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# *In Vitro* Antibacterial Activity of *Syzygium cumini* (Lomboy) Bark and Leaf Ethanolic Extract as a Component in Hand Sanitizer

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**ABSTRACT —** *In vitro* antibacterial activity of *Syzygium cumini* ethanolic leaf and bark extract as an active component of a hand sanitizer against *Staphylococcus aureus* and *Escherichia coli* have not yet been studied. Generally, the aim of this study was to determine the in vitro antibacterial activity of a hand sanitizer formulated with *S. cumini* leaf and bark ethanolic extracts against *S. aureus* and *E. coli*. Hand sanitizers containing increasing concentrations (25%, 50%, and 75%) of leaf, bark, and combined leaf and bark extracts combined with water, glycerin, and hydroxypropyl methylcellulose (HPMC), were assayed using the agar well diffusion method against *E. coli* and *S. aureus*, in 2 trials and 3 replicates. One-way ANOVA and post-hoc tests confirmed the significant differences at $p \leq 0.05$. The leaf-based hand sanitizer demonstrated significant antibacterial activity against S. aureus at the following concentrations: 25% (*mean* = 2.17, *sd* = 0.21), 50% (*mean* = 2.00, *sd* = 0.44), and 75% (*mean* = 2.37, *sd* = 0.49). This was comparable to the positive control (*mean* =1.67, *sd* = 0.06). The sanitizer with combined leaf and bark extracts exhibited significant antibacterial activity also at 25% (*mean* = 1.73, *sd* = 0.42), 50% (*mean* = 2.02, *sd* = 0.04), and 75% (*mean* = 2.13, *sd* = 0.08), and comparable to the positive control. No antibacterial activity was observed against *E. coli* with all leaf, bark, and combined extracts. These findings highlighted the potential of *S. cumini* leaf extracts in natural hand sanitizer formulation against Gram-positive bacteria, such as *S. aureus*.

***Keywords:*** *Syzygium cumini*, hand sanitizer, antibacterial activity, leaf and bark extract

## I. INTRODUCTION

Hand hygiene is widely recognized as one of the most important actions for reducing the spread of infectious diseases, particularly in hospitals. It generally refers to various methods of removing or killing microorganisms found on hands, such as hand washing or sanitizing. Hand sanitizers have been shown to lower infection rates and are especially beneficial in situations when access to water is limited. In addition to being useful in the absence of water, hand sanitizers have the advantage of providing strong antibacterial activity in a shorter period of time and eliminating the need to dry the hands, which could be another source of contamination.

Hand sanitizers remain a frontline defense against bacterial transmission, however, their variable effectiveness against non-multidrug-resistant bacteria, such as *Escherichia coli* and *Staphylococcus aureus* pose ongoing challenges in preventing hospital- and community-acquired infections [1]. *Staphylococcus aureus* is a common contaminant of the hands and skin, with studies showing a 9% prevalence in nasal carriers and 11.3% in hand contamination [2]. Similarly, *Escherichia coli*, a frequent pathogen on hands, has been found in 62% of cases on average, with higher contamination rates in low-income settings [3]. These high prevalence rates of *S. aureus* and *E. coli* on the skin and hands underscore their relevance as key organisms for investigating the effectiveness of hand sanitizers. This highlights the urgent need for new classes of antibacterial agents, with natural sources offering a promising alternative. Unlike synthetic antibacterial agents, plant-based have minimal side effects and exhibit substantial therapeutic potential for managing infectious diseases [4]. Indigenous medicinal plants, rich in flavonoids, alkaloids, and polyphenolic compounds, have long been recognized for their antiseptic, disinfectant, and antimicrobial properties [5]. Historically, plant extracts served as effective natural alternatives to combat bacterial infections.

Research indicates that plants such as *Syzygium cumini* are rich in various bioactive compounds, including alkaloids, flavonoids, tannins, saponins, steroids, and terpenoids which contribute to their antibacterial properties. Additionally, the leaves have been widely utilized in traditional medicine for the treatment of various ailments such as diabetes, constipation, stomachaches, fever, gastropathy, dermopathy, and to reduce blood discharge in feces [4]. These findings lead the researchers to harness the potential of

*S. cumini* as a sustainable and effective alternative in the development of new antibacterial agents, with hand sanitizer serving as its medium.

The current study aims to explore the potential of herbal formulations using *S. cumini* leaf and bark ethanolic extract as environmentally friendly, human-safe aseptic hand washing solutions using hand sanitizers as a medium. Many tropical plants, including those abundant in the Philippines, have demonstrated remarkable antimicrobial activity in previous studies, such as the work by Valle *et al*. (2015), which validated the bioactive properties of Philippine medicinal plants against drug-resistant bacteria [6]. The ecological advantage of utilizing *S. cumini*, commonly known in Hiligaynon dialect as Lomboy, in Tagalog as Duhat, or Java plum, is particularly noteworthy due to its abundance in the Philippines, eco-friendliness, and reported antibacterial potential. By bridging the general research gap on *S. cumini*, this study contributes to sustainable and accessible solutions for infection control.

## II. MATERIALS AND METHODS

### Data Collection Procedures

#### *Identification of the Plant Sample*

The identification of *S. cumini* was based on the taxonomic classification and descriptions provided in Quisumbing's 1978 publication. The taxonomic classification of the plant is as follows: Kingdom: Plantae, Phylum: Magnoliophyta, Class: Magnoliopsida, Order: Myrtales, Family: *Myrtaceae*, Genus: *Syzygium*, Species: *Syzygium cumini* (Quisumbing, 1978). It is characterized by its smooth, glossy, and leathery oval-shaped leaves, which are dark green on the upper surface and lighter underneath. The bark is thick and rough, with a grayish-brown color. Identification was confirmed through morphological comparison with Quisumbing's descriptions.

**Figure 2**
*Syzygium cumini* plant


*Note*. Photo of *Syzygium cumini* (a) tree and (b) bark taken from Villa Claro Compound, Sto. Niño Sur, Arevalo, Iloilo City. Own image.

#### *Collection of the Plant Sample*

The collection of *S. cumini* for this research study on its antibacterial properties was conducted under controlled conditions to ensure optimal sample quality. The plant materials were collected once, ensuring consistency in sample acquisition. The collection took place during dry weather conditions, avoiding periods of rainfall to prevent contamination or dilution of active compounds. Samples were obtained from plants located at least 100 meters away from any potential sources of environmental contamination and pollutants such as roads and factories. To maintain consistency in chemical composition, the collection was performed in the early morning at 6:00 A.M., when phytochemical concentrations of interest are expected to be at their peak. The collected plant materials were placed in sterile, labeled paper bags and transported immediately to the laboratory. After collection, the plant materials were authenticated at the Iloilo Provincial Environment and Natural Resources Office (PENRO) in FMS Compound, Parola, Port Area, Iloilo City.

#### *Extraction of Compounds from S. cumini leaves and bark*

The freshly collected *S. cumini* leaves and bark were properly washed with running tap water in order to remove coarse contaminants (i.e. dirt, small insects, etc.), then rinsed thoroughly with distilled water [7]. The parts were drained and cut into small pieces [7]. The leaves and bark were blended fresh using an electric blender separately. The resulting blended leaf and bark samples were weighed and then stored in a glass container.

***Ethanolic Extract Preparation***

The leaf and bark were extracted separately. For both the leaf and bark extracts, every 300g of the processed *S. cumini* extracts were mixed with 750 mL of 95% ethanol, a solvent most suitable for extraction due to its ability to dissolve not only many polar but also nonpolar compounds [8]. The solution was kept at room temperature for 72 hours before being strained through muslin cloth, and then further filtered using filter paper for clearer and more refined solution. The filtrate underwent rotary evaporation, and subsequently weighed before being subjected to drying in the oven set at a constant 40°C to recover the crude ethanolic *S. cumini* extract. Drying of the extracts was done continuously until the resulting product did not change its weight upon weighing.

***Preparation of Plant-based Hand Sanitizer***

A hand sanitizer base was prepared and formulated, combining the hand sanitizer base, glycerin, a thickening agent, hydroxypropyl methylcellulose (HPMC), and distilled water. The formulation was then transferred or poured into sterilized, screw-capped amber glass bottles and stored at refrigerator temperature for analysis [9]. Using an Eppendorf tube, the formulations were prepared at varying concentrations of *S. cumini* ethanolic extract, with a constant addition of the glycerin and hydroxypropyl methylcellulose (HPMC) mixture [10]. The researchers utilized a 10-100 uL micropipette to transfer and combine the mixtures. The obtained solution was mixed using a vortex mixer to achieve a homogenous and uniform formulation. The positive control, a commercially available hand sanitizer, was sourced within Iloilo City. The sanitizer that was sourced formulated exclusively with antibacterial properties without the added properties of fragrances, moisturizers, coloring, and those not involved in the antibacterial property of the hand sanitizer. The negative control used was the formulated mixture consisting of glycerin, a thickening agent, hydroxypropyl methylcellulose (HPMC), and distilled water.

***Antibacterial Assay of S. cumini***

The effectiveness of the hand sanitizer against specific clinical isolates were evaluated using established methods, including the well variant of the agar well diffusion test [11, 12, 13]. The agar well diffusion test was used to evaluate the antibacterial activity of the hand sanitizer. Normal saline was used as the diluent for preparing the bacterial suspension. This required the application of an inoculum equal to 0.5 McFarland with 1.5 x 108 CFU/mL. After inoculating with a swab, the test inoculum was allowed to stand at room temperature for 15 minutes. After incubation, both bacterial cultures were inoculated on Mueller Hinton Agar (MHA) for antibacterial susceptibility testing [14].

A sterile cotton swab was dipped into the standardized suspension, rotated several times, and pressed firmly against the inside wall of the tube above the fluid level to remove excess liquid. The dried surface of the MHA plate was inoculated by streaking the swab over the entire sterile agar surface. This streaking was done four times, rotating the plate approximately 60° each time. The rim of the plate was also swabbed in one swift direction to ensure even distribution of the inoculum [15].

Wells were aseptically created in the Mueller-Hinton Agar (MHA) plates using sterilized 0.2 µL yellow pipette tips with one tip used per well to avoid cross-contamination. To prevent bacterial growth beneath the wells, 50 µL of molten MHA was dispensed into each well and allowed to solidify, forming a uniform base layer. Using an automatic micropipette, 100 µL of each test solution was carefully dispensed into the wells to ensure precise and consistent volumes. Three wells contained the plant extract solutions at 25%, 50%, and 75% concentrations, respectively. The fourth well served as the negative control, receiving 100 µL of the hand sanitizer base which contained a mixture of HPMC, glycerin, and distilled water, while the fifth well served as the positive control, receiving 100 µL of the commercialized hand sanitizer. A standard size petri dish, 90 mm in diameter, was used. This provides sufficient surface area to accommodate five wells. Each of the five wells was placed at least 10 mm away from the edge of the plate and at least 30 mm away from each other. The zone of inhibition was measured using a standard measuring caliper, with millimeter and centimeter graduations. The zones of inhibition were assessed following a 24-hour incubation period at 37°C and results were recorded [12].

***Statistical Analysis***

The mean and standard deviation of the zones of inhibition were calculated and analyzed using One-way ANOVA to assess the differences in antibacterial activity among the various concentrations of the hand sanitizer. A post-hoc analysis using the Least Significant Difference (LSD) test was performed to identify specific group differences. Statistical significance was determined at a p-value of $\leq 0.05$, within a 95% confidence interval.

## III. RESULTS AND DISCUSSIONS

For *E. coli*, the hand sanitizer formulations containing *S. cumini* extracts at 25%, 50%, and 75% concentrations did not produce zones of inhibition significantly different from the negative control (glycerin, HPMC, and distilled water). In contrast, the positive control (commercial hand sanitizer) showed a clear and measurable zone of inhibition against *E. coli*. The positive control for hand sanitizer with leaf (*mean* =1.13, *sd* = 0.06), hand sanitizer with bark (*mean* = 1.03, *sd* = 0.11), and hand sanitizer with leaf and bark (*mean* = 1.00, *sd* = 0.00) confirmed the presence of antibacterial activity based on the zone of inhibition.

**Table 2**
In Vitro Antibacterial Activity of *Syzygium cumini* Bark and Leaf Ethanolic Extract in Hand Sanitizer against *E.coli*

| | Sanitizer | | | | | |
|---|---|---|---|---|---|---|
| | Leaf (A) | | Bark (B) | | Leaf and Bark (C) | |
| Hand sanitizer | Mean | SD | Mean | SD | Mean | SD |
| 25 % | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 50% | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 75% | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Negative control | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **Positive control | 1.13 | 0.06 | 1.03 | 0.11 | 1.00 | 0.00 |

In the case of *S. aureus*, a gram-positive bacterium, the *S. cumini* leaf-based hand sanitizer demonstrated notable antibacterial activity. The hand sanitizer with leaf at 25% (*mean* = 2.17, *sd* = 0.21), 50% (*mean* = 2.00, *sd* = 0.44), 75% (*mean* = 2.37, *sd* = 0.49), exhibited antibacterial activity against *S. aureus* when compared to the negative control, glycerin (mean = 0.00, SD = 0.00). All the concentrations were comparable to the positive control (*mean* = 1.67, *sd* = 0.06) activity. The *S. cumini* leaf and bark-based hand sanitizer demonstrated notable antibacterial activity, too. The hand sanitizer with leaf and bark at 25% (*mean* = 1.73, *sd* = 0.42), 50% (*mean* = 2.02, *sd* = 0.04), 75% (*mean* = 2.13, *sd* = 0.08), exhibited antibacterial activity against *S. aureus* when compared to the negative control, glycerin (mean = 0.00, SD = 0.00). All the concentrations were comparable to the positive control (*mean* = 1.67, *sd* = 0.06) activity.

**Table 3**
In Vitro Antibacterial Activity of *Syzygium cumini* (Lomboy) Bark and Leaf Ethanolic Extract in Hand Sanitizer against *S.aureus*

| | Zone of Inhibition (mm) | | | | | |
|---|---|---|---|---|---|---|
| | Leaf (A) | | Bark (B) | | Leaf and Bark (C) | |
| Hand sanitizer | Mean | SD | Mean | SD | Mean | SD |
| 25 % | 2.17 | 0.21 | 0.00 | 0.00 | 1.73 | 0.42 |
| 50% | 2.00 | 0.44 | 0.00 | 0.00 | 2.02 | 0.04 |
| 75% | 2.37 | 0.49 | 0.00 | 0.00 | 2.13 | 0.08 |
| *Negative control | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **Positive control | 1.67 | 0.06 | 1.63 | 0.20 | 1.80 | 0.25 |

A significant antibacterial effect of the hand sanitizer with 25%, 50%, 75% concentration of leaf and mixture of leaf and bark was observed against *S. aureus*. These findings indicate that antibacterial activity generally increases with concentration, with all tested concentrations showing significantly greater effects than the negative control.

In contrast, none of the extract treatments produced zones of inhibition against *Escherichia coli*, indicating that *E. coli* was resistant to the active compounds in the extracts.

These findings can be explained by the structural differences between Gram-positive and Gram-negative bacteria. *E. coli*, being Gram-negative, possesses an outer membrane that acts as a barrier against many antimicrobial compounds, which likely prevented the plant extracts from exerting their effect. On the other hand, *S. aureus* is Gram-positive and lacks this protective barrier, making it more susceptible to the phytochemicals present in the extracts [16]. Additionally, the superior performance of leaf extracts compared to bark extracts may be attributed to a higher concentration or more effective composition of active antibacterial compounds present in the leaves [17]. This finding is further supported by the study conducted by Upadhyay *et al*. (2020), which reported that seed and leaf extracts prepared using methanol and ethanol exhibited greater antibacterial activity than bark extracts [18]. The reduced zone of inhibition observed for bark extracts could be due to dilution effects when mixed with the base, as well as the inherently lower antibacterial potency of the bark.

In a similar study conducted by Da Rosa *et al*. (2024), hydroethanolic extracts of *S.cumini* demonstrated potent antibacterial effects against *S. aureus* [19]. This efficacy is attributed to the high content of phenolic compounds and flavonoids, identified through chemical characterization. Similarly, this study observed that leaf extracts exhibited the highest antibacterial activity against *S. aureus*, followed by the combination of leaf and bark, while bark alone showed no inhibitory effect. Both studies, in correlation to one another, emphasize the significant antibacterial activity of *S. cumini* leaf extracts against *Staphylococcus aureus*. The antibacterial activity observed in the *Syzygium cumini* leaf extract may be attributed to the presence of tannins and other phenolic constituents. Previous phytochemical analyses have demonstrated that *S. cumini* is particularly rich in polyphenolic compounds, notably gallic acid and ellagic acid derivatives, which are well-documented for their broad-spectrum antibacterial properties [20, 21]. These compounds are known to exert antibacterial effects by disrupting microbial cell walls, inhibiting enzymes, and interfering with microbial metabolism, thereby contributing to the significant inhibition observed against *S. aureus* in this study. The emphasis on phenolic content and extract optimization underlines a critical limitation of this study, namely, the absence of phytochemical profiling. So far, this is the first study on the formulation of hand sanitizer with ethanolic extract of leaf and combination of leaf and bark from *S. cumini* at concentration of 25%, 50%, and 75%.

This study is subject to several limitations that may influence the interpretation of results. The use of 95% ethanolic extracts may possibly have not extracted the active antibacterial compounds, potentially underrepresenting the extract's true efficacy, especially to *E.coli*. Additionally, the leaf and bark of *S.cumini* were harvested from Villa Claro Compound, Sto. Niño Sur, Arevalo, Iloilo

City. Finally, testing was conducted on a single strain each of *Escherichia coli* and *Staphylococcus aureus*, which restricts the generalizability of the findings.

Despite these limitations, the study contributes valuable insights into the use of *S. cumini* ethanolic leaf extract in natural hand sanitizer formulations, particularly for targeting Gram-positive bacteria like *S. aureus*. The findings support the exploration of plant-based antibacterial agents in hygiene products, offering a more sustainable and potentially safer alternative to synthetic chemicals. However, further research is needed to enhance its efficacy and ensure broader antibacterial coverage.

## IV. CONCLUSION

In the present study, it was found that *Syzygium cumini* extracts at concentrations of 25%, 50%, and 75% did not exhibit zones of inhibition against *Escherichia coli* and showed a significant difference from the negative control. In contrast, the positive control (commercial hand sanitizer) demonstrated a clear and measurable zone of inhibition against *E. coli.* For *S. aureus*, a Gram-positive bacterium, the hand sanitizers formulated with *S. cumini* leaf extract at all tested concentrations (25%, 50%, and 75%) showed notable antibacterial activity. These formulations exhibited clear zones of inhibition in comparison to the negative control and were comparable to the positive control. Additionally, the *S. cumini* leaf extract showed antibacterial effects at all tested concentrations against *S. aureus*, with stronger effects seen at higher concentrations. However, the hand sanitizers containing only *S. cumini* bark extract at the same concentrations did not show any antibacterial activity against *S. aureus.* Interestingly, formulations combining both leaf and bark extracts at 25%, 50%, and 75% concentrations did demonstrate antibacterial effects against *S. aureus* when compared to the negative control. Overall, this study provides scientific evidence supporting the antibacterial properties of hand sanitizers formulated with crude ethanolic extracts of *S. cumini* leaf, as well as combinations of leaf and bark, particularly against *S. aureus*.

## V. RECOMMENDATIONS

Further research is recommended to improve the formulation and concentration of *Syzygium cumini* leaf extract for optimal antibacterial effectiveness. Further investigations should aim also to study the mechanism of action of the extract, its stability within hand sanitizer preparations, and evaluate its antibacterial activity against a broader spectrum of pathogens. Moreover, further studies should be conducted to evaluate the user acceptability of the *S. cumini*-based hand sanitizer.

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