

In Vitro Antibacterial Activity of Waste Palm Cooking Oil Against *Staphylococcus Aureus*

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Abstract

Background: *Staphylococcus aureus* is a Gram-positive coccus which acts as a pathogen causing a wide range of infectious diseases. In recent years, several strains of *S. aureus* have been found to show resistance to several antibiotics. Waste cooking oil may be considered as an alternative antibacterial product, as it contains long-chain fatty acids whose antibacterial effectiveness against *S. aureus* has been known for years. In addition, oxidative biocides produced during the frying process have many targets for antibacterial activity in the cell and affect almost every biomolecule. Nonetheless, there is no literature that is able to prove the antimicrobial effects of the waste palm cooking oil. **Objective:** To examine the in vitro antibacterial effect of waste palm cooking oil against *S. aureus*. **Method:** a two-fold serial dilution method to set the minimum level of both inhibitory and bactericidal concentrations. **Conclusion:** This study showed that waste palm cooking oil did not show antibacterial effects against *S. aureus*, indicating that waste palm cooking oil is not possibly to be applied as an antibacterial agent against *S. aureus*.

Keywords: *Staphylococcus aureus*; waste palm cooking oil; minimum bactericidal concentration; minimum inhibitory concentration; antibacterial

Introduction

Infectious diseases are the top cause of health problems in developing countries, including Indonesia, and infection is associated with morbidity and mortality¹. *Staphylococcus aureus* is one of the bacteria that can cause various kinds of infectious disease, including meningitis, pneumonia, endocarditis, and necrotizing fasciitis, but it most often makes soft tissue and skin get infected². In the last decade, it was recorded that there was a significant increase of infection cases caused by methicillin-resistant *Staphylococcus aureus* (MRSA), affecting the cost of treatment and infection control. In recent years, several strains of *S. aureus* have been found to have developed resistance to antibiotics³. In 2014, the World Health Organization (WHO) reported data

from 85 member countries on antibiotic resistant by *S. aureus*, revealing that antibiotic-resistant *S. aureus* was identified in over 20% of cases in the WHO region, even exceeding 80% of cases in some reports⁴.

Therefore, it will be attracting to have the alternative products promoting antibacterial activity while affecting less side effects either in animals or humans. They are also needed since the bacteria start to be resistant to the antibiotics currently used. One of the alternative products with antibacterial activity being considered is waste palm cooking oil, which contains long-chain fatty acids⁵. For many years, it has been acknowledged that long-chain fatty acid promotes antibacterial activity. In Indonesia, many myths about healing wounds are still believed by many Indonesians to this day, and

one of these myths is that applying cooking oil to a body part that has a wound or burn can promote healing. In South Africa, linoleic acid and oleic acid are antibacterial components commonly utilized in the wound healing process of male circumcision⁶. In addition, long-chain unsaturated fatty acids have been considered bactericidal against imperative pathogenic microorganisms, in this case, including MRSA⁷. This study aims to evaluate the antibacterial activity found in the waste palm cooking oil against *S. aureus* by considering the minimum bactericidal concentration (MBC) and the minimum inhibitory concentration (MIC) for this substance.

Materials and Methods

Waste palm cooking oil

Waste palm cooking oil with various frying time was collected randomly from various households and food vendors in Banyuwangi, Indonesia. The waste palm cooking oil to be used in this study was prepared by dissolving waste palm cooking oil in Mueller Hinton Broth (CM0405, Oxoid, UK) and emulsifying with Tween 80 to ensure the sufficient dispersion into an emulsion. The tested sample shows that the last solvent concentration did not surpass 1%; as a result, bacterial viability was not affected by the presence of solvents⁸. The mixture was prepared using a vortex.

***S. aureus* strains**

S. aureus was provided by the Microbiology Laboratory, Faculty of Medicine, Universitas Airlangga. This study used *S. aureus* concentration of 1×10^8 CFU/ml, 0.5 McFarland standard.

Minimum inhibitory concentration and minimum bactericidal concentration

The antibacterial activities of the waste palm cooking oil were determined by using two-fold serial dilutions. The waste palm cooking oil were diluted with Mueller Hinton Broth to the following concentrations: 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, and 0.78% (v/v). To each tube, 1 ml of bacterial

suspensions and 1% Tween 80 as an emulsifier was added. In this study, the positive control comprised Mueller Hinton Broth medium with bacteria that have been tested, while the negative control comprised Mueller Hinton Broth with waste palm cooking oil and 1% Tween 80 which was incubated for 24 hours at 37°C. MIC can be defined as the lowest antimicrobial agent concentration inhibiting visually 99% development of microorganism. To determine the MIC, tube visual turbidity was investigated before and after the incubation process. Meanwhile, the experiment was performed in three circles in order to make sure the tested bacteria values.

Once MIC has been determined, the isolates from each tube were streaked on a nutrient agar plate (NAP) (CM0003, Oxoid, UK) using a sterile wire loop and incubated at 37°C for 24 hours. In this case, the absence or presence of bacterial development in agar plates before and after incubation can be used to determine the MBC. The lowest antimicrobial agent concentration that kills 99.9% of the early bacterial population indicates the MBC endpoint.

Results and Discussion

Bacterial strain

Colony identification was performed using the blood agar and Gram staining methods. The bacterial colonies were thawed and grown for two days on blood media agar at 37°C. Gram staining was used to see the shape of bacteria through observation under an light microscope using 1000 x magnification.

Contamination test of waste palm cooking oil samples

The contamination test was performed by streaking the study samples onto the NAPs and incubating at 37°C for 24 hours. Of the six samples collected randomly, two samples were contaminated with *Bacillus subtilis*, as demonstrated using the Gram staining method. This study used the four uncontaminated samples.

Minimum inhibitory concentration and minimum bactericidal concentration tests

Both MIC and MBC of waste palm cooking oil against *S. aureus* were determined using a serial dilution method. After the process of incubation at 37°C for 24 hours, the MICs were determined, as shown in Table 1. In this study, the MIC samples did not show a significant difference in turbidity and clarity before and after treatment. Thus, the MIC of waste palm cooking oil against *S. aureus* was absence.

Table 2 shows the MBC test results indicating the absence or presence of bacterial growth which is denoted as + or -, respectively. Suspensions from the tubes were streaked on a NAP and incubated at 37°C for 24 hours. *S. aureus* growth was obtained at all waste palm cooking oil concentrations. Thus the MBC of waste palm cooking oil against *S. aureus* was absence.

Table 1. Minimum inhibition concentrations (MIC) of waste palm cooking oil after 24 hours.

Dilution of waste palm cooking oil.		Concentrations % (V/V)							
		1.00	0.5	0.25	0.125	0.0625	0.03125	0.0156	0.0078
Sample No.	Set								
1	Set 1	+	+	+	+	+	+	+	+
	Set 2	+	+	+	+	+	+	+	+
	Set 3	+	+	+	+	+	+	+	+
2	Set 1	+	+	+	+	+	+	+	+
	Set 2	+	+	+	+	+	+	+	+
	Set 3	+	+	+	+	+	+	+	+
3	Set 1	+	+	+	+	+	+	+	+
	Set 2	+	+	+	+	+	+	+	+
	Set 3	+	+	+	+	+	+	+	+
4	Set 1	+	+	+	+	+	+	+	+
	Set 2	+	+	+	+	+	+	+	+
	Set 3	+	+	+	+	+	+	+	+

Negative sign (-) indicates there was no turbidity or absence of growth, while Positive sign (+) indicates there was turbidity or presence of growth.

Table 2. Minimum bactericidal concentrations (MBC) of waste palm cooking oil after 24 hours

Dilution of waste palm cooking oil.		Concentrations % (V/V)							
Sample No.	Set No.	1.00	0.5	0.25	0.125	0.0625	0.03125	0.0156	0.0078
1	Set 1	+	+	+	+	+	+	+	+
	Set 2	+	+	+	+	+	+	+	+
	Set 3	+	+	+	+	+	+	+	+
2	Set 1	+	+	+	+	+	+	+	+
	Set 2	+	+	+	+	+	+	+	+
	Set 3	+	+	+	+	+	+	+	+
3	Set 1	+	+	+	+	+	+	+	+
	Set 2	+	+	+	+	+	+	+	+
	Set 3	+	+	+	+	+	+	+	+
4	Set 1	+	+	+	+	+	+	+	+
	Set 2	+	+	+	+	+	+	+	+
	Set 3	+	+	+	+	+	+	+	+

Positive sign (+) indicates the presence of growth, while Negative sign (-) indicates the absence of growth

When the development of bacteria at various concentrations of waste palm cooking oil was evaluated after 24 hours, both MIC and MBC of *S. aureus* were absence at any concentration, indicating that waste palm cooking oil has neither bacteriostatic nor bactericidal activity (Table 1 and 2).

Although various bacterial infections have been for years treated with antimicrobials, its repetitive use may increase antimicrobial resistance⁹. Thus, alternative products with antibacterial activity have attracted attention. One of the alternative products being considered for this purpose is waste palm cooking oil because it contains long-chain fatty acids that have potential effectiveness against bacteria. The objective of this study is to assess the antibacterial effects of

waste palm cooking oil against *S. aureus* using MIC and MBC tests, which are commonly used to assess the antibacterial effects¹⁰. The MIC is considered the lowest antimicrobial concentration which is able to prevent the obvious growth of microorganism after the incubation for 24 hours. Furthermore, MIC tests are believed to be the ultimate procedure to decide the organism vulnerability to antimicrobial agents¹¹. The MBC, on the other hand, is considered the lowest antimicrobial concentration which is able to prevent the development of an organism and is determined by assessing the clear microbial inhibition zone (the zone without microbial growth) in the media by visual observation¹¹. The MIC and MBC are used to determine potential antimicrobial resistance and to make rational decisions in determining how successful an antimicrobial treatment is likely to be¹¹.

The bacterial strain selected for this study is *S. aureus*, a non-motile, Gram-positive coccus¹². Furthermore, it is one of the pathogens which is able to be source of various infectious diseases¹³. In addition, in recent years, several strains of *S. aureus* have been found to show resistance to several antibiotics³.

Waste palm cooking oil contains some long-chain fatty acids, namely: oleic (28.64%), palmitic (21.47%), linoleic (13.58%), stearate (13%), palmitoleic (7.56%), and other compositions (8.04%)⁵. It is obvious that, for many years, long-chain fatty acids have antibacterial activities. They serve as the main additive ingredient of antimicrobial food which prevent the development of undesirable microorganisms¹⁴. Long-chain fatty acids (C>16) have bactericidal effects for pathogenic microorganisms, including MRSA. Linoleic acid and oleic acid, for example, show antibacterial activity as well as synergistic effects on *S. aureus* inhibition⁶. Linoleic acid inhibits bacterial enoyl-carrier protein reductase (FabI) which is an encouraging target for antibacterial drugs since it is an important element of bacterial fatty acid synthesis¹⁵. Meanwhile, oleic acid was reported as a killer agent of *S. aureus*, and it has a natural protective effect against the primary adhesion stage in *S. aureus*¹⁶. Oxidative biocides, including hydrogen peroxide and chlorine, are the result of repeated frying processes and have multiple targets in cells and approximately in each biomolecule, including enzyme inhibition, oxidation of scavenger and thiol groups, peroxidation and disruption of membrane layers, disruption of energy production, disruption of protein synthesis, nucleoside oxidation, and, ultimately cell death¹⁷.

In this study, the MIC and MBC of waste palm cooking oil were determined using an in vitro serial dilution method, and this substance was found to have no antibacterial effect at any concentration. Thus, the hypothesis of this study was rejected. This is the first study in the literature to assess the antibacterial effect of waste palm cooking oil against *S. aureus*. Another study evaluated the antimicrobial effect of palm kernel oil and palm oil with the agar diffusion method

and obtained results similar to ours: Their results of this study show that the five microorganisms tested, including *S. aureus*, did not show any inhibition¹⁸. This could be because palm oil contains a diverse combination of fatty acids. Furthermore, it is presumably considered that these compounds make interaction since there is an action mechanism which finally decrease the antibacterial activity of their oils. In addition, the antibacterial activity of palm oil decreased because the release from triglycerides was incomplete during cleavage by lipase⁸.

In conclusion, waste palm cooking oil showed no antimicrobial activity against *S. aureus*. However, this study has a limitation: because the sampling was done randomly, the composition of each waste palm cooking was not known.

Conclusion

Based on the study result, the MIC of waste palm cooking oil against *S. aureus* was absence because there was no significant difference in clarity and turbidity at any concentrations. And, the MBC was also absent, because *S. aureus* at any concentration continued to grow. Thus, waste palm cooking oil has no antibacterial activity against *S. aureus*. Further research is needed by using a variety of bacteria and different types of oil to determine the inhabitation which potentially found in the waste palm cooking as an antimicrobial.

Ethical Clearance: This experimental study protocol had been approved by the Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

Conflict of Interest: The author declares that there is no financial conflict of interest in publishing these results.

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