

ORIGINAL ARTICLE

Combination of Red Coconut Coir (*Cocos nucifera L. var rubescens*) and Linezolid on Methicillin-Resistant *Staphylococcus aureus* in vitro Growth

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ABSTRACT

Introduction: Methicillin-Resistant *Staphylococcus aureus* (MRSA) bacterial infections may cause poor manifestations and even increase patients' morbidity and mortality. The use of antibiotics in the management of infections remains the main therapy. However, ever-increasing antibacterial resistance has prompted researchers to find the solution. This study aimed to determine the effect of a combination of red coconut coir (*Cocos nucifera L. var rubescens*) in various concentrations and linezolid 10 µ/ml on the growth of MRSA bacteria in vitro.

Methods: The antibacterial activity test method was well-diffusion test. Bacteria was plated in mueller hinton agar for 24 hours at 37°C. The treatment groups were red coconut coir extract and its combination with linezolid 10 µg/ml. The well-diffusion test results were statistically analyzed with the One-Way ANOVA and Tukey HSD post-hoc tests.

Results: Mean inhibitory zone diameter formed in the *C. nucifera L. var rubescens* extract test in various concentrations was 12.5 ± 0.36 - 16.2 ± 0.79 mm, while its combination with 10 µg/ml linezolid produced mean inhibitory zone of 15.1 ± 1.31-18.4 ± 0.46 mm. There was a significant difference between groups (p<0.05).

Conclusion: Increasing concentration of *C. nucifera L. var rubescens* extract was in line with the increase of mean diameter of the inhibitory zone. Extract and antibiotic combination tests showed higher mean diameter zones than the single test of materials. The combination of extract and linezolid has the potential to synergistically prevent antibacterial resistance.

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Introduction

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a strain of *S. aureus* that resistant to penicillin and its derivatives. This bacterium was first discovered in 1961.¹ In general, MRSA causes nosocomial infections.² Another study even suggested that community-acquired MRSA infection has now become a global problem.³ Until now, antibiotic therapy is still the main therapy in cases of infection due to bacteria so that antibacterial resistance is very likely to occur. According to WHO, antibacterial resistance can occur naturally and is accelerated by improper use in humans and animals.⁴ Related research in Indonesia conducted in 2014 revealed the incidence of antibacterial resistance around 700,000 cases per year.⁵ Researchers are trying to overcome this problem with new types of antibiotics as well as by combining antibiotics and

plant extracts.

Linezolid, a new type of antibiotic, has successfully overcome gram-positive infections in the last 10 years. The linezolid target is different from the previous antibiotic, which is in the 50S ribosome.² It also in different mechanism from coconut coir (*Cocos nucifera L.*) extract which has a target of action on cell walls. Researches stated that coconut coir can prevent the formation of biofilm,⁶ growth of gram bacteria,⁷ even MRSA with Minimum Inhibitory Concentration (MIC) of 156 µg/ml.⁸ It is because the extract contains phenol compounds that may cause intracellular leakage and inhibit the formation of peptidoglycan.^{9,10} Phenolic compound is secondary metabolite of plant which has the role as pathogen protector and plant's self-defense.¹¹ Indonesia has various species and subspecies of coconut and also known as the biggest coconut producer in the world in 2014.¹² This study used

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extracts of red coconut coir (*C. nucifera L. var rubescens*) because red coconut plant has the best resistance to pest attacks compared to other subspecies.¹³

The combination of antibiotics and plant extracts has not been much studied. However, a related study states that the differences in the targets of the two agents have the potential to have a synergistic effect in inhibiting bacterial growth.¹⁴ Combination of both substance are suspected can increase the spectrum of antibacterial activity, decrease toxicity, reach the antibacterial synergism, and prevent antibacterial resistance.¹⁵

Methods

This study was an experimental laboratory study using well-diffusion test. The material of *C. nucifera L. var rubescens* was obtained from Lumajang, East Java, Indonesia. Extraction was processed by maceration using 60% ethanol solvent. This experiment used 0.5 McFarland of MRSA from Microbiology Laboratory, Faculty of Medicine, Universitas Airlangga. The wells of 8 mm diameter were punched into the agar medium and filled with 100 µl of either plant extract, or combination of antibiotic and plant extract. The first 4 treatment groups contained single extracts of various concentrations (50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml), and the other 4 treatment groups contained combination of *C. nucifera L. var rubescens* extract and 10 µg/ml linezolid. Linezolid

10 µg/ml served as positive control and distilled water as negative control. All groups were incubated at 37°C for 24 hours. Inhibitory zone measurement (mm) was performed using calipers. Inhibitory zones obtained were processed statistically using IBM SPSS ver. 25 by one-way ANOVA with $p < 0.05$ considered as significant. This study has received ethical eligibility by the Health Research Ethics Committee, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia.

Results

The well-diffusion test results showed a combination of *C. nucifera L. var rubescens* extracts in various concentrations and 10 µg/ml linezolid carried out antibacterial activity (Figure 1). Single test results of *C. nucifera L. var rubescens* extract in concentrations of 50 mg/ml, 100 mg/ml, 150 mg/ml and 200 mg/ml were 12.5 ± 0.36 mm, 13.9 ± 0.27 mm, 15.0 ± 0.38 mm, and 16.2 ± 0.79 mm respectively. There was an increase in mean diameter of the inhibitory zone alongside a higher extract concentration and this also occurred in the combination group with 10 µg/ml linezolid which produced successive results of 15.1 ± 1.31 mm, 16.8 ± 1.31 mm, 17.6 ± 0.99 mm, and 18.4 ± 0.46 mm. Statistical analysis of the data showed a p value of 0.000, indicating a significant difference across the whole data (Table 1). Post-hoc Tukey HSD test revealed details of differences between data (Table 2).

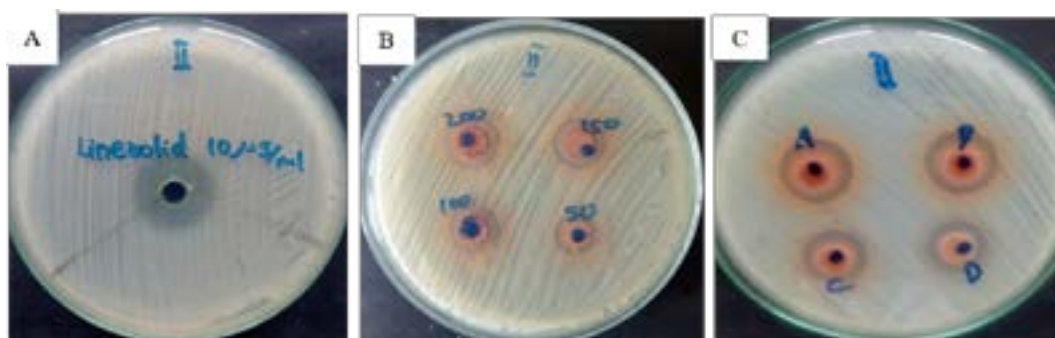


Figure 1. Well diffusion method of (A) Linezolid 10 µg/ml as positive control, (B) *Cocos nucifera L. var rubescens* extract in many concentration (50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml), (C) combination of each concentration of *Cocos nucifera L. var rubescens* extract with linezolid 10 µg/ml*.

*Left upper: 200 mg, right upper: 150 mg, left lower: 100 mg, right lower: 50 mg

Table 1. The mean of inhibition zone and the result of One-Way ANOVA test

Group	n	Concentration	Mean of Inhibition Zone (mm)	p	p (One-Way ANOVA test)
Linezolid 10 µg/ml (positive control)	4	10 µg/ml	14.8 ± 0.55	0.384	
Aquades (negative control)	4	-	0	.	
Single use extract of <i>C. nucifera L. var rubescens</i>	4	50 mg/ml	12.5 ± 0.36	0.650	0.000
		100 mg/ml	13.9 ± 0.27	0.240	
		150 mg/ml	15.0 ± 0.38	0.074	
		200 mg/ml	16.2 ± 0.79	0.246	
Combination extract of <i>C. nucifera L. var rubescens</i> and linezolid 10 µg/ml	4	50 mg/ml	15.1 ± 1.31	0.225	
		100 mg/ml	16.8 ± 1.31	0.613	
		150 mg/ml	17.6 ± 0.99	0.861	
		200 mg/ml	18.4 ± 0.46	0.666	

Table 2. The result of Tukey HSD post-hoc test

Group	n	Subset
Extract 50 mg/ml	4	a
Extract 100 mg/ml	4	ab
Linezolid 10 µg/ml (positive control)	4	bc
Extract 150 mg/ml	4	bc
Extract 200 mg/ml	4	bc
Extract 50 mg/ml + linezolid 10 µg/ml	4	cd
Extract 100 mg/ml + linezolid 10 µg/ml	4	cd
Extract 150 mg/ml + linezolid 10 µg/ml	4	d
Extract 200 mg/ml + linezolid 10 µg/ml	4	d

Note: the same alphabet shows the same data between groups

Discussion

Antibiotics are still the main therapy for bacterial infections. However, overuse or inappropriate use of antibiotic contributes to the occurrence of resistance. Several studies to overcome these problems were mostly performed to improve the quality of life of the patients. Linezolid is one of new antibiotics that can be a solution to antibacterial resistance problem. Linezolid is the first bacteriostatic in the oxazolidinone group. Its target of action is to inhibit protein synthesis in the ribosome RNA 23S subunit 50S subunit. Linezolid is widely used to treat resistant Gram-positive infections, such as MRSA or Vancomycin-Resistant *Staphylococcus aureus* (VRSA).¹⁶ These antibiotics have good potential in dealing with meningitis, endocarditis, and sepsis which are the complications of MRSA infection.¹⁷

Research on combinations of antibiotics and plant extracts, such as the coir extract of *C. nucifera L. var rubescens*, was also carried out. Our study demonstrates the synergy combination of coconut coir and linezolid could broaden the spectrum of antibacterial action. This shows a good therapeutic potential to overcome the problem of antibacterial resistance.⁷ Although the contents in coconut subspecies extracts, including coir extract of *C. nucifera L. var rubescens*, have not been specifically studied, a previous study found that *C. nucifera* coir itself contained phenol compounds.⁹ These compounds are secondary metabolites which are derivatives of the pentose pathway in plant metabolism.¹¹ Phenol compounds can induce intracellular leakage that damages bacterial cell walls¹⁸ by inhibiting some of peptidoglycan structures, inhibit the growth of gram-positive bacteria, such as strain of *S. aureus*,⁷ and protects plant bodies against pathogens or predators.¹⁹

Inhibitory zone formation indicates the presence of antibacterial activity in the tested material. Our results showed an increase in extract concentration followed by an increase in mean diameter of the inhibitory zone formed. This is caused by the difference of antibacterial active substances level which directly proportional to the concentration of the extract.²⁰ Diffusion of antibacterial active substances in the media,²¹ solvents used in the extraction process,²² as well as the sensitivity and size of bacterial inoculums, also influence the formation of inhibitory zone.²³ The expansion of antibacterial action spectrum was shown by a larger mean diameter of the

combination groups than the single groups.²⁴ Other related studies also suggested a significant reduction in *S. aureus* MIC in the combination of plant extracts and antibiotics compared to the single use of the material.¹⁴ The combination of antibiotics and plant extracts can provide a synergistic, additive or antagonistic response. It is called synergistic if the combination gives a better response than the single use.²⁵ The combination of antibacterial with different action targets shows a synergistic response.²⁶ *C. nucifera L. var rubescens* coir extract is rich in phenol compounds,¹⁰ which can initiate intracellular leakage and inhibit the formation of peptidoglycan,²⁷ along with linezolid which inhibits protein synthesis in the ribosome 23S subunit 50S.¹⁶ The well-diffusion test method was selected by taking into account the limitations of the researchers in carrying out visual evaluation if dilution method were used.

Conclusion

C. nucifera L. var rubescens extract and its combination with linezolid have antibacterial activity against the growth of MRSA. The higher the concentration, the greater the mean inhibitory zone diameter. An increase mean diameter of inhibitory zone in combined group indicates higher antibacterial activity, so it is expected

Conflict of Interest

The author stated there is no conflict of interest

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