# Antibacterial Activity of Lime Peel and Lemongrass Extract as Active Ingredients for Spray Hand Sanitizer

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**Abstract**

Hand sanitizers are widely used as an alternative way to maintain hand hygiene from the presence of pathogenic bacteria, such as *Staphylococcus aureus*. In general, excessive use of alcohol as the main ingredient in hand sanitizers might cause skin irritation. Utilizing antibacterial activity of plants as a component in hand sanitizer could be used as a substitution for alcohol. The aim of this study was to determine the potential antibacterial activity of lime peel and lemongrass extracts as active ingredients for hand sanitizer. Method used in this research included extraction using maceration, qualitative phytochemical test, antibacterial assay, and formulation of spray hand sanitizer and quality test of spray hand sanitizer. In this study alkaloid, flavonoid, saponin, tannin and terpenoid were found as phytochemical content of lime peel, while lemongrass extract contain fla vonoid, tanin, saponin and steroid. Compared to lemongrass extract, lime peel extract with concentration 40%showed the optimum inhibition zone of *S. aureus* and chosen as active ingredient in spray hand sanitizer formulation. Formulated spray hand sanitizer with lime peel extract was able to inhibit the growth of *S. aureus* bacteria on hand palm.

**Keywords:** antibacterial, lime peel, lemongrass, spray hand sanitizer, *Staphylococcus aureus*

## Introduction

It is undeniable that people frequently involve hands in their daily activities. *Staphylococcus aureus* is the most common opportunistic bacteria which inhabit human skin, including hand and its palm (Edmonds-Wilson *et al*., 2015). Because of their ability to produce enterotoxin as a major cause for food poisoning and their nature to be easily transmitted from hand, *S.aureus* might create a threat for human health (Harris *et al*., 2002)

Maintaining hand hygiene is commonly conducted by hand washing with soap and water. Modern life sees this habit is impractical and people switch to use hand sanitizer to keep hand hygiene. Alcohol is widely use as the main ingredient for hand sanitizers. Because excessive use of alcohol could cause skin irritation, an alternative active ingredients for alcohol substitution is important to be found. Grace *et al*. (2015), stated that the using of plant antibacterial compound could be applied as a substitution for alcohol in hand sanitizer formulation.

Lime and lemongrass are common cultivated plants in tropical countries including Indonesia. In daily life, lime peels are commonly considered as waste in culinary activities. Despite its presence as waste, Wardani *et al*. (2018) found that lime peel has antibacterial activity against *Staphylococcus aureus* due to its secondary metabolites compounds. Lime peel contains large numbers of flavonoids, more than its fruit. Aside from that, lemongrass is a spice that is often found in the kitchen and also shows great potential for antibacterial because of its flavonoids and tannins content (Manvitha and Bidya, 2010). Exploration of antibacterial compound from lime peel and lemongrass extract could provide important information related to subtitute alcohol from hand sanitizer formulation. The aim of this research was to study the potential antibacterial activity of lime peel and lemongrass extract to be used as an active ingredient in spray hand sanitizer.

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## Material and Methods

### *Materials*

Main materials used in this study were lime peel (*Citrus aurantifolia* (Christm.) Swingle) and lemongrass (*Cymbopogon citratus* (DC.) Stapf). Both were purchased from the Kranggan Market Yogyakarta.This research used raw extracts of lime peel and lemongrass, not a cosmetic grade such the essential oil of lime peel or lemongrass.Other materials needed were *Staphylococcus aureus*, ethyl acetate, ethanol 96%, Mueller Hinton Agar (MHA), NaCl 0.9%, rotary evaporator, blender, incubator, oven, glass jar, sprayer bottle, autoclave, microplate 96 well, cotton swab sterile disk papers, sterile cotton swab, ciprofloxacin, HPMC, Trietanolamin (TEA), and propylene glycol.

### Methods

#### *Extraction of Lime Peel and Lemongrass*

Lime peel and lemongrass were both extracted by maceration method. The dried lime peel was grinded to a fine powder. Two hundred and fifty (250) g of lime peel powder was soaked in 1000 ml of ethyl acetate in a glass jar and stored for 3 days. Two hundred and fifty (250) g of lemongrass powder was macerated with 1000 ml of ethanol 96% in a glass jar and stored for 3 days. Each extracts were filtered, then evaporated with evaporator at a temperature of 50°C. The concentrated extract was weighed to obtain the yield values.

#### *Qualitative Phytochemical Assays*

All of qualitative phytochemical assays were performed based on the methods mentioned in Kumar *et al.* (2011)

*Alkaloid Assay*

Zero point three (0.3) gram of extract was diluted to 5 ml HCl 2N, and boiled in water bath for 2-3 min. Zero point three (0.3) gram of NaCl was added when the extract had cooled down, and then filtered. Five ml of HCl 2N was added to the filtrate and divided into two parts, labeled as solution 1A and 1B. Solution 1A was added with 3 drops of Mayer reagent, solution IB was added with 3 drops of Wagner reagent, and solution 1C was a blank. The presence of sediment indicates alkaloids.

*Flavonoid Assay*

Zero point three (0.3) gram of extract was diluted to 5 ml 80% ethanol, then added view drops of $FeCl_3$ until color changed. Purple, blue, dark green discoloration indicates the presence of flavonoids.

*Tannin Assay*

Zero point three (0.3) gram of extract was diluted in hot water, stirred, and let cooled in room temperature. About three or four drops of 10% NaCl was added to the solution and filtered. The filtrate was divided into 2 parts : solution A and B. Solution A was a blank and solution B will be added with a view drops of $FeCl_3$, and color change was observed. Dark green color indicates the presence of tannin.

*Saponin Assay*

Zero point three (0.3) gram extract was added with 5 ml of aquadest in a test tube. The solution was shaken vigorously for 30 sec and observed for a persistent froth. A stable froth for 30 min with a height of 3 cm indicates the presence of saponin

*Steroid and Terpenoid Assay*

Zero point three (0.3) gram extract was added with 2 ml of chloroform and 2 ml of acetic anhydride, solution would then was cooled down and added with 1-2 drops of $H_2SO_{\$}$. The discoloration to green or blue indicates the presence of terpenoid, meanwhile red or purple discoloration indicates the presence of steroid.

#### *Antibacterial Activity Test*

The antibacterial activity of lime peel and lemongrass extracts were performed by disk diffusion method (Balouiri *et al.*, 2016). Fifteen (15) ml of Mueller Hinton Agar (MHA) were compacted on petri dish. *Staphylococcus aureus* bacteria from the culture stock was revived in NaCl 0.9% and equated to 0.5 McFarland. *S. aureus* was spread over the medium on petri dish with a sterile cotton swab. Twenty (20) µL of extracts was pipetted onto the disk paper and placed above the MHA medium. Petri dish that contain medium and inoculum

was incubated in a incubator at 37ºC for 24 h. Antibacterial activity was observed by measuring diameter of inhibition zone.

***Formulation of Spray Hand Sanitizer***

HPMC was weighed and dissolved in warm distilled water, then propylene glycol, TEA, and distilled water were added and stirred using a stirring rod until homogeneous. Chosen plant extract was put into the mixture and stirred again until homogeneous. This formulated spray hand sanitizer was poured into a spray bottle for further use. Formulation of spray hand sanitizer is shown in Table 1.

Table 1. Formulation of spray hand sanitizer

| Composition | Formula |
|---|---|
| HPMC | 0.1 g |
| Triethanolamine | 8 drops |
| Propylene glycol | 15 ml |
| *Lime peel Extract* | 40% |
| Aquadest | Ad 100 |

## Results

***Yield Percentage of Lime Peel and Lemongrass***

Crude extracts from lime peel (*Citrus aurantifolia)* and lemongrass (*Cymbopogon citratus)* were collected after evaporating all the solvents. Obtained total crude extract of *C. aurantifolia* was 8.97 g with yield percentage was 3.59%. Total crude extract of *C. citratus* was 17.93 g with yield percentage 7.17% (Table 2).

Table 2. Yield percentage of crude extracts

| Species | Weight of plant powder | Weight of crude extract (g) | Yield percentage (%) |
|---|---|---|---|
| *Citrus aurantifolia* | 250 | 8.97 | 3.59 |
| *Cymbopogon citratus* | 250 | 17.93 | 7.17 |

***Qualitative Phytochemical Assays***

Secondary metabolites of lime peel and lemongrass extracts can be determined through qualitative phytochemical assays. The presences of alkaloid, flavonoid, saponin, tannin, and terpenoid were detected in lime peel extract, meanwhile, flavonoid, saponin, tannin, and steroid were detected in lemongrass extract (Table 3).

Table 3. Qualitative phytochemical test of *C. aurantifolia* and *C. citratus*

| Phytochemical compounds | Species | |
|---|---|---|
| | *Citrus aurantifolia* | *Cymbopogon citratus* |
| Alkaloid | + | - |
| Flavonoid | + | + |
| Tannin | + | + |
| Saponin | + | + |
| Terpenoid | + | - |
| Steroid | - | + |

***Antibacterial Activity of Single Lime Peel and Lemongrass Extracts***

Based on the results, both of lime peel and lemongrass extracts were able to inhibit the growth of *S. aureus*. As shown in Table 4, all concentration of *Citrus aurantifolia* extract were able to inhibit *S. aureus*, with concentration of 40% was the optimum concentration in *S. aureus* inhibition. In contrast to *C. aurantifolia* results, extract of *C. citratus* was only able to inhibit test bacteria at the concentration of 100% (Table. 4)

Table 4. Antibacterial activity of *C. aurantifolia* and *C. citratus* against *Staphylococcus aureus*

| Extract | Concentration (mcg/20µL) | Inhibition zone (mm) |
|---|---|---|
| CA 20% | 4000 | 3 |
| CA 40% | 8000 | 9.6 |
| CA 60% | 12000 | 9.0 |
| CA 80% | 16000 | 9.3 |
| CA 100% | 20000 | 7 |
| CC 20% | 4000 | 0 |
| CC 40% | 8000 | 0 |
| CC 60% | 12000 | 0 |
| CC 80% | 16000 | 0 |
| CC 100% | 20000 | 7.3 |
| Ciprofloxacin (control +) | 20000 | 28.3 |
| Aquadest (control -) | 20000 | 0 |

CA: *Citrus aurantifolia*; CC: *Cymbopogon citratus*

***Antibacterial Activity of Combined Extracts***

To test whether combination of lime peel and lemongrass extracts were potent to inhibit *S. aureus*, both extracts was combined and grouped into 5 concebtration ratios to find the best inhibition activity against *S. aureus*. Table

Table 5. Antibacterial activity of C. *aurantifolia* and C. *citratus* combinations

| Combination | Extract | Concentration (mcg/20µL) | Inhibition zone (mm) |
|---|---|---|---|
| A | CA:CC = 20:80 | 20000 | 7.6 |
| B | CA:CC = 40:60 | 20000 | 7.3 |
| C | CA:CC = 60:40 | 20000 | 7.0 |
| D | CA:CC = 80:20 | 20000 | 7.0 |
| E | CA:CC = 50:50 | 20000 | 6.6 |

CA: *Citrus aurantifolia*; CC: *Cymbopogon citratus*

5 shows the best inhibition zone was obtained from combination A (20 CA: 80 CC).

Results in Table 4 and 5 show that the inhibition zone of single extract is slightly better than the combination extract. Statistical analysis with one-way ANOVA test showed that the significant level of *C. aurantifolia, C. citratus,* and its combinations are below 0.05, which means that there was a significant effect of the treatments of *C. aurantifolia, C. citratus,* and its combinations in inhibiting *Staphylococcus aureus* (Data not shown). Based on these results, single extract of lime peel with concentration of 40% was selected to be used as active ingredient in spray hand sanitizer formulation.

### *Antibacterial Activity of Formulated Spray Hand Sanitizer*

Formulated spray hand sanitizer as a product in this research was tesetd against *S. aureus* to study about its practical potency as desinfectant. Table 6 shows that the inhibition zone of formulated spray hand sanitizer to tested bacteria was 7 mm, while result from a commercial hand sanitizer was 0 mm. The inhibition zone of formulated spray hand sanitizer was not higher than inhibition zone of single lime peel extract on concentration 40% as showed previously. It could be suggested that the addition of other components to the hand sanitizer formula could cause further dilution to the initial 40% concentration of lime peel extract. As a consequence, inhibition activity to tested bacteria is reduced.

### *Quality Test of Spray Hand Sanitizer*

Formulated spray hand sanitizer was also organoleptically tested, which included perception of color, aroma, and texture of the product. It shown in Table 7 that spray hand sanitizer has yellow-brown color, lime citrus aroma, and liquid texture. In comparison, a commercial hand sanitizer used in this research showed a clear color with alcohol aroma and liquid texture.

Table 6. Antibacterial activity of spray hand sanitizer formulation

| Formulation | Inhibition zone (mm) |
|---|---|
| Spray Hand Sanitizer with *C. aurantifolia* extract concentration 40% | 7 |
| Commercial hand sanitizer | 0 |

Table 7. Organoleptic of formulated spray hand sanitizer

| Formula | Parameter | | |
|---|---|---|---|
| | Color | Aroma | Texture |
| SHS CA 40% | Yellow-brown | Lime Citrus | liquid |
| Commercial hand sanitizer | Clear | Alcohol | liquid |

SHS CA: Spray hand sanitizer of *C. aurantifolia*

Formulated hand sanitizer had a liquid form and made it easier to be sprayed using a spray bottle. It is shown that the spray pattern of formulated product was 9 cm, and expected to be spreaded thoroughly over the palms of hands. This liquid hand sanitizer was also homogeneous with a pH scale of 4.6 and complied with SNI 2588:2017 (Anonim, 2017) as shown in Table 8.

Table 8. Spray pattern, homogeneity, and pH of formulated spray hand sanitizer

| Formula | Parameters | | |
|---|---|---|---|
| | Spray pattern (cm) | Homogeneity | pH |
| SHS CA 40% | 9 | Homogenous | 4.6 |

**Discussion**

***Secondary Metabolites Compounds of Lime Peel and Lemongrass***

The polarity of a solvent affects the bioactive component that is attracted to (Umar *et al.*, 2016). In this research, ethyl acetate as a semi-polar solvent was able to extracts alkaloid, flavonoid, tannin, saponin, and terpenoid of lime peel, while ethanol 96% as a polar solvent was able to extracts flavonoid, tannin, saponin, and steroid of lemongrass. Both extracts have similar secondary metabolites, those are flavonoid, tannin, and saponin.

Flavonoid are commonly found in *Citrus* species. Wardani *et al.* (2018) found that more flavonoids were found in lime peel compared to the fruit, seeds, or lime juice. Naringin, hesperidin, nobiletin, and tageretin are the chemical compounds of flavonoids found in lime peel, which act as antibacterials (Adindaputri *et al.*, 2013). Flavonoids can damage bacterial cell membranes by making extracellular protein complexes that interfere the integrity of bacterial cell membrane, followed by the release of intracellular compounds (Nuria *et al.*, 2009).

The mechanism of tannin as antibacterial are by deactivating adhesion, inhibits work of enzymes, and ruining microbial cell function (Cowan, 1999). Saponin is different from other secondary metabolites. Saponin are known for their surfactant properties, because they dissolved in water and form a foamy solution. Saponin has ability to work as an antibacterial agent, by making cell membranes become unstable and resulting in lysis (Rahman *et al.*, 2017).

Alkaloid was detected in lime peel extract, but was not detected in lemongrass. Alkaloid is known as antibacterial due to its capability to interlacate bacteria DNA, resulting in inhibition of DNA and RNA synthesis (Karou *et al.*, 2005). The lipophilic nature of terpenoid compounds makes terpenoids react with the active side of the bacterial lipid constituents, increase their permeability, and lead to the lysis of bacterial cell membranes (Guimarães *et al.*, 2019). Steroid was detected in lemongrass, but not lime peel. Steroid also has antibacterial activity where it interacts with phospholipids as component of bacterial cell membrane, cause decrease in cell membrane integrity, resulting in leakage of bacterial liposomes (Lake *et al.*, 2019). All of phytochemical compounds detected from both lime peel and lemongrass are practically have antibacterial property.

***Antibacterial Potency of Lime Peel and Lemongrass***

Based on the results, antibacterial activity of lime peel and lemongrass extracts inhibitited the growth of *S. aureus* (Table 4). The presence of phytochemicals in lime peel and lemongrass extracts were such an important factor that played role in antibacterial activity. In general, the antibacterial properties of plant phytochemicals work by disrupting the bacterial cell membrane or inhibiting several virulence factors such as enzymes and toxins (Barbieri *et al.*, 2017).

On their research, Wardani *et al.*(2018) found that lime peel-ethyl acetate extract was succeeded to inhibit *S. aureus* bacteria with diameter of inhibition zone 12 mm (strong). In this research, various concentration of lime peel extracts were capable to inhibit *S. aureus*, with the most effective concentration was 40%. Diameter of inhibition zone of 40% of lime peel extract was 9.6 mm, considered as intermediate inhibition by Chandra *et al.*(2018). In contrast to lime peel, lemongrass extract only capable to inhibit *S. aureus* at the concentration of 100%. These antibacterial activities can be related to the presence of phytochemicals in it extracts. Lime peel extract performed a better antibacterial activity due to the presence of alkaloid, flavonoid, tannin, saponin, and terpenoid. Detected pytochemicals of lemongrass were only flavonoid, tannin, saponin, and steroid. The difference of phytochemical compounds owned by lime peel and lemongrass could be the main factor that differed their antibacterial activity against *S. aureus*.

Kumar *et al.* (2017), reported that combining different plants may increase the pharmacological and potential effects of the two plants, due to the interaction between bioactive compounds of those two extracts. In this research, compared to other variation

of concentrations, extract combination A showed the best inhibition zone against *S. aureus* (7.6 mm, Table 5). This antibacterial activity did not surpassed the antibacterial activity of *C. aurantifolia* 40% single extract (9.6 mm, Table 4). In that case, interaction of lime peel and lemongrass extract in their combination was called as "weak synergy" (Caesar and Cech, 2019).

***Antibacterial Activity of Formulated Spray Hand Sanitizer***

Because of its most potent inhibition to *S. aureus*, extract of *C. aurantifolia* at a concentration of 40% was used as active ingredients of spray hand sanitizer. It was shown in Table 6 that the formulated spray hand sanitizer performed 7 mm inhibition zone against *S. aureus*. The presence of *C. aurantifolia* extract in spray hand sanitizer formula was still able to inhibit *S. aureus* as test bacteria.

***Quality of Formulated Spray Hand Sanitizer***

As the final product of spray hand sanitizer, a quality tests were required to verify good properties of the product. Organoleptic observations are carried out by observing the visible appearance of the product such as color, aroma, and texture (Martono and Suharyani, 2018).

The final product of this research showed a yellow-brown color, with a typical lime aroma and liquid texture. Because of its liquid form, this formulated hand sanitizer was able to be sprayed with a bottle spray (Table 7). As shown in Table 8 that spray pattern of this product was 9 cm and assumed to spread thoroughly over the palms of hands. As a final product the formulated spray hand sanitizer, the pH was 4.6 and in accordance with SNI 2588:2017.

**Conclusion**

Lime peel (*Citrus aurantifolia*) extract has a more potent antibacterial activity than lemongrass (*Cymbopogon citratus*) extract or combinations of both extracts to inhibit *S. aureus*. Extract of lime peel has a potency to substitute alcohol as an active ingredient in non-alcohol based spray hand sanitizer.

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