Antibacterial Activity of Natural Paper from Banana Peel (*Musa paradisiaca* Linn.) with Additive Essential Oils

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Abstract. Several essential oils have been evaluated as antibacterial agent in the production of natural paper from banana peel (*Musa paradisiaca* Linn.), namely cinnamon, clove, lime and lemon oil. The purpose of the research was to examine the antibacterial activity of the papers with additive of those several essential oils against *Staphylococcus aureus* and *Escherichia coli*. Paper preparation was successfully carried out by alkalinization method, using 4% NaOH at a temperature of 100 °C for 1 h. The essential oils as antibacterial agent were added with concentration 2.5% to the pulp (1:40). The antibacterial activities were tested using Kirby-Bauer disc diffusion method. All of the paper produced meet the standard of ISO 6519:2016 about Basic Paper for Laminated Plastic Wrapping Paper within the parameter of pH and water content. The result of the research showed that all of the paper with essential oils additive has antibacterial activity against *S. aureus*, but only paper with cinnamon oil additive that have antibacterial activity against *E. coli*. The use of cinnamon oil gave the largest inhibition zone diameter both on *S. aureus* and *E. coli*. Paper with cinnamon oil additive had inhibition zone diameter 18 mm on *S. aureus* (strong category) and 9 mm on *E. coli* (medium category).

1. Introduction

Bacteria are the cause of many diseases suffered by the people of Indonesia, where one of the spreads is through food. Bacterial contamination in food can occur directly where contaminants are obtained from the place of life or origin of the food ingredients, or indirectly where contaminants are obtained through intermediaries. Wrapping paper unwittingly has become a bacterial contamination intermediary on food. So far, the raw material for making paper is wood cellulose. The more paper needs, the more wood is needed, so that over time more and more deforestation will occur. Banana peel has been reported to be an alternative raw material for making paper because of its high cellulose content. The resulting banana peel paper is reported to have fulfilled the SNI requirements on the parameters of pH, moisture content, grammage and brightness [1].

The way to weaken bacteria is by giving antibacterial compounds, a chemical agent that is able to inactivate bacteria by inhibiting bacterial growth (bacteriostatic) and killing bacteria (bactericidal) [2]. The mechanism of inhibition of bacteria by antibacterial substances can be done in 5 ways: 1) inhibiting microbial cell wall synthesis, 2) damaging the integrity of microbial cell walls, 3) inhibiting microbial cell protein synthesis, 4) inhibiting nucleic acid synthesis, and 5) damaging microbial cell nucleic acids [3].

Essential oil (also called volatile oil) is an aromatic oily liquid obtained from various plants.
(flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruit and roots). Essential oils are known to contain various chemical compounds, such as terpenes, aldehydes, alcohols, esters, phenolics, ethers, and ketones [4]. Essential oils are reported to have activity as antibacterial, antifungal, antiviral insecticides and antibiotics [5, 6]. Some essential oils, including cinnamon oil, clove oil, lime oil and orange oil, are reported to have antibacterial activity against S. aureus, B. subtilis, K. pneumoniae, P. vulgaris, P. aeruginosa and E. coli [7]. Cinnamon oil has the main component of E-cinnamaldehyde with a percentage reaching 94.67% [8]. The main component of clove oil is eugenol which is used to reduce pain [9]. While the main components of lime and orange oil are limonene and β-pinena [10]. By adding essential oils as antibacterial agents in making natural paper with banana peels as raw material, antibacterial paper is produced to prevent bacterial contamination of food.

2. Experimental

2.1. Materials

The material for making paper was Kepok banana peel, NaOH, tapioca, essential oil consisting of cinnamon oil, lemon oil, clove oil and lime oil. Material for a antibacterial activity assay was Mueller Hinton Broth, Mc. Farland, NaCl, potassium chloride and distilled water. A strain of gram-negative bacteria, E. coli ATCC 25922, and gram-positive bacteria, S. aureus ATCC 25923 was used. Distilled water are used as a negative control, tetracycline as a positive control for gram-positive bacteria, and chloramphenicol is positive control for gram-negative bacteria.

2.2. Method

2.2.1. Paper Production

Paper preparation carried out by alkaliization method, using 4 % NaOH at a temperature of 100 °C for 1 h. Bleaching process with 10 % hydrogen peroxide solution. The essential oils as antibacterial are added with concentration 2.5 % to the pulp (1:40). Essential oils used in this research are cinnamon oil, clove oil, lime oil, and lemon oil. Tapioca is added at concentration of 10 % as sizing agent.

2.2.2. Water Content and pH Measurement

The natural paper produced are tested for pH based on SNI ISO 6588-1.2010 and water content by dry-oven method based on SNI ISO 287:2010. All of the paper produced meet the standard of ISO 6519:2016 about Basic Paper for Laminated Plastic Wrapping Paper within the parameter of pH and water content.

2.2.3. Antibacterial Activity Assay

The antibacterial activities are tested using Kirby-Bauer disc diffusion method. All bacterial cultures were diluted with 0.9 % NaCl to 0.5 MacFarland. A bacterial suspension was swab on Mueller-Hinton Broth using sterile cotton bud. The culture was incubated at 37 °C for 24 hours. The diameter of inhibition zone was measured using calipers.

Paper disc from natural banana peel paper in some variation essential oil and put the disc in a petri dish with medium content bacteria inside. The petri dish was incubated at 30 °C for 24 hours. Inhibition Zone Diameter (IZD) was measured as a middle line start from the clear spot around the disc.

3. Result and Discussion

3.1. Physical and Chemical Characteristic of paper

Natural paper from banana peel has been succesfully produced by alkaliization method. The delignification process occurs with the addition of NaOH. Lignin contained in fiber sources (cellulose) will soften into strong fragments due to the transfer of hydrogen ions from hydroxyl groups in lignin to alkaline hydroxyl ions (Figure 1). Tapioca was added as sizing agent meanwhile essential oils was added as aroma agent as well as antibacterial agent. The appearance of paper produces as shown in Figure 2 are yellowish white with aroma according to the added essential oil. The physical and chemical properties of the paper produce shown in Table 1 and Table 2.
Figure 1. Delignification Reaction

Figure 2. Banana peel natural paper: a. without essential oil, b. with lemon oil, c. with cinnamon oil, d. with lime oil, e. with clove oil

Table 1. Physical properties of banana peel natural paper

| Banana peel natural paper | Grammage (g/m²) |
|---------------------------|-----------------|
| without essential oil     | 115.8           |
| with lemon oil            | 133.6           |
| with cinnamon oil         | 166.4           |
| with lime oil             | 158.4           |
| with clove oil            | 142.2           |

According to SNI 8218:2015, food packaging paper and cardboard have two levels of grammage. High grammage has a value of 255-500 g/m², while the low grammage is <225 g/m². The results showed that the paper produced meet the SNI standard with a low grammage paper category.
Table 2. Chemical properties of banana peel natural paper

|                        | Water Content (%) | pH  |
|------------------------|-------------------|-----|
| without essential oil  | 6.28              | 7.1 |
| with lemon oil         | 7.04              | 6.7 |
| with cinnamon oil      | 5.1               | 6.7 |
| with lime oil          | 6.02              | 6.4 |
| with clove oil         | 7.16              | 6.5 |

Based on Table 2, natural paper from banana peel (Musa paradisiaca Linn) produced has a range of pH 6-7 and the water content 5-7%. The standards of Basic Paper for Laminated Wrapping Paper on pH parameters ranging from 6-9 and water content parameter is a maximum of 8%. All of the paper produced meet the standard of ISO 6519:2016 about Basic Paper for Laminated Wrapping Paper within the parameter of pH and water content.

3.2. Antibacterial Activity

The antibacterial activity of the paper produced was indicated by the inhibition zone diameter (IZD) of S. aureus ATCC 25923 and E. coli ATCC 25922 (Figure 3).

In vitro research in this study shows that all four essential oils added as antibacterial agents in making paper from banana peels can inhibit the growth of S. aureus with different effectiveness, from medium to strong categories. Whereas in E. coli, only paper with cinnamon oil additive has antibacterial activity in the medium category (Table 3). This is consistent with several previous studies which showed high antibacterial activity of cinnamon oil both against gram positive bacteria and gram negative bacteria [10], while clove oil did not show activity against E. coli [6]. No inhibitory activity was observed in distilled water as a negative control. Tetracycline as a positive control for gram-positive bacteria, and chloramphenicol as a positive control for gram-negative bacteria each has inhibition zone diameter greater than 20 mm which shows very strong antibacterial activity. In comparison, commercial food wrapping paper was tested for both bacteria, showed bacterial contamination on commercial food wrapping paper.
Table 3. Inhibition Zone Diameter of papers against *S. Aureus* and *E. coli*

| Sample                  | *S. aureus* ATCC 25923 |         | *E. coli* ATCC 25922 |         |
|-------------------------|------------------------|---------|-----------------------|---------|
|                         | IZD (mm)               | Category| IZD (mm)              | Category|
| Paper without essential oil | 6.84                   | Medium  | 0.0                   | Unactive|
| Paper with clove oil    | 6.67                   | Medium  | 0.0                   | Unactive|
| Paper with lime oil     | 11.48                  | Strong  | 0.0                   | Unactive|
| Paper with lemon oil    | 8.86                   | Medium  | 0.0                   | Unactive|
| Paper with cinnamon oil | 18.0                   | Strong  | 9.0                   | Medium  |
| Commercial food paper  | 0.0                    | Unactive| 0.0                   | Unactive|
| Positive control        | 26.0                   | Very strong | 23.0               | Very Strong|
| Negative control        | 0.0                    | Unactive| 0.0                   | Unactive|

Antibacterial test results generally show that paper with essential oil additives has higher antibacterial activity against *S. aureus* than *E. coli* (Figure 4). This is related to the differences in the characteristics of the cell walls of the two bacteria. *S. aureus* and *E. coli* have different cell wall compositions. *S. aureus* is a gram positive bacterium whereas *E. coli* is a gram negative bacterium. About 90-95% of the cell wall of gram-positive bacteria consists of peptidoglycan, whereas other molecules, such as acids and proteins, are linked. Meanwhile gram-negative bacteria have a more complex cell wall with a peptidoglycan layer that is 2-3 nm thick [3]. An important characteristic of essential oils with their components is their hydrophobicity, which allows them to separate lipids from bacterial and mitochondrial cell membranes, disrupt the cell structure and make it more permeable [11, 12]. Because the gram-negative structure is more complex than gram-positive, hydrophobic essential oil molecules do not enter the gram-negative cell wall, so that essential oils are less able to influence the growth of gram-negative bacterial cells. This shows that essential oils on paper work as antibacterial agents by damaging the integrity of microbial cell walls.

**Figure 4.** Comparison of antibacterial activity of natural paper with several essential oils against *S. aureus* and *E. coli*

Cinnamon essential oil as antibacterial agent on the paper has a greater inhibitory effect on *S. Aureus* and *E. coli*. Paper with cinnamon oil additive had inhibition zone diameter 18 mm on *S. aureus* and...
(strong category) and 9 mm on *E. coli* (medium category). This shows that the chemical component in cinnamon oil is a source of antibacterial substances. Cinnamon oil is known to contain 38 components, with cinnamoneye as the main component [13,14], followed by benzaldehyde, benzoic acid and benzyl alcohol. The rest is found in very small quantities [10]. Antibacterial activity of products with cinnamon oil may be due to their main component, cinnamaldehyde, which has hydrophobic properties.

4. Conclusion
The result of the research showed that all of the paper with essential oils additive has antibacterial activity against *S. aureus*, but only paper with cinnamon oil additive that have antibacterial activity against *E. coli*. The use of cinnamon oil gave the largest inhibition diameter zone both on *S. aureus* and *E. coli*. Paper with cinnamon oil additive has inhibition diameter zone 18 mm on *S. aureus* (strong category) and 9 mm on *E. coli* (medium category). Cinnamon oil has a greater inhibitory effect on *S. aureus* and *E. coli*, because of its chemical component, namely cinnamaldehyde.

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