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Comparative Study of Antioxidant and Antibacterial Activities of Tropical Citrus Fruits (Juice and Peels)

Julfi Restu Amelia [1], Fildzah Hashfi Safitri[1], Giyatmi[1], Intan Nurul Azni[1,✉]

[1] Program Studi Teknologi Pangan, Universitas Sahid, Jakarta, INDONESIA.

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Corresponding Author:
✉ intannurulazni@gmail.com
(Intan Nurul Azni)

ABSTRACT

Tropical citrus fruits contain important bioactive compounds with health-promoting properties such as antioxidant and antibacterial properties. This study provided comparative data on the antioxidant and antimicrobial activity of tropical citrus (local lemons, imported lemons, and limes) juice and peel. Antioxidant activity of citrus fruits was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and expressed as IC50. Antimicrobial activity was evaluated against pathogens (E. coli, B. subtilis, S. aureus) using agar diffusion method. The results showed that very strong antioxidants were detected in domestic and imported lemon juice (33.45 ppm; 31.43 ppm) and peel (15.89 ppm; 44.50 ppm). However, lime juice and peel had stronger antioxidant activity (60.35 ppm and 59.54 ppm). The highest zone of inhibition was observed against S. aureus and the lowest was recorded against E. coli. The diameters of the zones of inhibition of local lemon, imported lemon, and lime juice against S. aureus were 26.40 mm, 27.95 mm, and 27.31 mm; E. coli was 15.64 mm, 16.84 mm, and 16.63 mm; and B. subtilis was 17.88 mm, 21.77 mm, and 21.24 mm, respectively. Tropical citrus juice showed higher activity against Gram-positive than Gram-negative bacteria tested. Local lemon, imported lemon, and lime peels did not inhibit E. coli, B. subtilis, and S. aureus.

1. INTRODUCTION

Tropical citrus fruits are among the tropical fruits belonging to the *Rutaceae* family and Citrus genus. These oranges are widely produced in Indonesia, with a productivity reaching 4-5 tons per month in 2019 (Anisa, 2020). One of the tropical citrus fruits found in Indonesia is the lemon, which comes in two types: locally produced lemon (local lemon) and imported lemon. Lemons are recognized as natural antioxidants due to their content of vitamin C, citric acid, essential oils, bioflavonoids, polyphenols, coumarin, and flavonoids. Local lemons are preferred over imported ones, as indicated by a study conducted by Nur Ulina (2020), showing higher attribute values (101.71 points) for local lemons compared to imported lemons (91.59 points) based on overall attributes (price, availability, taste, and aroma).

In addition to lemons, another tropical orange in Indonesia is the lime. Limes are utilized for various purposes, such as appetite enhancers, treatment for diarrhea, antipyretic, anti-inflammatory, antibacterial, and dietary supplement. Lime contains chemical compounds like citric acid, amino acids (tryptophan, lysine), essential oils (citral, limonene, phellandrene, lemon camphor, cadinene, geranyl acetate, linalyl acetate, acetylaldehyde, nonylaldehyde) (Kurnia Lestari *et al*., 2018). Limes also contain flavonoid compounds with antioxidant and antibacterial activities. Lime juice is known to contain essential oil limonene and 7% citric acid (Prastiwi & Ferdiansyah, 2017). Based on the above descriptions, both lemons and limes have the potential for antioxidant and antibacterial properties. However, a comparison of various types of tropical citrus fruits based on antioxidant and antibacterial activities is yet to be explored. The bacteria used in the research include *Escherichia coli* (*E. coli*), *Bacillus subtilis* (*B. subtilis*), and *Staphylococcus aureus* (*S. aureus*), representing Gram-negative, Gram-positive, and spore-forming bacteria, respectively. The objective of this study is to provide a comparative analysis of the antioxidant and antibacterial activities of the juice and peel of tropical citrus fruits, such as local lemons, imported lemons, and limes.

2. MATERIALS AND METHODS

This research compares the antioxidant and antibacterial activities of the juice and peel of local lemons, imported lemons, and limes. Antioxidant activity is determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, expressed as IC50, while antibacterial activity is evaluated using the agar diffusion method against pathogenic bacteria (*E.coli*, *B.subtilis*, and *S.aureus*). Each treatment is replicated twice.

2.1. Materials and Equipment

The materials used include local oval shaped yellowish-green local lemons, oval yellow imported lemons, greenish-yellow limes purchased from KedaiMart Official Shop E-commerce, test media (Nutrient Agar (NA), and Nutrient Broth (NB) Himedia brand), test bacteria (*Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*), and DPPH. The criteria for lemons used are locally produced and imported lemons with uniform elongated yellow shapes. Lemons and limes are obtained from e-commerce. The bacteria used in the research are *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*, obtained from PT Medifarma Laboratories. The equipment used includes a knife, filter cloth, citrus juicer, blender, calipers, UV-Vis spectrophotometer (Shimadzu UV-1700) with a wavelength of 517 nm, petri dishes, incubator, micropipette, and micropipette tips.

2.2. Methods

2.2.1. Lemon juice and lime juice extraction (Saragih & Herawati, 2017)

The extraction of lemon and lime juice is carried out using a method involving several stages as follows: sorting, washing, cutting, juicing, and filtering. Lemons and limes are washed and then cut in half using a knife. The cut lemons and limes are then juiced using a citrus juicer, followed by a filtration process of the lemon juice extract using a filter cloth, resulting in lemon and lime juice. From 1 kg of lemons and 1.5 kg of limes, 100 mL each is extracted.

2.2.2. Lemon peel juice extraction (Kusumawati, 2020)

Lemon peels are peeled, and the flavedo part is taken. The peels are washed, weighed (25g), and blended for 2 minutes with 150mL of water. Subsequently, the lemon peels are filtered using a filter cloth to separate the juice from the pulp.

2.2.3. DPPH antioxidant activity test (Tristantini *et al*., 2016; Rifkowaty & Martanto, 2015)

Five sample concentration variations are prepared for the comparative test, and 2 mL is mixed with 2 mL DPPH (1:1 volume ratio). The mixture is incubated for 30 minutes, and absorbance is measured at a wavelength of 517 nm. IC50 is determined based on the linear regression equation from the calibration curve, with the percentage of DPPH radical absorption inhibition as the y-axis and the concentration of the comparative test sample as the x-axis. IC is calculated by entering the 50% value into the linear regression equation as y, and then the x value is calculated as the IC50 concentration. Ascorbic acid is used as a reference. The antioxidant activity of the sample is determined by the extent of inhibition of DPPH radical absorption, calculated using the formula:

$$\% \text{ Inhibition} = \frac{\text{Initial absorbance - Final absorbance}}{\text{Final absorbance}} \times 100\%$$

2.2.4. Antibacterial activity testing (Indarto *et al*., 2019)

The antibacterial activity testing involves bacterial inoculation and antibacterial activity testing. Bacteria are inoculated using the pour plate method. Test bacteria must be regenerated from old to new medium before being used in antibacterial testing. Bacteria cultures are planted with a single loop in 10 mL of Nutrient Broth (NB) medium, then incubated for 48 hours. Afterward, 0.1 mL of the culture is poured into 250 mL of Nutrient Agar (NA) medium. The NA medium is poured into petri dishes, each containing approximately 20 mL of NA medium. Antibacterial activity testing is performed using the agar diffusion method with the cup-plate technique. The testing involves creating wells in the NA medium (well diameter ± 6.7 mm) containing test samples. Each dish is made with 3 wells using the tip of a micropipette, and each well is filled with the sample extract. Amoxicillin is used as a positive control, and sterile distilled water is used as a negative control. The NA medium with well-touched test solutions is then incubated at 37°C for 2 consecutive 24-hour periods under anaerobic conditions. After incubation, the inhibition zone is measured using calipers.

2.2.5. Data analysis

Antioxidant and antimicrobial activity data are presented in tables and graphs and analyzed descriptively using Microsoft Excel.

3. RESULTS AND DISCUSSION

3.1 Antioxidant Activity

Table 1. Antioxidant activities of local lemon juice and peel, imported lemon juice and peel, and lime juice and peel

Citrus type	Sample	Antioxidant Activity (ppm)
Local Lemon	Juice	33.45
	Peel	44.50
Imported Lemon	Juice	31.43
	Peel	15.89
Lime	Juice	60.35
	Peel	59.54

Local lemon juice and peel exhibit highly potent antioxidant activities of 33.45 ppm and 44.50 ppm, respectively. Imported lemon juice and peel also demonstrate strong antioxidant activities of 31.43 ppm and 15.89 ppm. Meanwhile, lime juice and peel show robust antioxidant activities of 60.35 ppm and 59.54 ppm (Table 1). Compounds are considered to have very strong antioxidant activity if the IC50 value is less than 50 ppm, strong if IC50 is between 50-100 ppm, moderate if IC50 is 101-150 ppm, and weak if IC50 is between 150-200 ppm (Molyneux, 2004). Both local and imported lemon juice and peel exhibit stronger antioxidant activities compared to lime juice and peel. This is attributed to lemons containing higher levels of antioxidant compounds than limes, such as lemon's vitamin C content of 60 mg, while lime has a vitamin C content of 27 mg per 100 grams (Ayu *et al*., 2017). The USDA Nutrient Database also states that lemon is richer in vitamin C and folate than lime, with 39 mg vitamin C and 20 µg folate in 100 grams of lemon juice, while 100 grams of lime contains 30 mg vitamin C and 10 µg folate, serving as antioxidants (Batubara, 2017). Imported lemon juice and peel exhibit stronger antioxidant activities compared to local lemon and lime. This aligns with Krisnawan's (2018) research, indicating that imported lemons have higher antioxidant levels than local lemons, both in the peel and fruit pulp.

The research results demonstrate that both local and imported lemon peels possess very strong antioxidant activities. The flavedo, the outer part of the peel bordering the epidermis containing chromoplasts and essential oil sacs, is used in the study. Lemon peel serves as a natural antioxidant due to its contents, including vitamin C, citric acid, essential oil, bioflavonoids, polyphenols, coumarins, and flavonoids (Hendra Krisnawan *et al*., n.d.). The antioxidant mechanism of flavonoids can be direct and indirect. Flavonoids function directly as antioxidants by donating hydrogen ions, neutralizing the toxic effects of free radicals. Flavonoids act as free radical scavengers, releasing hydrogen atoms from their hydroxyl groups. The lost hydrogen atom binds with free radicals, rendering them neutral. Flavonoids without hydrogen atoms undergo resonance, stabilizing the free radicals and preventing damage to lipids, proteins, or DNA (Barqy, 2021). Flavonoids also function indirectly by enhancing the expression of endogenous antioxidant genes through various mechanisms (Shinta & Kusuma, 2015). Monoterpene compounds present in lemon essential oil, such as limonene and γ-terpinene, are known for their excellent antioxidant activities (Conforti *et al*., 2007).

3.2. Antibacterial Activity

The research results in Figure 1 indicate that local lemon juice, imported lemon juice, and lime juice exhibit inhibition zones for *S. aureus* (Gram-positive cocci) of 26.40 mm, 27.95 mm, and 27.31 mm, respectively; for *E. coli* (Gram-negative bacilli) of 15.64 mm, 16.84 mm, and 16.63 mm, respectively; and for *B. subtilis* (Gram-positive bacilli) of 17.88 mm, 21.77 mm, and 21.24 mm, respectively. Greenwood classifies bacterial growth inhibition responses into categories, with inhibition zone diameters of <10 mm categorized as no inhibition, 10-15 mm as weak, 16-19 mm as moderate, and >20 mm as strong (Ramadheni *et al*., 2017). Based on this classification, it is evident that local lemon juice, imported lemon juice, and lime juice exhibit strong inhibitory effects against *S. aureus*. However, against *E. coli*, local lemon juice, imported lemon juice, and lime juice show moderate inhibitory effects. The data also indicates that imported lemon juice and lime juice exhibit strong inhibition against *B. subtilis*, whereas local lemon juice only shows moderate inhibition.

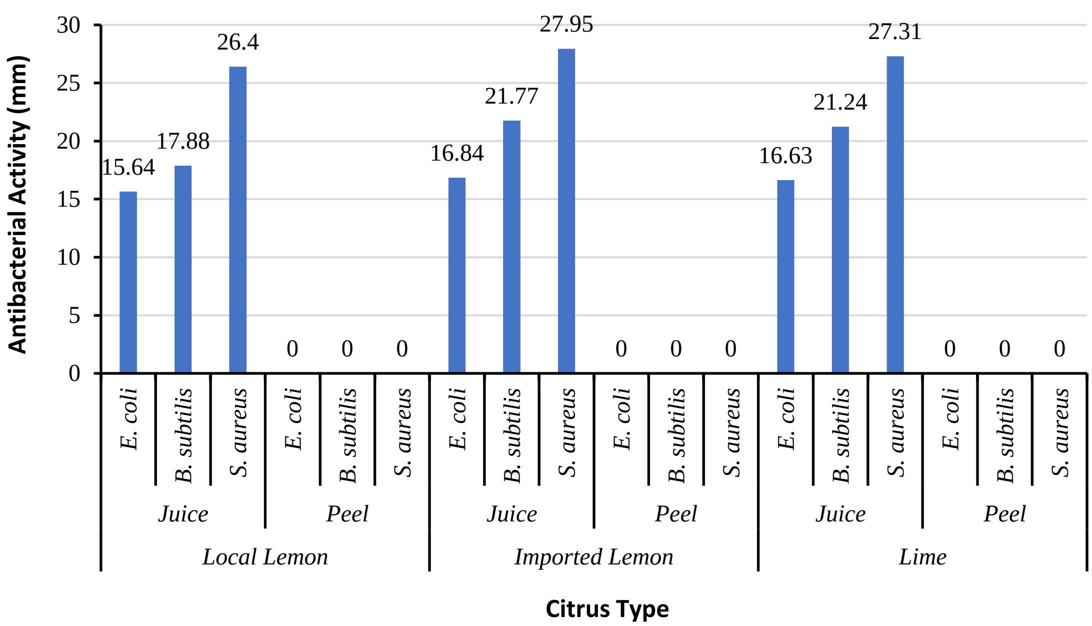
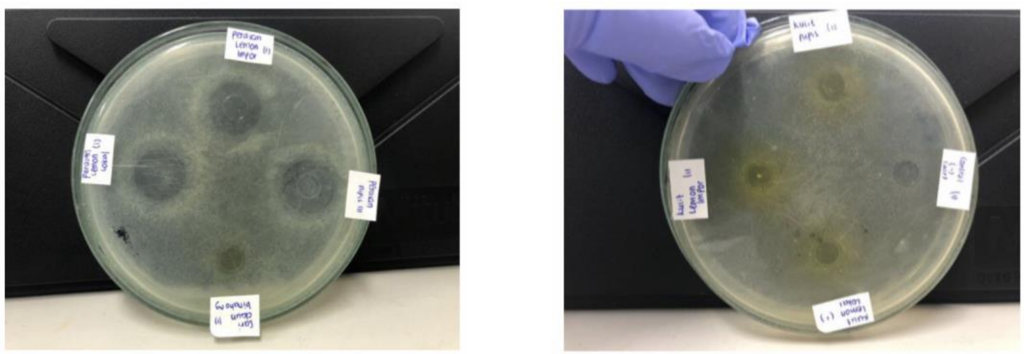


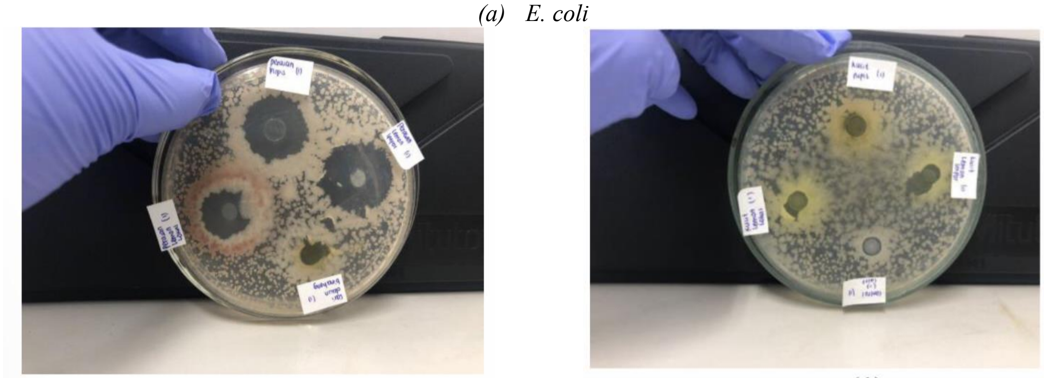
Figure 1. Antibacterial activity of different citrus fruit types (juice and peel) against *E. coli*, *B. subtilis*, and *S. aureus*

The research findings also show that lemon exhibits greater inhibitory effects compared to lime against *S. aureus*, *E. coli*, and *B. subtilis*. This is attributed to lemon having higher levels of bioactive components, such as citric acid. Izza & Rahayu (2019) reveals that lime has a citric acid content of 1.1901%, while lemon has a content of 1.3590%. The citric acid compounds in lemon have the ability to inhibit bacterial growth and act as antibacterial agents. Izza & Rahayu (2019) state that lemon has a higher tannin content than lime, and tannins exhibit antibacterial activity by inactivating microbial cell adhesins and enzymes, and disrupting protein transport within bacterial cell membranes. Tannins also target peptidoglycan in cell wall formation, resulting in imperfect cell wall formation, leading to bacterial cell lysis due to osmotic and physical pressure, and ultimately causing bacterial cell death (Ngajow *et al*., 2013).

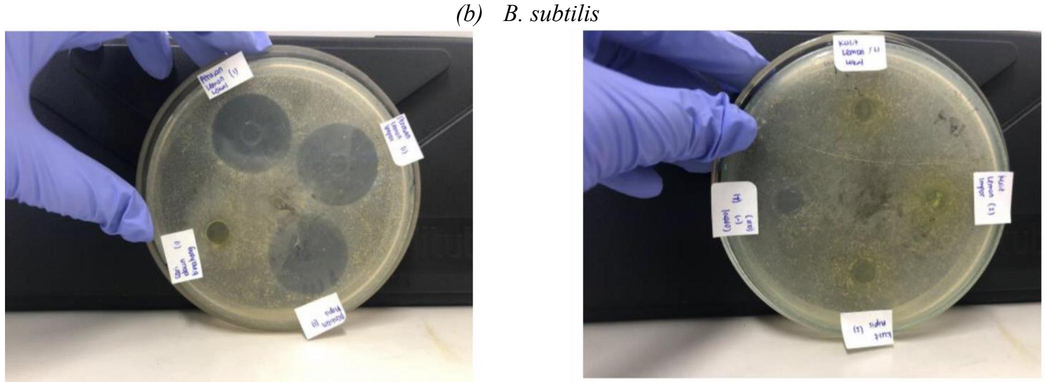
The research results in Figure 2 also indicate that the inhibition activity against Gram-positive bacteria (*S. aureus* and *B. subtilis*) is higher than against the tested Gram-negative bacteria (*E. coli*). This discrepancy is due to the distinct characteristics of the cell walls of Gram-positive and Gram-negative bacteria. The cell wall of Gram-positive bacteria contains peptidoglycan, teichoic acid, and teichuronic acid. The cell wall of Gram-negative bacteria consists partly of polysaccharides and a small amount of peptidoglycan located between the outer membrane and inner membrane of the cell wall. The outer layer of the Gram-negative bacterial cell wall is a component consisting of phospholipids and some proteins commonly referred to as the outer layer. The bacterial cell wall that is most easily denatured is the one composed of polysaccharides compared to the one composed of phospholipids. Based on this, it can be observed that Gram-positive bacteria undergo denaturation more quickly than Gram-negative bacteria. This is also because the cell wall of Gram-positive bacteria contains teichoic acid, which is a polar polymer structure. Polar antibacterial compounds, such as flavonoids, can easily penetrate polar peptidoglycan compared to the nonpolar lipid layer, resulting in a higher level of inhibition activity against Gram-positive bacteria than Gram-negative bacteria (Rostikawati, 2020).



(a) E. coli



(b) B. subtilis



(c) *S. aureus*

Figure 2. Inhibition zones of local lemon juice (left) and peel (rght), imported lemon juice and peel, and lime juice against (a) *E. coli*, (b) *B. subtilis*, and (c) *S. aureus*

The research data also indicates that antibacterial activity against cocci bacteria is higher compared to bacilli bacteria, as evidenced by greater inhibitory activity against *S. aureus* than *B. subtilis* and *E. coli*. *E. coli* possesses a capsule containing a mucous layer composed of polysaccharides and glycoproteins, serving a protective function for the cell. Bacillus sp. bacteria are spore-forming bacteria with a capsule structure containing polypeptides derived from D-glutamic acid. Bacteria with capsules protect themselves from phagocytosis through surrounding polysaccharides (Pratiwi, 2017), making them more resistant to antibacterial compounds or antibiotics.

The research results also demonstrate that lemon juice and lime juice exhibit antibacterial activity against *S. aureus*, *E. coli*, and *B. subtilis*. This is due to the bioactive compounds present in both fruits. Lemon contains alkaloids, saponins, flavonoids at moderate concentrations, and tannins at high concentrations (Batubara, 2017). Lime also contains bioactive compounds, such as alkaloids, terpenoids, saponins, flavonoids, tannins, and steroids, capable of inhibiting bacterial growth through various inhibitory mechanisms, such as damaging cell walls, disrupting bacterial cell membrane integrity, altering molecular structures of proteins and nucleic acids, and inhibiting bacterial enzyme activity (Djoenaidi, 2017).

The mechanism of alkaloids as antibacterials involves disrupting components that make up peptidoglycan in bacterial cells, preventing the formation of a complete cell wall and causing cell death (Miftahendarwati, 2014). Flavonoids also function as antibacterials by inhibiting bacterial nucleic acid synthesis and bacterial motility. Flavonoids work by interfering with hydrogen binding in nucleic acids, inhibiting DNA and RNA synthesis. Flavonoids can prevent bacterial growth by disrupting cell membrane stability and bacterial energy metabolism. This instability results from changes in the hydrophilic and hydrophobic properties of the cell membrane, reducing membrane fluidity and causing bacterial cell death (Hendarwati, 2014). Flavonoids can also inhibit protein and nucleic acid synthesis processes in bacteria by damaging nucleic acids and denaturing proteins, causing disruptions in protein and nucleic acid synthesis processes and total cell damage (Rostikawati, 2020).

Research results (Razak *et al*., 2013) show that lime has the ability to inhibit the growth of *Staphylococcus aureus* bacteria in vitro due to chemical contents such as essential oil and phenols, which are bactericidal. The results state that higher lime concentrations result in better inhibitory effects. The bactericidal ability of phenols can denature proteins and damage bacterial cell membrane, disrupting active transport functions, selective permeability functions, and bacterial protein arrangement control.

The antibacterial activity testing results of local lemon peel, imported lemon peel, and lime peel indicate that all three samples are unable to inhibit the growth of *E. coli*, *B. subtilis*, and *S. aureus*. This is evident from all inhibition zone diameters being less than 10 mm. These findings differ from the study by (Wardani *et al*., 2018), which shows antibacterial activity in lime peel at concentrations of 25, 50, and 75%. Lime peel contains flavonoids with antibacterial properties. However, in that study, lime peel was extracted using ethanol and ethyl acetate solvents. Ethanol can dissolve flavonoid compounds, and ethyl acetate can extract flavonoid compounds. Flavonoids are polar compounds capable of disrupting bacterial cell wall permeability (Wahyudi, 2013). In this study, lemon peel was only extracted with water at a concentration of 14% and exhibited inhibition zones below 10 mm. This aligns with Sriwarthini's (2014) study, which tested the antibacterial activity of kamboja stem bark extract with ethyl acetate, ethanol, and water solvents at concentrations of 30, 45, and 60% against *Bacillus cereus*, *Staphylococcus aureus*, Shigella dysentriae, and Pseudomonas aeruginosa. The results show that samples dissolved in water at all three concentrations had the smallest inhibition zone diameters compared to ethyl acetate and ethanol, i.e., below 10 mm.

4. CONCLUSIONS

Local and imported lemon juice and peel exhibit remarkably strong antioxidant activities, while lime juice and peel demonstrate strong antioxidant activities. Consequently, both local and imported lemon juice and peel possess stronger antioxidant activities when compared to lime juice and peel. Local lemon juice, imported lemon juice, and lime juice demonstrate strong inhibitory effects against *S.aureus* and moderate inhibitory effects against *E.coli*. Imported lemon juice and lime juice exhibit strong inhibition against *B.subtilis*, whereas local lemon juice only demonstrates a moderate effect. Lemon juice shows a greater inhibitory capacity compared to lime juice against *S.aureus, E.coli*, and

B.subtilis. The inhibitory activities of lemon juice and lime juice are higher against Gram-positive bacteria (*S.aureus* and *B.subtilis*) compared to the tested Gram-negative bacteria (*E.coli*). Antibacterial activity against cocci bacteria is higher compared to bacilli bacteria, evident by a more substantial inhibitory effect against *S.aureus* compared to *B.subtilis* and *E.coli*. Local lemon peel, imported lemon peel, and lime peel do not inhibit the growth of *E.coli*, *B.subtilis*, and *S.aureus*.

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