EFFECTIVENESS OF ANTI-BACTERIAL EXTRACT FROM KULIT JERUK NIPIS (Citrus aurantiifolia) IN REDUCING THE NUMBER OF Escherichia coli BACTERIA ON PLATES

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ABSTRACT

Bacterial contamination of Escherichia coli on eating utensils, especially plates, is a serious health challenge, particularly in areas with low hygiene standards. The use of natural ingredients such as lime peel extract (Citrus aurantiifolia) as an antibacterial agent can be an environmentally friendly solution. This study aims to test the effectiveness of lime peel extract in reducing the number of Escherichia coli bacteria on plates. The study was conducted using an experimental method with a Static Group Comparison design. Plate samples were obtained from a restaurant in West Kota District. A total of 18 plate swab samples were taken and tested at the Gorontalo Ministry of Health Polytechnic Microbiology Laboratory. The samples were divided into three groups: 6 samples without treatment, 6 samples with 5% extract concentration, and 6 samples with 7% concentration treatment. The results showed that the average number of E. coli bacteria on untreated plates was 11.24 CFU/cm². After treatment with a 5% concentration, this number decreased to 1.08 CFU/cm² (90.43%). At a concentration of 7%, the E. coli count decreased significantly to 0.15 CFU/cm² (98.66%). Statistical analysis showed a significant difference between the control and treatment groups (p-value < 0.05), with the highest effectiveness at a concentration of 7%. In conclusion, lime peel extract is effective as a natural antibacterial agent, especially at a concentration of 7% with a reduction rate of up to 98.66%, and has the potential to be used as an environmentally friendly solution for maintaining the cleanliness of eating utensils.

Keywords : Antibacterial, Lime Peel, Escherichia coli, Eating Utensils, Sanitation.

INTRODUCTION

According to the WHO, cases of foodborne diseases caused by pathogenic bacteria such as Escherichia coli (E. coli) remain a significant threat in many countries, especially in developing regions. The WHO reports that worldwide, approximately 600 million cases of foodborne diseases occur each year, resulting in around 420,000 deaths, many of which occur in countries with low hygiene standards, [1].

The use of clean eating utensils that are free from microbial contamination is an important aspect of maintaining public health. Escherichia coli (E. coli) bacteria are one of the pathogens commonly found on unclean eating utensils. Infection caused by E. coli can lead to serious digestive disorders, such as diarrhea, which can result in dehydration and other more severe complications, especially in vulnerable groups such as children and the elderly, [2].
The availability of tableware is closely related

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to hygiene. Even tableware that has been washed is not necessarily guaranteed to be clean. Therefore, maintaining the cleanliness of tableware must be done thoroughly, from the washing process to storage., [3].

Food safety issues in Indonesia are reflected in the high number of food poisoning cases, which continue to increase every year. Based on data from the Ministry of Health (Kemenkes), in 2021 there were 70 cases of food poisoning, while the Food and Drug Supervisory Agency (BPOM) recorded 50 cases in the same year. In 2022, the number of cases increased to 3,514. This increase continued into 2023, with 4,792 cases of food poisoning recorded from January 1 to October 16. The head of the Food Safety Working Group at the Ministry of Health's Directorate of Environmental Health, Cucu Cakrawati Kosim, said that the number of cases in 2023 had increased by more than 1,000 cases compared to the previous year, [4].

One notable case was the food poisoning incident that affected more than 500 students at Brawijaya University in February 2023. This incident came under scrutiny because the food consumed was found to be contaminated with Escherichia coli (E. coli) bacteria. This incident occurred during a student camp in Malang, where the victims experienced symptoms of diarrhea, nausea, and vomiting. Findings from the local Health Department confirmed that the food served at the event was indeed contaminated with E. coli, highlighting the importance of implementing strict hygiene standards in food processing to prevent similar cases from recurring, [5].

Based on data from the Gorontalo City Health Office in August 2024 in Gorontalo City, specifically in the West Kota District, Buladu Village. Seven people were reported to have suffered food poisoning after attending an event. The case resulted in several victims experiencing mild dehydration, but there were no fatalities. This underscores the importance of good hygiene practices in food processing, especially on a large scale, [6].

This case highlights the importance of implementing hygiene practices, especially in environments that frequently serve large numbers of people, such as restaurants. Restaurant owners have a significant responsibility to maintain the cleanliness and quality of the food served to consumers. Regular monitoring and education of restaurant managers and employees are essential to prevent similar cases from occurring in the future, [7].

The province of Gorontalo, with its abundant natural resources, has the potential to become a center for the development of plant-based products. If the antibacterial effectiveness of lime peel extract against E. coli is proven to be significant, this could open up new economic opportunities for the local community through the development of natural lime-based sanitation products. The use of lime peel as a natural antibacterial agent could be an environmentally friendly alternative to reduce the use of synthetic chemicals in the sanitation of eating utensils, [8].

The city of Gorontalo, known for its natural wealth, has the potential to utilize lime peel (Citrus aurantiifolia) due to its bioactive compounds, such as flavonoids, limonene,

ascorbic acid, citric acid, and essential oils, which have been proven to have antibacterial properties. The active compounds in lime are capable of fighting Escherichia coli (E. coli) bacteria by creating an acidic environment through citric acid, which damages the bacterial cell wall, disrupts membrane stability, and inhibits E. coli metabolism, which generally grows at neutral pH. Additionally, the flavonoids and essential oils in lime function as antimicrobial agents that disrupt the structure and function of bacterial cell walls and plasma membranes, thereby inhibiting bacterial growth or even killing bacterial cells, [9]

The local use of lime peel extract in various regions of Indonesia shows variations in its effectiveness, which is thought to be influenced by environmental factors and extraction methods. In addition, with increasing public awareness of the importance of using natural products that are safe for health and the environment, this research is highly relevant. This research will not only contribute scientifically to the development of natural antibacterial agents but also provide practical solutions for the community in maintaining the cleanliness of their daily eating utensils, (Ria, 2020).

Previous research by Sari & Asri, (2022), shows that lime peel extract is effective in inhibiting the growth of several pathogenic bacteria. However, specific research on the effectiveness of this extract against Escherichia coli on the surface of eating utensils, especially in the Gorontalo region, is still very limited. In Gorontalo City, plates remain the primary eating utensil commonly used in various eating establishments, such as restaurants, cafes, and food stalls. For example, at Restaurant X, plates are used daily to serve food to customers. This widespread use of plates underscores the importance of maintaining the cleanliness of plates as the primary eating utensil in these locations.

METHOD

This study is an experimental study that aims to test the effectiveness of lime peel extract in reducing the number of E. coli bacteria on tableware. The research design used is Static Group Comparison, in which there is a treatment group given lime peel extract at concentrations of 5% and 7%, and a control group without lime peel extract treatment. This design allows for an objective analysis of the effectiveness of lime peel extract. The sample consisted of 9 plates taken from a restaurant, which were then divided into three treatment groups. The first group consisted of 3 control plates without treatment, the second group consisted of 3 plates treated with 5% concentration extract, and the third group consisted of 3 plates treated with 7% concentration extract.

Swab samples of the plates were taken over two days, with 9 plates used each day, consisting of 3 control plates, 3 plates treated with 5% extract, and 3 plates treated with 7% extract. Thus, the total number of samples used in this study was 18, consisting of 9 samples on the first day and 9 samples on the second day.

The tools used are plates, gloves, masks, swabs, petri dishes, measuring pipettes, colony counters, compact dryers, and cool boxes, sterile cotton swabs (water swabs), sterile gloves, small letter markers, Bunsen burners or spirit lamps, sample collection forms, small scissors,

cellophane tape, sterile alcohol swabs for disinfection, lime peel extract, E. coli-positive well water samples, plastic samples, label paper, 75% alcohol, phosphate buffer solution as a sample transport medium, and a blender.

Preparing lime peel:

1. Separate the lime peel from the flesh. Then cut the peel into small pieces so that it is easier to process.
2. Blend the lime peel pieces using a blender, adding a little sterile water (if necessary).
3. Saring cairan hasil perasan menggunakan kain muslin atau saringan halus untuk memisahkan ampas dari cairan ekstrak.
4. Re-filter using filter paper or a finer sieve to ensure that the extract liquid is pure and free of sediment.

Preparation of lime peel extract using the maceration method:

1. Cut the lime peel into small pieces.
2. Weigh 30 grams of lime peel.
3. Add 450 ml of ethanol as a solvent, with a ratio of 1:15 (30 grams of material : 450 ml of solvent).
4. Stir the mixture manually until evenly mixed.
5. Perform the maceration process for 48 hours.
6. Place the maceration process in a tightly closed container at a room temperature of around 25°C.
7. After maceration is complete, filter the solution using Whatman no. 01 filter paper.
8. The filtrate obtained is then evaporated by periodically opening the container lid and left at a room temperature of 25°C for 48 hours.

Soaking dishes in well water positive for E. coli:

1. The plates to be used are soaked overnight in well water that has been proven to contain E. coli bacteria to ensure contamination.
2. After soaking, the plates are ready for swabbing.

Sample Collection and Preparation Procedure:

1. Use sterile gloves to collect samples from utensils that are still soaking.
2. Randomly select utensils to be inspected from the soaking area.
3. Prepare the utensil inspection record form and group them as needed.
4. Prepare sterile cotton swabs and test tubes containing phosphate buffer solution and seal them tightly.
5. Insert the sterile cotton swab into the test tube, then press it against the wall of the test tube to remove excess liquid, then remove it and use it to swab.
6. The swabbing procedure is carried out according to the type of tableware: for plates, swabbing is done on the inner surface in a zigzag pattern.
7. Each surface area is swabbed three times in a row, and one cotton swab or one swab is only used for one group of tableware being inspected.
8. This process is repeated until all utensils in the group have been swabbed, with one cotton swab used for only one type of utensil.
9. Before and after swabbing each utensil, the test tube containing the sterile cotton swab and phosphate buffer solution must be sterilized by heating it over a Bunsen burner and then tightly resealed with cotton.

10. Cellotape labels are attached to each test tube, complete with sample information for control samples, treatment samples one or two.
11. The test tubes are arranged neatly on the test tube rack, followed by examination of the E. coli bacteria count using the Compact Dry method.

Procedure for treating lime peel extract:

1. Lime peel extract is prepared in two spray bottles with concentrations of 5% and 7% as follows: a) dilute 5 ml of 5% concentration extract with 100 ml of distilled water using a pipette; b) dilute 7 ml of 7% concentration extract with 100 ml of distilled water using a pipette.
2. Spray the extract solution onto the surface of the plate with 3 sprays.
3. Remove any remaining extract solution from the surface of the plate.
4. Wipe the plate utensils and repeat the same procedure as before wiping for the untreated sample.

In laboratory analysis, E. coli can be identified using the Compact Dry method, which is a practical and efficient dry medium-based culture method. Compact Dry is designed to detect and count the number of bacterial colonies in a sample, including E. coli. In this medium, growing E. coli will produce blue or metallic green colonies due to the activity of the β-glucuronidase enzyme, while non-E. coli coliforms will appear reddish-purple. This identification utilizes a color differentiation reaction that occurs due to the chromogenic compounds contained in the Compact Dry medium.

The procedure for checking the number of germs on tableware such as plates in the laboratory is carried out using Compact Dry:

1. Prepare a test tube containing sterile cotton swabs and phosphate buffer solution as a transport medium for the previously wiped tableware samples.
2. Open the Compact Dry Plate.
3. Take 1 ml of solution using a pipette and place it in the Compact Dry.
4. Close the Compact Dry Plate again and label it with sample codes C1, P1a, P1b, and so on.
5. Incubate the Compact Dry container properly at the appropriate temperature and time for the organism being tested.
6. The colonies that grow on the medium are observed after incubation. If the incubation results show blue or metallic green colonies, this indicates the presence of Escherichia coli bacteria in the tested sample.
7. The appropriate guidelines are used to identify microorganisms and interpret the results.

After analysis using the Compact Dry method, the number of E. coli colonies growing on the medium was counted for each sample. These results were then used to determine the level of bacterial contamination on the surface of tableware in the form of plates. To obtain a more representative E. coli count, further calculations were made based on the area of the plate wiped, using the following formula:

$$\text{Jumlah } E.\ coli / \text{cm}^2 = \frac{\text{Jumlah koloni } E.\ coli \times 10}{\text{Luas bidang usap (cm}^{2)}}$$

Where the number 10 comes from the volume of phosphate buffer solution (in ml) used to extract bacteria from the surface of the plate. After analysis, the results of the E. coli bacterial count on the plate will be interpreted based on the standards set out in Minister of [11] where the safe limit or eligibility requirement is 0 CFU (Colony Forming Unit). Next, a comparison was made between the number of E. coli on plates that were not treated (control) and plates that had been treated with lime peel extract at various concentrations. This comparison aimed to evaluate the effectiveness of lime peel extract in reducing the number of E.coli on tableware, so that the extent to which the extract could act as an effective antibacterial agent could be determined.

RESULTS AND DISCUSSION

This study was conducted at the Microbiology Laboratory of the Gorontalo Ministry of Health Polytechnic, with samples taken from plates at a restaurant in the West District of Gorontalo City. In the initial stage, researchers conducted preliminary tests to detect the presence of E. coli bacteria on the surface of the plates. The preliminary test results showed that no E. coli bacteria were found, only coliform bacteria. This indicated that the plates were not sufficiently contaminated with E. coli for optimal antibacterial effectiveness testing. As a solution, the researchers used an alternative method by soaking all plate samples in well water that had been proven to contain E. coli. The soaking was carried out for 24 hours so that the entire surface of the plates was exposed and evenly contaminated with E. coli. This well water was taken from a resident's house in Bonebolango Regency, Gorontalo Province, which is known to have water quality with a fairly high level of bacterial contamination. This step was taken to ensure that all samples had a uniform level of E. coli contamination before being treated with lime peel extract, so that the effectiveness test could be carried out validly and consistently.

After soaking, the plates were divided into three treatment groups, namely: the control group, which was immediately wiped and tested for E. coli levels without additional treatment; the 5% extract treatment group, which was sprayed with 5% lime peel extract (5 ml of extract diluted with 100 ml of distilled water) before being wiped and tested for E. coli levels, and a 7% extract treatment group that was sprayed with 7% lime peel extract (7 ml of extract diluted with 100 ml of distilled water) before being wiped and tested for E. coli levels.

The lime peel extract was made using the maceration method for 4 days or 2×48 hours using ethanol as a solvent. The extract was applied by spraying it on the surface of the plates after soaking, according to the concentration of each treatment. After all treatments were completed, laboratory tests were conducted to measure the remaining E. coli bacteria on the surface of the plates. The laboratory test results were then analyzed to determine the effectiveness of each treatment in reducing the number of E. coli on the plates.

Univariate Analysis

1. Analysis of E. coli bacteria counts in dishware swab samples.

The test results showed differences in the number of E. coli bacterial colonies that grew in the control group (without extract), the 5% extract treatment group, and the 7% extract treatment group. The test results data can be seen in Table 1 below:

Table. 1
E. coli Bacteria Count in Swab Samples of Tableware

No Sample	Day	Treatment	*E.Coli Count* (CFU/ cm^2)	Average *E.Coli* (CFU/ cm^2)
1	Day 1	Without Extract	1,30	11,24
2			0,85	
3			0,23	
4	Day 2		24,91	
5			23,10	
6			17,04	
7	Day 1	5% extract	0,57	1,08
8			0,06	
9			0,00	
10	Day 2		3,45	
11			1,64	
12			0,74	
13	Day 1	7% extract	0,17	0,15
14			0,00	
15			0,00	
16	Day 2		0,51	
17			0,17	
18			0,06	

Source: Analysis results, 2025

Based on Table 1, it can be seen that the average number of E. coli on untreated plates was 11.24 CFU/cm², the average number of E. coli on plates treated with 5% extract was 1.08 CFU/cm², and the average number of E. coli on plates treated with 7% extract was 0.15 CFU/cm².

The group without extract treatment shows that under normal conditions, without antibacterial treatment, E. coli bacteria are still present in fairly high numbers on tableware. There were significant differences between samples, with the highest value reaching 24.91 CFU/cm² (sample 4) and the lowest value 0.23 CFU/cm² (sample 3), indicating variation in bacterial contamination between samples.

This difference in contamination levels was also observed significantly between the first and second days of sampling. On the second day, the level of E. coli contamination tended to be higher than on the first day. This was determined based on direct observations in the field, where on the second day there was increased activity around the well water source, such as washing food ingredients, washing eating utensils, and the presence of livestock pens located close to the well. These activities are strongly suspected to be one of the causes of the increased level of E. coli bacterial contamination in the well water used to soak eating utensils.

Previous research supports these findings [12] The study revealed that human activity around water sources has the potential to increase the level of bacterial contamination, particularly E. coli. E. coli contamination in well water can originate from livestock manure

or human fecal contamination that enters water sources through rainwater runoff or liquid waste. Also, Diantoro (2023) states that increased activity around water sources can worsen well water quality, given the possibility of cross-contamination between water sources and polluted surrounding environments.

Therefore, activities that took place on the second day, such as washing food ingredients and eating utensils, as well as the proximity of livestock pens to wells, were significant risk factors in increasing E. coli levels in well water, which then affected the level of contamination in eating utensils soaked in that water.

High contamination in control samples soaked in well water directly affected the results of surface swab tests on eating utensils. Control samples soaked in well water with high E. coli levels produced fairly high contamination levels on eating utensils before being treated with 5% and 7% lime peel extract. Because the level of E. coli contamination in the well water used to soak the eating utensils was very high, on the second day, the eating utensils tested showed higher levels of E. coli on their surfaces, even before treatment was carried out. This resulted in the swab test results on tableware treated with 5% and 7% lime peel extract not showing a significant decrease compared to the first day. This condition reinforces the importance of applying antibacterial agents such as lime peel extract to reduce the number of bacteria.

This is in line with the results of research by [13] which states that there is no decrease in the number of bacteria in the control sample if it has not been treated with antibacterial extract from lime peel or other antibacterial substances. In other words, without special treatment, eating utensils remain at high risk of becoming a medium for the spread of bacteria.

Table 1 shows that after treatment with 5% extract, the average number of E. coli decreased to 1.08 CFU/cm². This value is much lower than the condition without extract, indicating that lime peel extract has an antibacterial effect on E. coli. The lowest decrease was seen in samples 9 and 8, where the number of bacteria fell to 0.00 CFU/cm². However, there were still quite high values in some samples, for example in sample 10 with 3.45 CFU/cm².

After treatment with 5% extract, the reduction effectiveness was 90.43%. Although this is quite effective, the results show that some samples still contained E. coli. This is because the content of chemical compounds that can reduce or inhibit bacterial growth at this concentration is quite low, so it cannot completely reduce the number of bacteria on the surface of tableware to 0 CFU/cm², based on the quality standards for tableware according to Ministry of Health Regulation No. 14 of 2021, which requires E. coli = 0 CFU/cm². According to research conducted by Mahmudi et al., (2021), Lime peel extract with a concentration of 1% to 5% has not been able to completely inhibit or kill bacterial growth by 100%, but it has shown effectiveness in reducing the number of bacteria present.

Table 1 shows that at a concentration of 7%, the number of E. coli bacteria decreased further to an average of 0.15 CFU/cm², with almost all repetitions showing very low numbers, where in samples 14 and 15 the number of bacteria reached 0.00 CFU/cm²,

while in repetition 16 the highest value was 0.51 CFU/cm². These results indicate that at a concentration of 7%, lime peel extract is able to provide a more optimal antibacterial effect compared to a concentration of 5%. The effectiveness of the 7% concentration reached 98.66%. At a concentration of 7%, the bacterial count can decrease dramatically so that it almost meets the quality standards for eating utensils, which require that the E. coli bacterial count on eating utensils be 0 CFU/cm². At this concentration, the chemical compound content in the extract is quite high, so even though the difference is only about 2% compared to the previous extract treatment, its effectiveness in reducing the number of bacteria still shows significant results.

This decrease indicates that even though the difference in concentration appears small, the active ingredient content is able to work more optimally in inhibiting bacterial growth. This finding is also in line with research conducted by Ivo, (2019), which shows that the use of lime peel extract in concentrations of 6% to 11% has been proven effective in reducing the number of germs to less than 1 CFU/cm². This proves that the higher the concentration of the extract, the greater the antibacterial potential produced.

1. Analysis of the effectiveness of reducing E. coli bacteria levels by administering lime peel extract at concentrations of 5% and 7%.

After conducting a univariate analysis of E. coli bacteria counts based on treatment groups, an analysis of the effectiveness of reducing bacteria counts through the administration of lime peel extract was carried out. This effectiveness assessment was calculated as a percentage reduction in the number of bacterial colonies after treatment with 5% and 7% concentrations of the extract. The use of extracts with concentrations of 5% and 7% showed differences in the level of effectiveness in reducing bacterial counts on tableware. The data on the effectiveness of applying these extracts can be seen in Table 2.

Table. 2
Effectiveness of reducing E. coli bacteria in dishware wipe samples

No Sample	Day	Treatment	*E.Coli Count* (CFU/ cm²)	Average *E.Coli* (CFU/ cm²)
1	Day 1	5% extract	56,52	90,43
2			93,33	
3			100	
4	Day 2		86,13	
5			92,89	
6			95,68	
7	Day 1	7% extract	86,95	98,66
8			100	
9			100	
10			97,95	
11	Day 2		99,26	
12			99,67	

Source: Analysis results, 2025

Based on Table 2, it can be seen that the effectiveness of reducing E. coli levels on tableware after treatment with 5% and 7% lime

peel extract was significantly different. This effectiveness was measured in terms of the percentage reduction in the number of E. coli after treatment.

In the treatment group with 5% extract, the effectiveness of E. coli reduction varied between 56.52% and 100%, with an average effectiveness of 90.43%. This shows that the use of 5% lime peel extract is effective enough in reducing the number of E. coli on eating utensils, although in some repetitions, lower effectiveness was still found, such as in the first sample, which only reached 56.52%. However, in the other two samples (samples 2 and 3), the effectiveness reached 100%, meaning that no E. coli bacteria were detected after treatment.

Meanwhile, in the treatment group with 7% extract, the effectiveness of E. coli reduction was higher and more consistent, ranging from 86.95% to 100%, with an average effectiveness of 98.66%. These results indicate that lime peel extract with a higher concentration is capable of providing a stronger and more stable antibacterial effect. Most samples showed an effectiveness of over 97%, and in three samples (8th, 9th, and 12th), the effectiveness reached 100%, which means that E. coli was completely eliminated after treatment.

Overall, the results of this study indicate that lime peel extract has antibacterial properties against E. coli on tableware. The higher the concentration of extract used, the higher its effectiveness in reducing the number of bacteria. A 7% concentration proved to be more effective than 5%, as it resulted in a more stable reduction in bacteria, approaching 100% in almost all repetitions. This shows that lime peel extract has the potential to be used as a natural ingredient in reducing the risk of pathogenic bacterial contamination.

Bivariate Analysis

In the bivariate analysis in this study, researchers used ANOVA (Analysis of Variance) to evaluate the decrease in the number of E. coli bacteria on tableware between the control group (without treatment) and the group treated with lime peel extract at concentrations of 5% and 7%. Previously, the data had been tested for normality using the Kolmogorov-Smirnov test, and the results showed that the data were normally distributed. After that, a data homogeneity test was performed using the Levene test and Post-hoc LSD, and the results were considered relevant because they were able to provide an informative picture of the differences between groups.

Table 3.

ANOVA Test Results: Differences in E. coli Bacteria Levels in 3 Experiments

Treatment	Average E. coli Levels	*P-Value*
Without Extract	11,24	0,023
5% Extract	1,08	
7% Extract	0,15	

Source: Analysis results, 2025

Based on Table 3, it can be seen that the results of the E. coli level difference test in the three treatment groups show that there is a significant difference between the group that was not given the extract and the group that was given lime extract at concentrations of 5% and

7%.

The average E. coli level in the group without extract treatment was 11.24 CFU/cm², while in the group given 5% lime peel extract, there was a drastic decrease to 1.08 CFU/cm². Furthermore, in the treatment group with 7% lime extract, the E. coli level decreased further to 0.15 CFU/cm², showing higher effectiveness than the treatment with 5% extract.

The results of the ANOVA statistical test showed a p-value = 0.023 ($p < 0.05$). This proves that the application of lime peel extract has a significant effect on reducing the number of E. coli bacteria on the surface of tableware. In addition, the effectiveness of bacterial reduction increases with increasing extract concentration. This can be seen in the results of the treatment group with 7% extract, which is close to the quality standard.

These findings are reinforced by the results of previous studies conducted by Sari & Asri, (2022) and Retno Retno Tri Astuti et al., (2022), which show that the antibacterial effectiveness of orange peel extracts, both lime and lemon, increases with the increase in the concentration of the extract used. This increase in effectiveness is related to the higher content of active compounds such as flavonoids and tannins, which are known to inhibit the growth of various types of bacteria, including Shigella, Salmonella typhi, and Escherichia coli. These compounds work by damaging the structure of bacterial cell walls, inhibiting protein synthesis, and disrupting bacterial enzymatic functions, thereby inhibiting or even stopping bacterial growth.

Uji *Post-hoc* LSD (*Least Significant Difference)*

The LSD (Least Significant Difference) post-hoc test aims to identify significant differences between each treatment group, thereby providing a deeper understanding of the relationship between groups in this study.

Table. 4
LSD Test Results for each Treatment Group

Treatment		***P-Value***
Without Extract	5% Extract	0,021
	7% Extract	0,013
5% Extract	7% Extract	0,817

Source: Analysis results, 2025

Based on Table 4, the LSD (Least Significant Difference) test results show that there are significant differences between the group without extract treatment and the groups given 5% and 7% lime peel extract, with p-values of 0.021 and 0.013 (<0.05), respectively. This indicates that the administration of lime peel extract significantly reduced the level of E. coli on eating utensils compared to the untreated group.

However, the comparison between the 5% and 7% extracts resulted in a p-value of 0.817, which means that there was no significant difference between these two concentrations in reducing E. coli levels. In other words, although the 7% extract showed a greater reduction, its effectiveness was not statistically different from that of the 5% extract.

The LSD test itself was used in this study to determine whether there were significant differences between treatment groups after conducting an ANOVA test, thereby identifying

which groups had significantly different effects. These results indicate that the application of lime peel extract, at both 5% and 7% concentrations, is effective in reducing E. coli on eating utensils, but increasing the concentration from 5% to 7% does not provide a significant additional effect.

When compared to the quality standards set out in Minister of Health Regulation (Permenkes) No. 14 (2021), which requires E. coli = 0 CFU/cm², the use of this extract still does not fully meet the standards in all samples. This indicates that although lime peel extract has potential as an antibacterial agent, its effectiveness is not yet optimal and requires further development to achieve the expected standards.

Several factors may contribute to the discrepancy between the research results and the standard quality criteria, Ani et al., (2024), mentioning that extract concentration is one of the main factors, where the content of antibacterial active compounds such as flavonoids and tannins increases with increasing concentration. However, the concentration used in this study, although effective, may not be high enough to provide maximum results. In addition, the duration of contact between the extract and the surface of the eating utensils also has an effect, where longer contact times can increase the extract's ability to kill bacteria more effectively. The application method used also plays an important role. Soaking, for example, tends to be more effective than spraying because the solution can evenly coat the surface of the cutlery, thereby increasing its effectiveness. Environmental factors, such as temperature and humidity, also affect the performance of lime peel extract as an antibacterial agent. An unsupportive environment, such as excessively low temperatures or high humidity, can reduce the ability of the active compounds in the extract to inhibit bacterial growth.

To overcome these obstacles and increase the effectiveness of lime peel extract, several steps can be taken. Hujjatusnaini et al., (2021) and Dinata et al., (2023), Recommendations: First, increase the concentration of the extract or combine it with other antibacterial agents, such as vinegar or hot water, to maximize its antibacterial potential. Second, use the immersion method rather than spraying to allow for longer contact time and more even distribution of the extract on the surface of eating utensils. Third, extend the duration of application to increase its bactericidal power. Finally, conducting tests under various environmental conditions, such as variations in temperature and humidity levels, to evaluate its effectiveness in a wider range of scenarios.

Lime peel contains various active compounds that have antibacterial properties, such as flavonoids, tannins, saponins, phenols, alkaloids, and essential oils. Each of these compounds has a different mechanism of action in inhibiting bacterial growth. Among these compounds, flavonoids and tannins are the components with the highest concentration in lime peel, making them more effective as antibacterial agents, [20].

Flavonoids in lime peel have broad biological activity, including the ability to inhibit bacterial growth and function as antiviral, antibacterial, and anti-inflammatory

agents. As antimicrobials, flavonoids work by disrupting the vital functions of microorganisms, such as bacteria or viruses, by dissolving and binding to extracellular proteins and integral proteins. As a result, the cell walls of microorganisms become weak and prone to rupture because they are unable to withstand cytoplasmic pressure, [21].

On the other hand, tannins, which are complex polyphenolic compounds with high molecular weight, also play an important role as antibacterial agents. Tannins work by reacting with bacterial cell membranes, inactivating important enzymes, and damaging the function of bacterial genetic material, [22].

Poor hygiene of eating utensils can support the growth and spread of germs, thereby increasing the risk of disease and poisoning. Therefore, maintaining the hygiene of eating utensils is very important to prevent contamination by pathogenic germs and other harmful substances. Bacterial contamination through unclean eating utensils can affect food quality and consumer health. This study aims to explore the potential of lime peel extract as a natural ingredient that can inhibit bacterial growth on eating utensils. Although the results of the study show that lime peel extract is not yet fully effective in killing E. coli completely according to quality standards, the use of this extract still contributes significantly to reducing the number of bacteria on eating utensils. Thus, lime peel extract can be a natural alternative that helps reduce bacterial contamination on eating utensils, although its effectiveness still needs to be improved through further development.

CONCLUSION

Lime peel extract at concentrations of 5% and 7% is effective in reducing the number of E. coli bacteria on tableware. However, the LSD test results show that the difference in effectiveness between the 5% and 7% concentrations is not statistically significant, meaning that although the 7% extract has a higher average reduction effectiveness (98.66%) compared to the 5% extract (90.43%), increasing the concentration from 5% to 7% does not yield a statistically significant difference. Therefore, the most effective reduction is still achieved at a concentration of 7% with an effectiveness of up to 98.66%.

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