Test for the antibacterial inhibition of kaffir lime leaf (Citrus hysteric D.C) extract against pathogen bacteria in improving food safety

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Abstract. Kaffir lime leaf (Citrus hystrix) is a plant from the citrus tribe that has long been known by community as flavor ingredient. To support its use and increase its application in supporting food safety, a test the inhibition of on kaffir lime leaf extract against pathogenic bacteria, namely Gram Negative Bacteria (Escherichia coli, Salmonella typhimurium) and Gram Positive bacteria (Staphylococcus aereus, P. aeroginosa). Making kaffir lime leaf extract (Citrus hystrix) was done by weighing 150g of lime leaf powder, then immersing in 96% ethanol solution and leaving for + 3 days. Kaffir lime leaf extract was dissolved with sterile distilled aquades to obtain a concentration of 5%, 10% and 15%. The antibacter ial activity of kaffir lime leaf extract was tested by diffusion method using disc paper to determine of the bacterial growth inhibition area. The results showed that kaffir lime extract had antibacterial activity inhibition of 12,78 mm of S. aereus, 9 mm of E.coli, 7,12 mm of S. typhimurium and 9,3 mm of P. aeroginosa. Kaffir lime leaf extract has inhibition effectiveness for gram positive bacteria Staphylococcus aereus and gram negative bacteria E. coli, Salmonella typhimurium, P. aeroginosa. Thus, kaffir lime leaf extract can be used as a decontaminant agait these 4 type of bacteria, especially Staphylococcus aereus which has a strong inhibitory power, so it can maintain quality and increase the safety of mead based foods.

1. Introduction

Food safety is a public need in maintaining the quality / preventing food / processed products from being contaminated or contaminated by pathogenic bacteria and not causing or causing foodborne illness and infection. In an effort to prevent food from being contaminated by pathogenic bacteria, spices form plants are used as natural preservatives, namely kaffir lime leaves (Citrus hystrix DC).

Kaffir lime leaves (Citrus hystrix DC) is a plant from the citrus tribe that has long been recognized by the public as a natural flavor ingredient in food products (peanut brittle, potato chili sauce, fish meat sauce, etc.) and beverages. The leaves of Citrus stomach (Citrus hystrix DC) contain 1.8% tannin compounds; steroids, triterpenoids, 1-1.5% essential oils, alkaloids, polyphenols, flavonoids so that they can be used as antibacterial agents [1] In addition, kaffir lime leaves (Citrus hystrix DC) also have several chemical components, namely 81,49%, citronellal; citronellol 4.22%; linalol 3.69%; geraniol 0.31%; other components 6.29% [2].
Kaffir lime leaf extract with a concentration of 1% and 2% has antibacterial activity against Escherichia coli with MIC and MBC values <0.0625 and has compounds of the terpenes group. According to [3], that the compounds of the terpenes group from kaffir lime leaf extract have antibacterial activity by damaging cell membranes and inhibiting growth and killing pathogenic bacteria by disrupting cell walls [3].

Pathogenic bacteria are usually found in basic ingredients for processed food products, processing equipment and processed products, namely spore-forming bacteria, including Staphylococcus aureus, and Bacillus cereus (Gram-positive bacteria) and Salmonella typhimurium, Escherichia coli (Gram-negative bacteria). Staphylococcus aureus also plays a role in food safety because it can cause mastitis in dairy cows and has the potential to contaminate cow's milk products [4] as well as flora bacteria found in food products, including poultry, fish, bread, cakes and salads; Bacillus cereus is found in meat, fish, milk, vegetables; Salmonella typhimurium is found in poultry, raw eggs, vegetables, fruits; Escherichia coli is a foodborne bacteria that can cause various lower digestive tract disorders in warm-blooded animals [5], also found in spinach, cucumber, cheese, beef, fish, milk and beverages but in some strains such as O157: H7 can cause food poisoning.

These bacteria often make contamination of food and beverages, causing disease, infection and food poisoning and have an impact on product quality degradation. For this reason, in inhibiting the growth of pathogenic bacteria and increasing food safety, kaffir lime leaf extract is needed as an antibacterial. The research objective was to test the antibacterial inhibition of kaffir lime leaves extract against pathogenic bacteria.

2. Methods
2.1. Material
The materials used are kaffir lime leaves and their extracts. Staphylococcus aureus bacteria (FNCC 0047), Bacillus cereus (IFO 13690), Salmonella typhimurium (IFO 12529), Escherichia coli (IFO 3301) obtained by the Food and Nutritional Culture Collection (FNCC) Center for Inter-Study UGM. The medium used was Muller-Hinton Agar (MHA).

2.2. Method
The extract of kaffir lime leaves (Citrus hystrix) was carried out by weighing 150 grams of kaffir lime leaf powder. This powder is then immersed in a 96% ethanol solution and left to stand for ± 3 days. The results obtained are filtered and evaporated for three days. This process aims to evaporate the ethanol to obtain a thick extract from the kaffir lime leaves. Kaffir lime leaves extract was weighed as much as 0.5 g; 1.0 g; 1.5 g was then dissolved with 10 mL of distilled water to obtain a concentration of 5%, 10%, 15%, of the kaffir lime leaf extract.

2.3. Media making
Making Muller-Hinton media according to what is stated on the label, namely by dissolving 38g of agar in 1 liter of distilled water, and then heating it to dissolve completely. The agar solution was sterilized in autoclave at 1210°C for 15 minutes. At 450°C temperature, then poured into a 20 ml petri dish and allowed to solidify.

2.4. Research methods
In this study, the antibacterial activity of kaffir lime leaf extract was tested against the pathogenic bacteria Staphylococcus aureus, Bacillus cereus, Salmonella typhimurium, Escherichia coli in vitro using the paper disc diffusion method. The parameter measured is the diameter of the inhibition area (DDH) in millimeters (mm).
2.5. **Antibacterial test**

Determination of antibacterial activity was carried out by the paper disc diffusion method. The batteries used were obtained from FNCC PAU UGM-Jogyakarta and had been rejuvenated in Muller-Hinton media and incubated at 37°C for 24 hours. Bacteria that grow from the culture are taken as much as one ose and dissolved in physiological 0.9% NaCl as much as 4.5 ml, so that they have a turbidity according to close to the turbidity of the main solution of bacteria \((4 \times 10^9)\) is diluted three times until a bacterial colony suspension is obtained \((4 \times 10^6)\). Bacterial suspension is used as inoculum.

2.6. **Inhibition power diameter**

A total of 0.5 ml of bacterial suspension was poured into a petri dish filled with solid Muller-Hinton agar, the bacterial suspension was leveled and allowed to stand for a while, the excess liquid was piped, and then the media was allowed to dry. On the media was placed disc paper that had been saturated with the test solution for kaffir lime leaf extract at a concentration of 5%, 10%, 15%, respectively. The spelling of each antibacterial activity test was carried out aseptically in a laminar air flow cabinet with three repetitions, then incubated at 37°C for 24 hours. A reading of the total inhibitory power diameter (DDH) when the zone of inhibition formed around the disc paper shows a clear zone. Partial inhibition zone, that is, if the growth of several colonies in the inhibition zone is still visible. Zero zone of inhibition if there is no zone of inhibition around the disc paper. The diameter of the drag zone can be calculated using the caliper in mm.

3. **Research design**

The research design used a factorial design. The first factor was the concentration of kaffir lime leaf extract (5%, 10%, 15%) while the second factor was the isolates of *Staphylococcus aureus*, and *Bacillus cereus* (Gram Positive bacteria) and *Salmonella typhimurium*, *Escherichia coli* (Gram Negative bacteria). Each treatment has three replications. If there is a significant difference in each treatment, Duncan's multiple distance test is performed [6].

4. **Results and discussion**

In this study, the antibacterial activity test in kaffir lime leaf extract was carried out in vitro by using the diffusion method to determine the antibacterial activity contained therein. The results of measurements carried out on the diameter of the inhibition area (DDH), using white lime leaf extract against the bacteria *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhimurium*, *Escherichia coli* are presented in Table 1.

| Kaffir Lime Leaves Extract (*Citrus hystrix DC*) Concentration | DDH    |
|--------------------------------------------------------------|--------|
| 15%                                                          | 13.75a |
| 10%                                                          | 12.41b |
| 5%                                                           | 9.73c  |

Note: Different superscripts in the direction of the column show significant differences \((P <0.05)\)

The results in Table 1 show that kaffir lime leaves extract is very effective in inhibiting the growth of pathogenic bacteria, namely *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhimurium*, *Escherichia coli* at a concentration of 5%, 10% and 15%. The higher the administration of kaffir lime leaves extract, the greater the diameter of the bacterial inhibition (DDH), this means that the antibacterial activity of kaffir lime leaves extract is greater. According to [7], the higher the concentration, the higher the active substance
content so that the antibacterial activity will be greater and vice versa, the lower the concentration, the less the active substance content so that the antibacterial activity will decrease.

Table 2 shows the difference in the diameter of the inhibitory power (DDH) which is influenced by several things including the size of the inoculum, the thickness of the agar plate and the diffusion power of the test solution and the sensitivity of bacteria to the lime leaf extract solution. The media used in this study is the Mueller Hinton Agar (MHA) media which is a good medium for the growth of pathogenic bacteria in the antibacterial inhibitory activity test using the disc diffusion method and is a standardization of the Clinical and Laboratory Standards Institute (CLSI) in testing antibacterial activity using the diffusion method. disc. The content of kaffir lime leaves extract as a test solution is known to have antibacterial activity, namely tannin and flavonoid compounds whose working principle is to damage cell walls in order to inhibit bacterial growth. The alcohol groups in flavonoid compounds damage bacterial cells by taking advantage of differences in the polarity of the lipids that make up cells [8].

Table 2. Effect of treatment combinations between concentrations of kaffir lime leaves extract (Citrus hystrix DC) and pathogenic bacterial isolates on the diameter of inhibition power (DDH) formed.

| Kaffir Lime Leaves Extract (Citrus hystrix DC) Concentration (%) | Types of Bacteria | DDH (mm) |
|---|---|---|
| 15% | S.aureus | 16.47a |
| 10% | S.aureus | 14.82c |
| 5% | S.aureus | 12.78d |
| 15% | B. cereus | 15.81b |
| 10% | B. cereus | 13.49c |
| 5% | B. cereus | 9i |
| 15% | S. typhimurium | 12.58e |
| 10% | S. typhimurium | 9.35b |
| 5% | S. typhimurium | 7.12k |
| 15% | E. coli | 11.38f |
| 10% | E. coli | 10.63g |
| 5% | E. coli | 9.11i |

Note: Different superscripts in the direction of the column show significant differences (P <0.05)

The antibacterial activity of kaffir lime leaves extract against four pathogenic bacterial isolates consists of two Gram-negative bacteria, namely Staphylococcus aureus, Bacillus cereus and two Gram-positive bacteria, namely Salmonella typhimurium, Escherichia coli which are presented in Table 3. The antibacterial activity of Gram-positive bacteria Staphylococcus aureus, Bacillus cereus showed the results of measuring the inhibitory power diameter (DDH) were greater than Gram negative bacteria, namely Salmonella typhimurium, Escherichia coli. Gram-negative bacteria Salmonella typhimurium, Escherichia coli have a high cell wall and lipid content of address [9] 11-22% and a multilayer cell wall structure consisting of lipoproteins, outer membrane phospholipids, and lipopolysaccharides, making it difficult to penetrate the cell walls of Gram negative bacteria. by antibacterial substances compared to Gram-positive bacteria [10];
[11]; [12]. Gram-positive bacteria have a peptidoglycan layer on the outside and less act as an effective permeability defense [13].

Table 3. Effect of kaffir lime leaves extract on inhibition diameter of four bacterial isolates of Staphylococcus aureus, Bacillus cereus, Salmonella typhimurium, Escherichia coli

| Types of Bacteria               | DDH  |
|---------------------------------|------|
| Staphylococcus aureus           | 16.27<sup>a</sup> |
| Bacillus cereus                 | 14.09<sup>b</sup> |
| Salmonella typhimurium          | 11.54<sup>c</sup> |
| Escherichia coli                | 9.51<sup>d</sup> |

Note: Different superscripts in the direction of the column show significant differences (P <0.05)

5. Conclusion

Extra kaffir lime leaves (Citrus hystrix DC) is effective against pathogenic bacteria and can be used as a decontamination for Gram-positive bacteria, namely Staphylococcus aureus which has the largest diameter of inhibition to maintain quality and improve food safety for meat-based food ingredients.

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