

## **Research Article**

### ***Anti-Methicillin-Resistant Staphylococcus aureus (MRSA) of Ethanol Extract of God's Crown (Phaleria macrocarpa) Leaves, Peel, and Fruit Flesh***

### ***Anti-Methicillin-Resistant Staphylococcus aureus (MRSA) Ekstrak Etanol Daun, Kulit dan Daging Buah Mahkota Dewa (Phaleria macrocarpa)***

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#### **ABSTRACT**

*Methicillin-resistant Staphylococcus aureus (MRSA) is a pathogen that becomes the main concern since it is a multidrug-resistant organism and causes high morbidity and mortality. This study aimed to investigate the antibacterial activity of ethanolic extract of Phaleria macrocarpa leaves, peel, and fruit flesh on MRSA. This study was an experimental laboratory study with a post-test only control group design to assess the antibacterial activity of ethanolic extract from leaves, peel, and fruit flesh of Phaleria macrocarpa against MRSA ATCC 43300 and MRSA clinical isolates using a disk diffusion method. Extracts from the leaves, peel, and flesh of Phaleria macrocarpa had potential as an antibacterial agent against MRSA ATCC 43300 at a concentration of 40%, although not yet equivalent to antibiotic control. The ethanol extract of Phaleria macrocarpa peel at a concentration of 30-40% had potential as an antibacterial agent against MRSA clinical isolates, although not yet equivalent to antibiotic control. Further research needs to be performed so that Phaleria macrocarpa extract can be a potential source of subsequent antibacterial development against MRSA.*

**Keywords:** Ethanol extract, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Phaleria macrocarpa*

#### **ABSTRAK**

*Methicillin-resistant Staphylococcus aureus (MRSA) sebagai salah satu patogen yang menjadi perhatian utama karena merupakan organisme yang resisten terhadap berbagai antibiotik dan menjadi penyebab tingginya morbiditas dan mortalitas. Penelitian ini bertujuan untuk menyelidiki aktivitas antibakteri ekstrak etanol daun, kulit dan daging buah Phaleria macrocarpa terhadap MRSA. Rancangan penelitian yang digunakan adalah experimental laboratory dengan desain post-test only with control group design untuk menguji aktivitas antibakteri ekstrak etanol dari daun, kulit buah dan daging buah Phaleria macrocarpa terhadap MRSA ATCC 43300 dan isolat klinis MRSA dengan metode disk diffusion. Ekstrak dari daun, kulit dan daging buah Phaleria macrocarpa memiliki potensi antibakteri terhadap MRSA ATCC 43300 pada konsentrasi 40%, meski belum setara dengan kontrol antibiotik. Ekstrak etanol kulit Phaleria macrocarpa pada konsentrasi 30-40% memiliki potensi antibakteri terhadap MRSA isolat klinis, meski belum setara dengan kontrol antibiotik. Perlu dilakukan penelitian lanjutan, agar ekstrak Phaleria macrocarpa dapat menjadi sumber potensial pengembangan antibakteri berikutnya terhadap MRSA.*

**Kata Kunci:** Ekstrak etanol, *Methicillin-resistant Staphylococcus aureus*, *Phaleria macrocarpa*

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## INTRODUCTION

Globally, infectious diseases still become a serious problem, mainly because of the widespread antimicrobial resistance (1). For centuries, they have been among the leading causes of death and rising health-care costs, especially in developing countries. Infectious diseases become difficult to cure because of limited antibiotic options for therapy. Methicillin-resistant *Staphylococcus aureus* (MRSA) is a pathogen that gets the main concern because it causes various infections of life-threatening diseases, such as skin infection, pneumonia, health-care-associated infection, bacteremia, and sepsis (2,3). Methicillin-resistant *Staphylococcus aureus* is a multidrug resistance organism (MDRO) that resists to beta-lactams, aminoglycosides, macrolides, tetracyclines, and fluoroquinolones (multidrug) (4). Methicillin-resistant *Staphylococcus aureus* causes high morbidity and mortality in humans and animals. In January 2017, World Health Organization (WHO) stated that MRSA is one of the 12 deadliest drug-resistant bacteria (5).

The exploration of natural products from the medicinal plant is a promising alternative option against MRSA (2). *Phaleria macrocarpa* (*P. macrocarpa*), one of the medicinal plants in tropical countries, is used traditionally as anti-inflammation, antioxidant, and antimicrobial (6). Almost all parts of *P. macrocarpa*, including fruit, seeds, stems, and leaves, can be used as medicine (7). The antimicrobial activities of *P. macrocarpa* extract have been reported to fight against eight bacterial strains, namely *Bacillus cereus*, *Bacillus subtilis*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumonia*, *Micrococcus luteus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. All parts show weak to moderate antibacterial activity with inhibition zones ranging from 9.3 to 23.3 mm (8,9). All parts of *P. macrocarpa* are known to have antibacterial active compounds, such as flavonoid, alkaloid, saponin, and tannin (10). The potential antibacterial activity of the ethanolic extract of *P. macrocarpa* leaves, peel, and fruit flesh against MRSA has not been studied yet. This study aimed to investigate the antibacterial activity of ethanol extract of *P. macrocarpa* leaves, peel, and fruit flesh against MRSA.

## METHODS

### Research Design

An experimental laboratory study with a post-test only control group design was performed to determine the antibacterial activity of ethanol extract of *P. macrocarpa* leaves, peel, and fruit flesh against MRSA. The four concentrations of each *P. macrocarpa* leaves, peel, and fruit flesh extracts (10%, 20%, 30%, and 40%), positive control (Vancomycin), and negative control (distilled water), with three replications, were challenged to the MRSA isolates in vitro. This research has been reviewed by the Research Ethics Commission of Faculty of Medicine, Jenderal Soedirman University, and obtained an approval No. 3952/UN23.07.5.1/PP.1/2019 under exempted status.

### Extraction of *P. macrocarpa* Leaves, Peel, and Fruit Flesh

The leaves, peel, and fruit flesh *P. macrocarpa* obtained from Banyumas regency were extracted in the Pharmaceutical Biology Laboratory, Faculty of Health Science, Jenderal Soedirman University using the maceration method. Each dried leaves, peel, and fruit flesh were mashed using a blender, followed by putting

into a macerator and filled with ethanol solvent 1:1 (w/v) for 2x24 hours. Then, the result was filtered and collected in an Erlenmeyer so that the ethanol extract filtrate was free from impurities. The ethanol extract filtrate was then evaporated using a rotary evaporator at 40°C with a speed of 120 rpm. The extract obtained was made into four concentrations, including 10%, 20%, 30%, and 40% (8).

### Preparation of Bacterial Suspension Test

The MRSA isolates used in this study were MRSA ATCC 43300 and MRSA clinical isolates obtained from the collection of the Microbiology Laboratory, Faculty of Medicine, Jenderal Soedirman University. The strains were subcultured in the Mannitol Salt Agar (MSA) medium and incubated for 24 hours at 37°C. The growth of isolated colonies was observed. The *Staphylococcus aureus* clinical isolates were identified by Gram staining, catalase test, and coagulase test. Subsequently, the MRSA isolates were detected by cefoxitin disc according to the Clinical Laboratory Standard International (CLSI, 2019). The bacterial colonies were taken from MSA medium and then put into 5 ml physiological NaCl.

### Antimicrobial Susceptibility Test of *P. macrocarpa* against MRSA

Anti-MRSA activity of the *P. macrocarpa* extract was determined using the disk diffusion method (11) in the Microbiology Laboratory, Faculty of Medicine, Jenderal Soedirman University. Plates were prepared by pouring Mueller Hinton Agar (MHA) media in solid form. The lawn bacteria were made on MHA media by dipping a cotton swab into NaCl containing MRSA, then dispersed to ensure robust cell growth. Plates were then allowed to dry for 5 minutes.

The paper disc (diameter of 6 mm) was dipped into each concentration of extract. The paper discs were drained until no drip was obvious and then left for 30 minutes. The extract-soaked paper discs were then placed on the inoculated MHA plates and allowed the incubation for 30 minutes at room temperature to ensure proper extract diffusion. The plates were incubated at 37°C for 16-18 hours. The inhibition zones/clear zones were observed around the paper disc in diameter (mm) produced by the extract.

### Data Analysis

The results were analyzed descriptively to determine which of the concentration of extract that has the anti-MRSA potential.

## RESULTS

The results of the antibacterial activity of each extract against MRSA ATCC 43300 and MRSA clinical isolates are shown in Table 1. The table shows the antibacterial activities for each extract according to a clear zone ranged from 0-9 mm.

**Table 1. Antibacterial activity results for each extract against MRSA ATCC 43300 and MRSA clinical isolates**

No	Extracts	Concentration (%)	Mean (mm)	
			MRSA ATCC 43300	MRSA clinical isolates
1.	Leaves	10	0	0
		20	0	0

**Table 1. Antibacterial activity results for each extract against MRSA ATCC 43300 and MRSA clinical isolates (Cont.)**

No	Extracts	Concentration (%)	Mean (mm)	
			MRSA ATCC 43300	MRSA clinical isolates
2.	Peel	30	6	0
		40	9	0
		control (+) vancomycin	18.33	22.33
		control (-) aquadest	0	0
		10	0	0
		20	7	7.66
		30	7	8
		40	7.66	8
3.	Fruit flesh	control (+) vancomycin	18.67	21.67
		control (-) aquadest	0	0
		10	0	0
		20	0	0
		30	0	0
		40	6.66	0
		control (+) vancomycin	18.33	22
		control (-) aquadest	0	0

Table 1 shows that extracts of leaves, peel, and fruit flesh of *P. macrocarpa* had antibacterial activity against MRSA ATCC 43300 and MRSA clinical isolate at a concentration of 40%: 9 mm and 0 mm; 7.66 mm and 8 mm; 6.66 mm and 0 mm. Leaves and fruit flesh extracts in 10%, 20%, 30%, and 40% concentrations showed no antibacterial activity against MRSA clinical isolate. Peel extract had antibacterial activity against both MRSA ATCC 43300 and clinical strains in 20%, 30%, and 40%: range 7-8 mm.

## DISCUSSION

Medicinal plants are economical, have antibacterial activity, and no side effects (11). *Phaleria macrocarpa* is a medicinal plant originating from Papua Island, Indonesia, and has been mostly used traditionally as a medicinal plant in Indonesia to cure many diseases (8). The biological activity of *P. macrocarpa* serves as a valuable resource for antibiotic development. Research about the antibacterial activity of this plant against *S. aureus* has been widely reported, but those against MRSA are still very rare (7-9,11,12).

The extraction of this medicinal plant was using 96% ethanol solvent. Most compounds in plants that have antimicrobial activity could be extracted by ethanol solvent. Ethanol has the capability in the high extraction

process for all compounds (12). The study by Astriyani *et al.* showed that ethanol is a better solvent than distilled water to extract the antibacterial compound from *P. macrocarpa* leaf extract.

This antibacterial susceptibility assay was conducted using the disk diffusion method. The antibacterial activity showed that the width of the inhibition zone was directly proportional to antibacterial activity, the stronger the antibacterial activity, the bigger the extent of the inhibition zone. The higher the concentration of the antibacterial compound, the faster the microbial cells are killed, and their growth is inhibited (13).

The inhibition zone was revealed due to the antibacterial compounds from *P. macrocarpa* extract, i.e. flavonoid, saponin, alkaloid, and tannin (10). Flavonoids damage bacterial cell walls. Saponin affects the bacteria cell walls that affect like soap, which is antiseptic and has a metabolic effect on bacterial cells. Alkaloid inhibits bacterial growth by inhibiting the formation of protein and bacterial DNA (14). The antibacterial mechanism of tannin is related to its ability to activate microbial cell adhesins and enzymes and to interfere with the cellular transport performance of these components against MRSA cells (12).

Methicillin-resistant *Staphylococcus aureus* is the main cause of infections of skin tissue, bone, joints, abscesses, bacteremia, and infections associated with decreased therapeutic efficiency of available antimicrobial agents because of its resistance to all penicillin and methicillin derivatives. Vancomycin is the drug chosen for MRSA therapy (3). In this study, vancomycin was used as a positive control that inhibited the growth of MRSA with a diameter range of 18.33-22.33 mm.

The result of this study showed that ethanol extract of leaves, peel, and fruit flesh from *P. macrocarpa* has antibacterial activity on MRSA ATCC 43300. But only ethanol extract of peel has antibacterial activity against both MRSA ATCC 43300 and MRSA clinical isolates. The recommendation for further research is to increase the concentration of the extract to obtain a dose with an inhibitory activity equivalent to cefoxitin as a standard antibiotic.

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