ANTIBACTERIAL ACTIVITY OF KECOMBRANG FRUIT SIMPLICIA EXTRACT (Nicolaia speciosa) AGAINST GRAM POSITIVE BACTERIA Staphylococcus aureus FNCC 0047 IN VITRO

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ABSTRACT

The survival of microorganisms depends on the environment. Unhealthy environment causes food intoxication problems resulting in food poisoning. One alternative that can be done to prevent the occurrence of food poisoning by using traditional medicines made from natural ingredients, especially kecombrang fruit simplicia extract. This study aims to determine the activity of kecombrang fruit simplicia extract against Staphylococcus aureus FNCC 0047 bacteria. The design used was a Completely Randomized Design (CRD) which was arranged with 10 treatment factors and repeated 3 times, resulting in 30 experimental units. The factors studied were the concentration of the kecombrang fruit simplicia extract consisting of concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%. The variable observed in this study was the antibacterial activity of S.aureus FNCC 0047 with the well-diffusion method. The data obtained were analyzed by parametric analysis of one way analysis of variance (ANOVA) at the level of 5% then significant results were continued with Duncan Multiple Range Test (DMRT) at the level of 5%. Kecombrang fruit simplicia extract has antibacterial activity against the growth of Gram positive S. aureus FNCC 0047 bacteria with the most effective concentration in inhibiting the growth of S.aureus FNCC 0047 bacteria is 40%. In this case, the extract of the kecombrang fruit simplicia has the ability to inhibit the growth of S. aureus FNCC 0047 bacteria.

Keywords: Antibacterial activity, Kecombrang fruit simplicia extract, S. aureus FNCC 0047

Introduction

Microorganisms are very small living things. It depends on the surroundings which are formed by nature and human intervention and can affect human life [1]. Humans always live interacting with the environment both in a healthy environment and in an unhealthy environment. Poor human lifestyles can cause an unhealthy environment, leading to more interactions with bacteria, viruses, fungi, and various forms of parasitic life. This is also supported by the condition of dusty air, warm and humid temperatures that cause microbial fertility [2].

An unhealthy environment can cause food intoxication. Intoxication occurs when hygiene aspects are not considered in the processing of food ingredients, resulting in food poisoning (poisoning through food). One of the bacteria that often causes food poisoning is S. aureus. S. aureus is capable of producing enterotoxins which are toxic substances that affect the small intestine, causing excessive secretion of fluid into the intestinal cavity. Although not all S. aureus strains produce enterotoxins but all strains have the potential to cause poisoning [3]. The Food and Nutrition Culture Collection (FNCC), UGM Center for Food and Nutrition...
Studies have isolated and identified about 500 bacterial isolates from various sources namely fermented foods, vegetables and fruits, processed meat products and gastrointestinal materials, one of which is the *S. aureus* FNCC 0047 bacteria. *S. aureus* bacteria FNCC 0047 are pure bacteria that have been isolated and characterized by microbiologists and are free of contamination [4].

Research on antimicrobial activity with natural ingredients has been carried out mainly from various types of local spice plants. Plants that are often used as natural sources of antimicrobials generally have bioactive compounds such as saponins, flavonoids and 25 types of phenolic compounds. Kecombrang is a group of zingiberaceae which has long been known and used by the community as a food flavor, as well as traditional medicines [5,6,7,8]. Based on community experience, the use of kecombrang fruit as a traditional medicine is applied by the people of Central Java by using a stew of kecombrang as a medicine for stomach ache and further processing into a kecombrang fruit syrup product. Another use is as a cleansing wound on the skin and red eyes. The experience associated with traditional medicine is certainly still traditionally empirical. Therefore, research is needed to prove the truth.

Each part of the kecombrang plant contains different dominant bioactive compounds [8,9,10]. The active compounds dominant in the kecombrang fruit are alkaloids, flavonoids and phenols [7;11]. The part of the stem, rhizome and leaves of kecombrang is dominated by other different compounds [9]. The use of kecombrang fruit is still rarely studied, so an idea emerged to utilize natural ingredients found in kecombrang fruit which can be used as bioactive alkaloid compounds, flavonoids and phenols as antimicrobials [11]. Bioactive components can be obtained from plant extraction. The extraction process can be carried out by various methods including maceration, percolation, soxhlet, reflux, digestion, and simplicia [1].

**Research Methodology**

**Materials and Treatment Design**

The materials used in this study are, kecombrang fruit, *S. aureus* bacteria FNCC 0047, Aquadest, Nutrient Agar (NA), Nutrient Broth (NB), MSA, Lamb blood, H2O2, Crystal violet, Lugol, 95% alcoholic acid, and Fuschine water. The tools used in this study are knives, fruit chopper machines, cabinet dryers "Teguh Wijaya Teknik", Erlenmeyer "Pyrex", "ACIS BC-500" digital scales, glassware, spatulas and filter cloth (250 mesh), Laminers, "All American" autoclave, 37°C "Memmert" incubator, shaker, "Pyrex" measuring cup, petri dish, vortex, stirrer, "Gilson" micropipette, blue tip, eyebrow needle, pyramid lamp, "LG" refrigerator, spatula, mortar, sorong term and other laboratory equipment for analysis. This study uses an experimental method with Completely Randomized Design (CRD). The treatment that was tried consisted of 1 factor with 10 treatment levels, repeated 3 times against the test bacteria to obtain 30 experimental units.

**Research Analysis**

The research includes several stages, namely simplicia powder making [11], making simplicia extract [12,13], preparation of research tools, preparation of agar media, preparation of test bacteria, identification of test bacteria, analysis of antibacterial activity of well diffusion
method [9,11] and determination of MIC (Minimum Inhibitory Concentration) of simplicia extract kecombrang fruit [11].

Result and Discussion

Colonial Morphology

The bacterial morphology can be divided into two, namely colony morphology and cell morphology. Examination of the morphology of the *S. aureus* FNCC 0047 bacterial colonies can be done by observing bacterial growth in nutrient agar (NA) after bacteria are grown for 2 x 24 hours on a petri dish. The morphology of microorganism colonies was assessed from several things, including form, smell, size, edge of colonies, surface, color and elevation [11]. Observations of morphology of bacterial colonies of research trials are presented in Table 1. and Figure 1.

Table 1. The results of morphological observations of colonies bacterial test *S. aureus* FNCC 0047

| No. | Colony morphology | Research observation result | Observation result [11] |
|-----|------------------|----------------------------|-------------------------|
| 1   | form             | Round, clustered           | round, 1-2mm            |
| 2   | smell            | Bad Smell                  | Bad smell               |
| 3   | type             | medium                     | medium                  |
| 4   | side             | flat                       | flat                    |
| 5   | surface          | glowing                    | glowing                 |
| 6   | color            | White yellowish            | Dark yellow to brown    |
| 7   | Elevation        | Convex                     | Convex                  |

Figure 1. Morphology of *S. aureus* FNCC 0047 colonies on agar nutrient media (primary data processed, 2018).

Based on direct observations, the morphology of *S. aureus* FNCC 0047 colonies is in accordance with [14] statement, that colonies formed by *S. aureus* bacteria in nutrient growth media to be round, convex, opaque, shiny, soft consistency, and colored brown to yellowish brown but the young colonies of bacteria are colorless.
Cell Morphology

Cell morphology examination is done by testing Gram stain. Gram staining is to find out the bacteria tested including in Gram positive or Gram negative bacteria with the help of a microscope [15]. The results of the Gram staining method under a microscope with 1000x magnification carried out in this study are shown in Figure 2.

![Figure 2](image1.png)

(a) Gram staining of *S. aureus* in the literature [15]; (b) The direct observation of Gram stain of *S. aureus* FNCC 0047 researchers (primary data processed, 2018).

In Figure 2 (b) it is shown that the test bacteria used by researchers have purple characteristics, shaped like kokus, clustered and formed strands like grapes. These characteristics are in accordance with the statement [16], that *S. aureus* bacteria have cell morphological features in purple, kokus-shaped, and group like grapes on the Gram staining method. The direct observation of the test bacteria by the Gram staining method of the researcher was in accordance with the results of Gram staining in the literature (Figure 2a). Based on the results of Gram staining, the identification of the type of bacteria detected was *S. aureus* FNCC 0047 with a description of the purple cell morphology, kokus-shaped, as well as groups like grapes as infectious microorganisms in invasive skin and soft tissue [16]. Thus, bacteria test can be used to test the antibacterial activity of kecombrang fruit simplicia extract.

Biochemical Test

The biochemical testing of *S. aureus* FNCC 0047 was carried out by conducting catalase and oxidase tests. The catalase test results of *S. aureus* FNCC 0047 bacteria can be seen in Figure 3.

![Figure 3](image2.png)

Figure 3. Air bubbles as a result of catalase test of *S. aureus* FNCC 0047 bacteria (primary data processed, 2018).

Based on Figure 3, the results of testing the catalase of *S. aureus* FNCC 0047 bacteria obtained positive results which were marked by the formation of air bubbles in the glass of the object being observed. The air bubbles formed prove that the *S. aureus* FNCC 0047 bacteria can produce the catalase enzyme which is able to hydrolyze hydrogen peroxide (H₂O₂) into...
water (H₂O) and gas bubbles (O₂). This is consistent with the research conducted by [15], regarding the identification and characterization of *Staphylococcus* sp. and *Streptococcus* sp.

The oxidase test is carried out to determine the presence or absence of oxidase enzymes in bacteria by using paper oxidase which can be seen from the color changes that occur on paper oxidase. The results of oxidase testing carried out on test bacteria using paper oxidase are presented in Figure 4.

![Figure 4. Oxidase test results on *S. aureus* FNCC 0047 (primary data processed, 2018)](image)

Figure 4 shows that the *S. aureus* FNCC 0047 bacteria used in this study cannot change the color of the oxidase paper to purplish blue which shows negative oxidase. This is consistent with the research conducted by [17], that *S. aureus* has a negative oxidase result, because it does not have an oxidase enzyme.

*Staphylococcus aureus* test FNCC 0047 with MSA Media and Lamb Blood

The *S. aureus* FNCC 0477 test was carried out using the modified Mannitol Salt Agar (MSA) media with 5% added lamb blood from the total volume of MSA media. The addition of 5% lamb blood aims to determine the strength of the test bacteria in lysing blood. The test results of *S. aureus* FNCC 0047 bacteria can be seen in Figure 5.

![Figure 5. *S. aureus* FNCC 0047 test results on blood MSA media (primary data processed, 2018).](image)

Figure 5 shows only the discoloration of the MSA media by adding lamb blood to the area around the bacterial colonies from black red to pink but does not form a clear zone around the bacterial colonies. This shows that the *S. aureus* FNCC 0047 bacteria used in this study cannot lyse lamb blood and enter into the category of γ-hemolysis. γ-hemolysis is defined as the absence of lysis in red blood cells.

According to [18], *S. aureus* pathogens have the following characteristics: medium size, yellowish white, and have colonies with patterns of hemolysis (clear zone) on blood agar. Figure 5 shows only the discoloration of the MSA media by adding lamb blood to the area around the bacterial colonies from black red to pink but does not form a clear zone around the bacterial colonies. This shows that the *S. aureus* FNCC 0047 bacteria used in this study cannot lyse lamb blood and enter into the category of γ-hemolysis. γ-hemolysis is defined as the absence of lysis in red blood cells.
Antibacterial Activity of Kecombrang Fruit Simplicia Extract with the Well Diffusion Method

Research conducted to determine the antibacterial of kecombrang fruit simplicia extract to *S. aureus* FNCC 0047 test bacteria in vitro showed inhibitory zone diameter of kecombrang fruit simplicia extract against *S. aureus* FNCC 0047. Extraction of kecombrang fruit simplicia in this study was conducted using maceration method, so that the extract of the kecombrang simplicia was obtained with a concentration of 100%, the extraction results were then diluted using aquadest solvents to obtain a concentration of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% and 90%.

The overall results showed that all concentrations of kecombrang simplicia could inhibit the growth of *S. aureus* FNCC 0047 bacteria. The lowest concentration of 10% kecombrang fruit simplicia had been able to inhibit the growth of *S. aureus* FNCC 0047 bacteria with a clear zone in the diameter of the inhibition zone. 7.26 mm. At a concentration of 20% and 30% of the kecombrang fruit simplicia, the inhibition zone diameter was 7.54 mm and 7.51 mm. The best inhibition occurred at a concentration of 40% by obtaining an inhibitory zone diameter of 7.83 mm, but for concentrations of 50% to 100% it showed a decrease in the gradual effectiveness of kecombrang fruit simplicia compared with a concentration of 40%. This condition can be caused by the speed factor of diffusion of antibacterial compounds on the media so that the higher the concentration of antibacterial compounds causes the antibacterial compound to become thicker and thus affects the speed of diffusion 4. The average value of the inhibition zone diameter of the kecombrang fruit simplicia concentrates in the *S* test bacteria *aureus* FNCC 0047 can be seen in Figure 6.

Based on Figure 6 shows that at a concentration of 40% is an effective and efficient concentration in inhibiting the growth of *S. aureus* FNCC 0047 bacteria compared to other concentration treatments with the strength of inhibition reaching 7.83 mm. Treatment can be regarded as effective concentration when the concentration reaches the most extensive inhibition zone compared to other concentration inhibition zones [19]. While efficient concentration is the concentration with the best antimicrobial power with fewer resources [20]. According to [21], the antibacterial provisions are with the formation of 20 mm or more resistance area means very strong, the resistance area 10 - 20 mm means strong, 5-10 mm
means medium, and the resistance area 5 mm or less means weak. Based on the results obtained and the underlying literature, it can be concluded that the kecombrang simplicia extract has a moderate antibacterial effect on the *S. aureus* FNCC 0047 bacteria, because the average diameter is in the range of 5-10 mm.

In general, the diameter of the inhibitory zone tends to increase in proportion to the increase in extract concentration. However, in this study there was a decrease in the diameter of the inhibition zone at an extract concentration of 50% to 100%. Similar results also occur in the results of [22], which states that the diameter of the inhibition zone does not always increase in proportion to the increasing concentration of antibacterial compounds, this is possible because of differences in the speed of diffusion of antibacterial compounds in the agar media and the different types and concentrations of antibacterial compounds gives a different diameter of the inhibition zone at a certain length of time. Another literature mentions also when low extract concentrations are more effective than concentrations above them because they are influenced by the thickness of the extract solution, making it easier for the diffusion process in experimental media where bacteria grow around it [23].

*Microbial Inhibitory Concentration* (MIC) Analysis in this study based on Bloomfield method 1. The method uses linear regression techniques by plotting between Ln kecombrang fruit simplicia concentration on the X axis with the square of the inhibitory zone on the Y axis. Linear regression arising from the formula applied in this study is $y = 22.34x - 9.783; R^2 = 0.1981$. Determine the MIC value of the research that has been done by multiplying the value of $X (Y = 0)$ with the method constant 0.25. MIC values obtained based on this study can be seen in Table 2.

Table 2. MIC values based on linear regression between the concentration of simplicia of kecombrang fruit and the diameter of the inhibitory zone

| Bacteria      | Linear regression | $R^2$ | $X(Y=0)$ | MIC (%) |
|---------------|-------------------|-------|----------|---------|
| *S. aureus*   | $Y = 22.34X - 9.783$ | 0.1981 | 2.2834  | 0.5708  |

Based on the results of the MIC analysis of the [24] method, MIC results were obtained with a number of 0.5708% or 0.6%. Minimum Inhibition Concentration (MIC) is the lowest concentration (ingredient content in one solution) of an antibacterial which inhibits the growth of a microorganism after incubation [25]. These results can be explained that the kecombrang simplicia at a concentration level of 0.6% can inhibit bacterial growth *S. aureus* FNCC 0047.

The diameter of the inhibitory zone arising from the addition of kecombrang fruit simplicia extract to the test bacteria in the nutrient medium so that there are bioactive compounds or active substances contained in the kecombrang fruit. Active substances with antimicrobial effects contained in kecombrang fruit include phenols, flavonoids and alkaloids [11]. Alkaloids are an organic base containing elements of Nitrogen (N) generally derived from plants, which have strong physiological effects on humans. The usefulness of alkaloid compounds in the field of pharmacology is to stimulate the nervous system, increase blood pressure, and fight microbial infections [26]. The mechanism of action of alkaloids as antibacterial is by disrupting the constituent components of peptidoglycan in bacterial cells, so that the cell wall layer is not formed intact and causes cell death [27]. In this study, alkaloid became the most effective active substance in the inhibition mechanism of *S. aureus* FNCC.
because in its mechanism alkaloids can interfere with the components that make up the cell wall of bacteria which cause more bacterial cell death compared to living bacterial cells, resulting in inhibition bacterial growth.

Based on its mechanism of action, antimicrobials can be divided into five groups, namely antimicrobials that interfere with microbial cell metabolism, inhibit the synthesis of microbial cell walls, inhibit protein synthesis of microbial cells, inhibit the synthesis or damage of nucleic acids of microbial cells, and disrupt the permeability of microbial cell membranes. Underlying, the mechanism of inhibition of dominant bioactive compounds (phenols, flavonoids and alkaloids) contained in kecombrang fruit simplicia extract works on the second mechanism of action by inhibiting the synthesis of microbial cell walls, resulting in damage to microbial cell walls.

Conclusion

Kecombrang fruit simplicia extract (*N. speciosa*) has antibacterial activity against the growth of Gram positive *S. aureus* FNCC 0047 bacteria with the most effective concentration of 40%. The MIC (Minimum inhibitory concentration) value of the kecombrang fruit simplicia extract against the growth of Gram positive *S. aureus* FNCC 0047 bacteria was 0.6%.

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