to synthetic antibiotics amid rising antimicrobial resistance. Despite known individual antimicrobial properties, limited evidence exists on their combined effects against *Escherichia coli*. The study aimed to determine and compare the antibacterial activity of individual and combined ethanol extracts and assess whether their interaction is synergistic or indifferent. A quantitative experimental design was employed using the Kirby–Bauer disk diffusion method. Ethanol extracts of calamansi peels, lemongrass leaves, and their combination were tested against a standardized *E. coli* culture with positive and negative controls, each in triplicate. Zones of inhibition (mm) were measured and analyzed using mean, standard deviation, one-way ANOVA, and Tukey's HSD at a 0.05 significance. The results revealed mean zones of inhibition of 17.33 mm for calamansi ethanol extract, 18.00 mm for lemongrass ethanol extract, and 18.33 mm for the combined ethanol extract. Although the combined extract exhibited the highest mean inhibition, statistical analysis indicated no significant difference among the three plant treatments, suggesting an indifferent interaction rather than synergistic effect. The findings highlight the potential of calamansi peels and lemongrass leaves as natural antibacterial agents and support their application in eco-friendly disinfectant formulations. Future studies should determine minimum inhibitory concentrations and conduct phytochemical profiling to strengthen product development.

Keywords: Calamansi, lemongrass, antibacterial activity, *Escherichia coli*, indifferent interaction

Introduction

Calamansi (*Citrus microcarpa*) or known as Philippine lime is a famous citrus variety fruit grown mainly in Southeast Asia and in the Philippines. Cultivation of this fruit spans thousands of hectares, particularly in areas like MIMAROPA, Central Luzon, and Ilocos Region. Processing calamansi in the Philippines produces large amounts of peel waste, because producers generally focus on extracting juice and often discard the peels (Ramoran, 2024). Due to high demand for calamansi products in the Philippines, it produces significant solid waste, consisting of thrown away peels.

Calamansi peels which are usually thrown away are known for its strong citrusy scent that contributes to its natural antibacterial and antifungal properties (Tenoc et al., 2025). In addition to its antimicrobial properties, calamansi peels contains rich bioactive compounds such as flavonoids, phenols, and essential oils that exhibit antibacterial activity (Karthikeyan et al., 2023; Munekata et al., 2023). Husni et al. (2021) finds that calamansi fruit peels and leaves essential oils have antibacterial properties and can fight off against certain gram-negative bacteria. Begafria et al. (2025) reported significant inhibition of bacterial growth following exposure to methanolic and aqueous extracts of calamansi peels. The reutilization of calamansi peels therefore presents an opportunity to reduce agro-industrial waste while developing sustainable, value-added antibacterial products.

Lemongrass (*Cymbopogon citratus*) is a perennial tropical grass characterized by its tall stature of at least 2 meters, narrow leaves, and distinctive lemon-like aroma, thriving in warm and humid environments and widely cultivated in tropical regions. It is a low-maintenance crop that is easy to cultivate and extensively utilized in culinary, medicinal, and agricultural applications, with its strong scent attributed to high concentrations of essential oils primarily in its leaves (Terra et al., 2017).

Lemongrass is increasingly recognized in the global and regional markets because of the rising demand for its bioactive compounds. Lemongrass leaves contain bioactive compounds such as citral (including geranial and neral), limonene, linalool, flavonoids, and phenolic compounds that are effective against certain types of Gram-negative bacteria (Gaba et al., 2020; Isabelly et al., 2025). Along with myrcene, it also enhances the plant's ability to support its applications in medicinal, food preservation, and health therapies (Barjees et al., 2024). It is well documented for its outstanding antibacterial, antifungal, and antioxidants activities and its effective inhibitory effects against pathogenic microorganism, highlighting its potential as antibacterial agent (Hazifa et al., 2022; Al-Hamdani et al., 2022).

In the Philippines, *Escherichia coli* (*E. coli*) remains a major public health concern due to its association with inadequate and poor water, sanitation, and hygiene (WASH) conditions which affect school children, studies in Metro Manila public schools reporting diarrhea rates up to 28% (Sangalang et al., 2022). Due to the increasing health risks associated with poor hygiene and sanitation in schools, the Department of Education implements the Water, Sanitation, and Hygiene in Schools (WinS) Program through DepEd Order No. 10, s 2016 (DepED, 2016). The program provides comprehensive policy and guidelines to promote proper hygiene and sanitation in schools ensuring a clean environment in and around school for the health and safety of the students. In this, the development of plant-based natural antibacterial agents from locally available sources may support sanitation and hygiene initiatives in schools.

The rise of antimicrobial resistance (AMR) has become a global health concern, as many bacteria are increasingly resistant to conventional antibiotics. Misuse and overuse of antibiotics in humans and animals have accelerated this problem, reducing the effectiveness of standard treatments (Tiwari et al., 2025). Multiple sources document concrete mortality data: bacterial AMR caused 4.95 million deaths worldwide in 2019, with 1.27 million directly attributable to AMR, ranking it the third leading cause of death globally (Ahmad et al., 2025; Ranjbar & Alam, 2023).

Reported outbreaks of diseases caused by *E. coli* related to urinary tract and gastrointestinal infections and the rise of antimicrobial resistance (AMR) underscores the needs for a natural antibacterial agent amid rising antibiotic resistance in bacteria. This situations highlights the urgent need for plant-based alternatives, as natural extracts with antibacterial properties may provide safer, cost-effective, and sustainable options to combat resistant pathogens.

Combining plant extracts is often studied because different phytochemicals can interact to improve antimicrobial effectiveness, broaden the spectrum of activity, or reduce the risk of resistance. A combination can produce an indifferent interaction, where the overall activity is comparable to the individual extracts without significant enhancement or antagonism, or a synergistic effect, where the combined activity is significantly greater than the sum of each extract alone. Understanding these interactions is important in natural product research, as it helps identify more potent and efficient formulations from medicinal plants.

Although there are studies regarding their antimicrobial properties, there is a limited literature and study examining the individual and combined antimicrobial activity of calamansi peels and lemongrass leaves extracts. To date, limited studies have investigated the synergistic antibacterial activity of extracts from calamansi peel and lemongrass leaves specifically against *Escherichia coli*. Jeong et al. (2023) reported that combining plant extracts may enhance antimicrobial activity compared to single extracts. Though this has not been thoroughly researched and done between calamansi peels and lemongrass leaves.

This study aimed to address this gap by evaluating the individual and combined antibacterial activities of calamansi peels extracts and lemongrass leaves ethanol extracts against *Escherichia coli* using disk diffusion method. The purpose of this study is to generate evidence on the synergistic antibacterial effects of the locally available plant materials and to contribute to the knowledge of scientist on the development of natural plant-based antimicrobial agents from agro-waste. The findings of this study may contribute to public health initiatives, antimicrobial research, and sustainable utilization of agricultural waste in the Philippines.

This study also supports several United Nations Sustainable Development Goals (2015), particularly SDG 3 (Good Health and Well-Being), SDG 12 (Responsible Consumption and Production), and SDG 6 (Clean Water and Sanitation). By using agricultural waste products such as calamansi peels and exploring plant-based antimicrobial agents, this research promotes sustainable resource use while addressing public health concerns related to contamination, limit the use of synthetic chemical preservatives, and provide safe, natural alternatives that promote better health and responsible resource use.

The specific objectives of the study were (1) determine the inhibition zone diameter (mm) produced by the individual ethanol extracts from calamansi peels, lemongrass leaf, and their combination against *Escherichia coli*, (2) assess whether the combined extracts exhibit synergistic or indifferent antibacterial activity compared to the individual extracts, (3) determine if there is a significant difference in the mean zones of inhibition using Analysis of Variance (ANOVA) and Tukey's HSD test, and (4) propose potential applications or commercial products based on the results obtained.

Methods

Research Design

This study employed a quantitative experimental research approach to examine and compare the antibacterial activity of ethanolic extracts from calamansi peels (*Citrus microcarpa*), lemongrass leaves (*Cymbopogon citratus*), and the combined against *Escherichia coli*. The independent variable was the type of extracts used (calamansi peels ethanol extracts, lemongrass leaves ethanol extracts, and combined extracts), while the dependent variable was the diameter of the zones of inhibition measured in millimetres (mm).

Locale of the Study

The experiment was conducted at San Mariano National High School – Main located in San Mariano, Isabela. Laboratory procedures, including the preparation of plant extracts and antibacterial assays, were performed in coordination with the Department of Science and Technology – Region II (DOST-R02) laboratory, where appropriate facilities and equipment for microbiological analysis were available. The selected locale provided accessible plant materials such as Calamansi peels and Lemongrass leaves, as well as a controlled laboratory environment necessary to ensure the accuracy and reliability of the experimental procedures.

Participants/Test Organism

The test organism used in the conduct of the study was *E. coli,* a Gram-negative bacterium commonly used as an indicator organism in microbiological and public health research. A pure culture of *E. coli* was obtained and maintained at the DOST-R02 laboratory. The bacterial suspension was standardized to a 0.5 McFarland turbidity standard prior to inoculation to ensure uniform bacterial concentration across all experimental treatments.

Research Instrument

The various research instruments used to evaluate the antibacterial activity of the calamansi peels and the lemongrass leaves against *Escherichia coli* (*E. coli*) includes basic laboratory glassware and equipment such as the knife/scissor and Erlenmeyer flask are used for the preparation and handling of plant extracts and bacterial cultures. An extraction apparatus, including the blender or grinder and filtering materials (filter paper) is utilized to obtain extracts from the calamansi peels and the lemongrass leaves. Culture media and Petri dishes are used to cultivate *E. coli* and observe bacterial growth, while an incubator provided suitable conditions for bacterial incubation. Measuring instruments such as the digital weighing scale and liquid measuring cups are also used to ensure the accurate measurement of the needed materials. Additionally, we used the 95% ethanol as the extraction solvent, it dissolved and extract bioactive compounds from the plants.

Research Procedures

Preparation of Plant Extracts

Fresh calamansi peels and lemongrass leaves were washed thoroughly with clean water to remove surface dirt and contaminants. The plant materials were dried and then chopped into smaller pieces. Each plant material was weighed using a digital scale. For the individual treatments, 15 g of each plant material was used. For the combined treatment, 7.5 g of calamansi peels and 7.5 g of lemongrass leaves were mixed prior to extraction. Ethanol maceration was the extraction method employed following the general plant extraction procedure described by Guevarra (2005) stated at Teresita and F. Manuel (2014), which allows the diffusion of bioactive compounds from plant materials into the solvent. Ratio of 1:10 w/v is respectively used for the ethanol maceration of all the plant materials in three treatments (calamansi, lemongrass, and combined) for greater extraction of volatile compounds in the plant materials.

Each sample was soaked in a 150 mL of ethanol in labelled Erlenmeyer flasks and allowed to undergo maceration for more than 24 hours for better extraction of compounds. The resulting mixture was filtered using sterile filter paper to obtain the filtrates. The extracts were stored in sterile, labelled containers until use. The process was used for all the samples.

Antibacterial Assay (Disk Diffusion Method)

The antibacterial activity of the plant extracts was evaluated using the Kirby–Bauer disk diffusion method in accordance with the guidelines of the Department of Science and Technology Regional Standards and Testing Laboratory of Region 2 (DOST-RSTL II). Because the exact step-by-step procedural specifics were beyond the technical scope of the school's facilities, the standardized disk diffusion assay was outsourced performed by DOST RSTL II. The external laboratory handled the preparation of agar plates, uniform inoculation with the standardized *Escherichia coli* suspension, and the proper impregnation and placement of sterile 6 mm paper disks. Five experimental treatments were evaluated in the assay: (1) calamansi peel ethanol extract, (2) lemongrass leaf ethanol extract, (3) combined ethanol extracts of calamansi peel and lemongrass leaves, (4) a positive control, and (5) a negative control. Each treatment was tested in triplicate. After standard incubation at 37°C, the zones of inhibition surrounding each disk were observed and measured in millimeters by the DOST-R02 laboratory personnel to indicate the antibacterial activity of the extracts.

Measurement of Zones of Inhibition

After incubation, the diameter of clear zones of inhibition surrounding each disk was measured in millimeters using a ruler or digital caliper by the DOST-R02 personnel. Each measurement was taken at least twice to minimize error, and any variations were noted. The results for all test samples, including individual and combined extracts, were systematically recorded in a table and provided to the researchers. These data were then prepared for statistical analysis to compare the antibacterial effectiveness of the different treatments and to assess potential effects.

Data Analysis

The data collected in this study were analyzed through descriptive and inferential statistical methods to determine the antibacterial effectiveness of the ethanol extracts of calamansi peels, lemongrass leaves, and their combination against *Escherichia coli*, while antimicrobial activity was identified according to Guevarra's criteria:

Table 1.
Inference on the Results of the Zone of Inhibitions

Zone of Inhibition	Inferences
<10 mm	Inactive
10-13 mm	Partially Active
14-19 mm	Active
>19 mm	Very Active

The mean and standard deviation were computed to describe the average antibacterial activity and the variation among the treatments. A One-Way Analysis of Variance (ANOVA) was employed to determine the significant differences in the mean zones of inhibition among the five treatment groups. When the ANOVA results indicated a statistically significant difference, Tukey's Honestly Significant Difference (HSD) post-hoc test was applied to identify which specific treatments differed significantly from one another. All statistical analyses were conducted at a 0.05 level of significance to ensure the reliability and validity of the comparisons among treatments.

Results and Discussion

Antimicrobial Activity of Calamansi, Lemongrass, and Combined Extracts Against *Escherichia coli*

The antibacterial activity of the Calamansi, Lemongrass, and Combined Extracts against *Escherichia coli* was determined by measuring zones of inhibition (mm) using the disc diffusion method. The efficacy of the extracts was evaluated based on the diameter of the zones surrounding each sample, where a larger zone indicates higher antibacterial potency.

Table 2 summarizes the antimicrobial activity of the ethanol extracts against *Escherichia coli* as determined by the disc diffusion method. The calamansi peel ethanol extracts produced zones of inhibition measuring 18 mm, 15 mm, 19 mm, and a mean of 17.33 mm, which were interpreted as Active. The lemongrass leaves ethanol extracts yielded zones of 19 mm, 17 mm, 18 mm, and a mean zone of 18 mm, also classified as Active. The combined ethanol extracts from calamansi peels and lemongrass leaves showed zones of 19 mm, 20 mm, and 16 mm, displaying a mean of 18.33 mm, likewise interpreted as Active. In comparison, the positive control produced markedly the highest zones (mean = 34 mm) compared to the others, and was interpreted as Very Active, while the negative control consistently measured 6 mm, which corresponds to the diameter of the paper disk itself, indicating the absence of antibacterial activity beyond the disk and was interpreted as Inactive. Overall, the findings indicate that both individual ethanol extracts are effective against *E. coli*, while the combined extract yielded a slightly greater numerical inhibition than the individual extracts, suggesting an indifferent interaction between the bioactive compounds without significant enhancement or antagonism.

Table 2
Antimicrobial Activity of Extracts Against Escherichia coli

Sample Name	R1	R2	R3	Mean (mm)	Interpretation
Calamansi Peels Ethanolic Extracts	18	15	19	17.33	Active
Lemongrass Leaves Ethanolic Extracts	19	17	18	18.00	Active
Calamansi + Lemongrass Ethanolic Extracts	19	20	16	18.33	Active
Positive Control	35	33	34	34.00	Very Active
Negative Control	6	6	6	6.00	Inactive

Note: >19 mm = Very Active; 14-19 mm = Active; 10-13 mm = Partially Active; and <10 mm = Inactive

The ethanol extracts of calamansi peels and lemongrass leaves demonstrated clear antibacterial activity against *E. coli*, producing mean inhibition zones of 17.33 mm and 18.00 mm, respectively, and both were classified as Active. These outcomes align with or exceed those reported in comparable studies. For instance, ethanol extracts from calamansi leaves produced a mean inhibition zone of 5.73 mm against *E. coli* at 40% ethanol concentration (Oktasila et al., 2020), while calamansi peels extract have showed zones of 14.4 mm at 24 hours exposure against *E. coli* (Tenoc et al., 2025). The lemongrass leaves extracts performed in line with established literature on *Cymbopogon citratus*, which has demonstrated antibacterial activity against *E. coli* using the disc diffusion method (Hamdia & Al-Hamdani, 2022; Nyamath & Karthikeyan, 2018). The three extract treatments indicate that the observed effects likely arise from bioactive components present in the plant materials rather than measurement variability. Based on existing literature, the primary compounds presumed to be responsible for this include flavonoids and phenolic acids in calamansi peels (Venkatachalam et al., 2023), and citral, the dominant component in lemongrass leaves. These components disrupt bacterial cell membranes, causing the bacteria to stop their growth and multiplication. The positive control validated the susceptibility of the test organism, while the negative control confirmed that the extraction solvent did not contribute to the observed inhibitions.

Statistical Analysis of Antimicrobial Activity of Calamansi, Lemongrass, and Combined Extracts Against *Escherichia coli*

A one-way ANOVA using Welch's approach was conducted to identify whether there were statistically meaningful differences in the antibacterial activity among the five treatment groups: Calamansi peel extract, Lemongrass leaf extract, their combination, and a positive and negative control.

Results from the analysis confirmed a significant difference among the treatments ($F = 496.3$, $p < 0.001$). Since the p-value is below the 0.05 threshold, the null hypothesis (H_o) was rejected. Post-hoc Tukey HSD comparisons indicated that the three plant-derived extracts were statistically equivalent, as seen by the similarity of their superscript (b), with means of 17.33 ± 2.08 mm (Calamansi), 18.00 ± 1.00 mm (Lemongrass), and 18.33 ± 2.08 mm (Combined extracts). In contrast, the negative control differed significantly from all extract treatments.

Table 3

One-Way ANOVA Summary of Antibacterial Activity Against Escherichia coli

Treatments	N	Mean (mm)	SD	F	*p*	Decision
Calamansi Peels Ethanol Extracts	3	17.33^b	2.08	496.3	<.001	Reject H_o
Lemongrass Leaves Ethanol Extracts	3	18.00^b	1.00			
Calamansi + Lemongrass Ethanol Extracts	3	18.33^b	2.08			
Positive Control	3	34.00^a	1.00			
Negative Control	3	6.00^c	0.00			

Post-hoc comparison placed all three extract treatments (calamansi peels, lemongrass leaves, and their combined extracts) in the same statistical group (labeled by ^{b}), demonstrating that their antibacterial potencies are statistically equivalent. Although a previous Philippine comparative study reported that lemongrass leaf extract generally outperforms calamansi peels extract against *E. coli* when tested individually (Andreasson et al., 2016), the statistical analysis in the present study demonstrated no significant difference between the two individual extracts. Furthermore, the statistical equivalence among the treatments shows that while the combined extract exhibited a slightly higher mean zone of inhibition (18.33 mm) than the individual extracts, the difference is not statistically significant, suggesting an indifferent interaction rather than synergy. The findings of this study reinforce the potential of these locally abundant plant materials in the Philippines as sources of natural antimicrobial agents, particularly amid the rising antibiotic resistance of given pathogens.

Conclusion and Recommendations

This study demonstrated that ethanol extracts from calamansi peels (*Citrus microcarpa*) and lemongrass leaves (*Cymbopogon citratus*) exhibit measurable antibacterial activity against *Escherichia coli*. Both extracts inhibited bacterial growth when tested individually, while the combined extract produced a comparable level of inhibition. Statistical analysis showed no significant difference between the antibacterial effects of the individual and combined extracts, indicating that the interaction between the two plant materials results in an indifferent rather than a synergistic effect.

Based on these findings, calamansi peels and lemongrass leaves can be considered potential natural sources of antibacterial agents. The utilization of calamansi peels, which are commonly discarded as agricultural waste, also highlights the potential for sustainable use of local plant resources in the development of plant-based sanitation products. The observed antibacterial activity provides a scientific basis for the proposed formulation of a calamansi–lemongrass disinfectant spray for surface sanitation.

Further research should focus on the phytochemical characterization of the extracts to identify which specific bioactive compounds are responsible for the antibacterial activity. Determination of the minimum inhibitory concentrations and utilization of other bacterial pathogens are also recommended for additional studies to further validate their antimicrobial potential. Moreover, the proposed disinfectant formulation spray should be further evaluated in terms of stability, safety, and practical effectiveness under real-world conditions.

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Conflict of Interest

The authors declare no conflicts of interest. The individuals and organizations providing support had no role in the study design, data collection, analysis, interpretation, or the decision to publish these findings.

Artificial Intelligence (AI) Declaration Statement

OpenAI's ChatGPT was utilized strictly for language editing and structural refinement during the preparation of this manuscript. The authors rigorously reviewed and validated all AI-generated suggestions and take full responsibility for the accuracy, integrity, and final content of the paper.