



Original Research



The potential of ethanol extract of white pomegranate leaves (Punica granatum L) as anti-bacterial

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HIGHLIGHTS

We were found that the extract of white pomegranate leaves was able to inhibit the growth of positive Gram bacteria strains MRSA and the extract was unable to inhibit the growth of Escherichia coli bacteria strain ESBL.

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ABSTRACT

Treatment of infections using penicillin-derived antibiotics such as methicillin has been found to cause antibiotic-resistant bacteria. This bacteria could produce a beta-lactamase enzyme to form a resistant strain. Research on antibacterial activity continues to develop. Pomegranate (*Punica granatum L.*) was one of the herbal plants whose fruit has long been used for the treatment and prevention of various diseases. This study aimed to determine the potential inhibition of white pomegranate leaf extracts (*Punica granatum L.*) on the growth of Gram-negative bacteria including *Escherichia coli* Extended-Spectrum Beta-Lactamase (ESBL) strain and Gram-positive bacteria *Staphylococcus aureus* Methicillin-Resistant *Staphylococcus aureus* (MRSA) strain. White pomegranate leaf extract macerated with ethanol 96%, evaporated to obtain pure extracts made with a concentration of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and with 100% tested with invitro diffusion method. It was found that the extract of white pomegranate leaves with 30% (10.00 ± 0.0) concentration was able to inhibit the growth of positive Gram bacteria strains MRSA and the extract was unable to inhibit the growth of *Escherichia coli* bacteria strain ESBL.

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1. INTRODUCTION

Compounds that work by inhibiting bacterial growth or causing bacterial death called antimicrobials. The use of excess antimicrobials, incorrect dosages¹ can lead to resistance. This bacterium has the defense power of antibiotics by mutating the active and binding sites, forming trans membrane proteins known as efflux and plasmid proteins that encode the antibiotic-resistant gene.^{2,3} Gram-negative bacteria in the beta-lactam class were one of the bacteria that can perform that ability. Bacteria produce beta-lactamase enzymes that can break the beta-lactam ring so that the antimicrobial becomes inactive.¹ The resistance of beta-lactamase producers is formed mainly by inappropriate use of antibiotics.^{1,4} Bacteria that produce the beta-lactam enzyme called Extended-Spectrum Beta-Lactamase (ESBL) such as *Escherichia coli*,¹ *Staphylococcus aureus* strain Methicillin-resistant *Staphylococcus aureus* (MRSA).^{3,5} Bacteria have been resistant to penicillin-derived antibiotics such as methicillin, so it is necessary to choose the right drug.^{1,6}

Bacteria that are resistant to antibiotics need proper treatment.^{6,7} Treatment of infectious diseases using herbal plants that have been used because it has active compounds that act as antibacterial compounds such as pomegranate leaves.⁸ Pomegranate (*Punica granatum* L.) herbal plants that have long been used as a fruit treatment and prevention of various diseases, including cancer-preventing diseases,^{9,8,10} cardiovascular disease,^{11,3} inflammation,¹² and protection against ultraviolet radiation.^{13,9}

Pomegranate has various types, namely red, white, black pomegranate. Red pomegranate leaf extract has a natural antibacterial power against *Escherichia coli*.¹² There is no research on white pomegranate leaves against Gram-negative bacteria that produce the beta-lactam enzyme. This study aimed to determine the potential of white pomegranate leaf extract (*Punica granatum* L.) on inhibiting the growth of Gram-negative bacteria including the extended Spectrum Beta-Lactamase (ESBL) and Gram-positive strains of Methicillin-Resistant *Staphylococcus aureus* (MRSA) strains.

2. MATERIAL AND METHOD

This research is experimental. White pomegranate leaves were taken in Kayu Putih Village, Oebobo District, Kupang, East Nusa Tenggara. White pomegranate leaves are taken, washed thoroughly with water, and air-dried for 5 days, then mashed until leaf powder is found. White pomegranate leaf powder weighed 300 grams, then macerated first with 70% ethanol in a ratio of 1:10 and the second 1:5. Ethanol extract was thickened with a rotary evaporator, dissolved with 5% dimethyl sulfoxide (DMSO) made in series of concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%.

Test bacteria *Staphylococcus aureus* strain Methicillin-Resistance *Staphylococcus aureus* (MRSA) was compared with Mc Farland 0.5. A total of 100 µl of test bacteria was leveled on Muller Hinton Agar (MHA) medium and then made into wells with a diameter of 8 mm using the well diffusion method. Extracts of white pomegranate leaves that have made various concentrations are put into the well as much as 50 ul, then incubated at 37^o C for ± 24 hours. Amoxicillin 1 mg/ml is used as a control. The observed parameter was the diameter of the inhibition zone of each treatment.¹⁴ Each concentration of white pomegranate leaf extract was repeated three times and then the average was calculated.

3. RESULTS AND DISCUSSION

The measurement results of the inhibition zone formed for the extract of white pomegranate leaves with concentrations ranging from 10%, 20%, 30%, 40%, 50% against *Staphylococcus aureus* bacteria strain Methicillin-Resistance *Staphylococcus aureus* (MRSA) can be seen in [table 1](#).

Tabel 1. The inhibition zone formed of extract white pomegranate

Concentration (mg / ml)	The mean diameter of the zone of inhibition against <i>S. Aureus</i> Bacteria \pm SD (mm)			The average diameter of the zone of inhibition against <i>E. Coli</i> bacteria \pm SD (mm)		
100	14.50	\pm	0.50	0	\pm	0
90	13.83	\pm	0.29	0	\pm	0
80	13.67	\pm	0.58	0	\pm	0
70	11.50	\pm	0.50	0	\pm	0
60	10.83	\pm	0.29	0	\pm	0
50	9.83	\pm	0.29	0	\pm	0
40	10.33	\pm	0.58	0	\pm	0
30	10.00	\pm	0.00	0	\pm	0
20	0.00	\pm	0.00	0	\pm	0
10	0.00	\pm	0.00	0	\pm	0

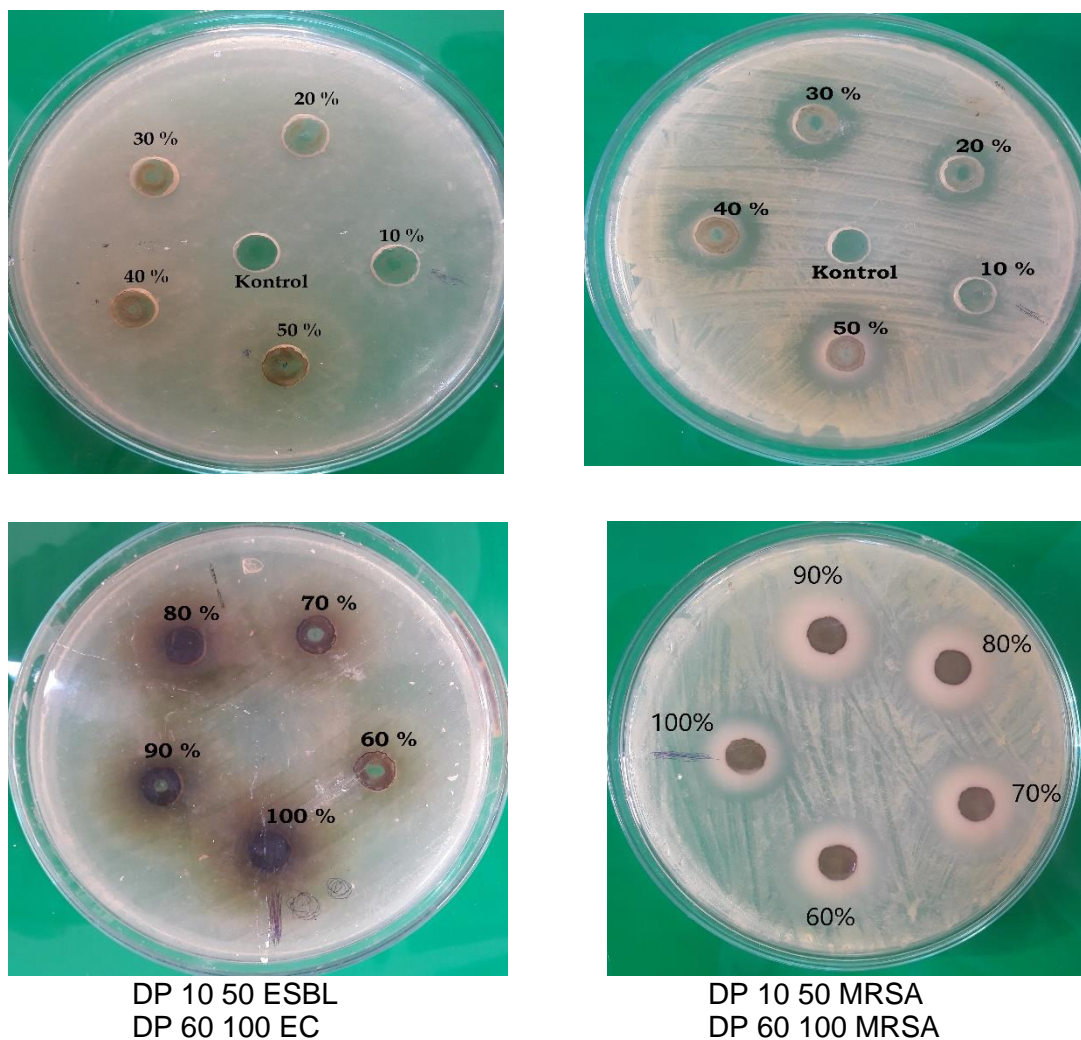


Figure 1. The inhibition zone formed of extract white pomegranate to ESBL and MRSA

White pomegranate leaf extract was tested with bacteria and measured by the clear zone formed around the extract. This shows (Figure 1) the inhibitory white pomegranate leaf extract has antibiotic or antimicrobial properties when tested with *Staphylococcus aureus* strain of Methicillin-Resistant *Staphylococcus aureus* (MRSA), although it gives different sensitivity results.

The difference in anti-bacterial sensitivity is influenced by the structure of the bacterial cell wall.^{1,15,16} This corresponds to the morphology of the *Staphylococcus aureus* bacteria that are included in Gram-positive bacteria, which tends to be more sensitive because of the structure of the simple cell wall compared to Gram-negative, so it makes it easier for anti-bacterial compounds to enter into cell.¹⁷ *Staphylococcus aureus* cell wall structure consists of peptidoglycan with little lipid and cell walls containing polysaccharides (teichoic acid).³ Teichoic acid is a water-soluble polymer, which functions as a positive ion to exit or enter. This water-soluble indicated that the cell wall of Gram-positive bacteria is more polar.¹⁵ Anti-microbial compounds found in white pomegranate leaves are part that is polar so that makes it easier penetrates the polar layer of peptidoglycan.³

White pomegranate leaf extract has the smallest inhibitory zone at a concentration of 30% which is 10 mm, then increasing to a concentration of 100% 14.50 mm. The concentration of white pomegranate extract which is effective as an antibacterial against *Staphylococcus aureus* bacteria was analyzed with a P-value 0.000 so that there is an effect of white pomegranate extract on the growth of *Staphylococcus aureus* (MRSA) bacteria.

The difference in inhibition of each concentration is influenced by the levels of the extract. According to small inhibitory zones indicate lower antibacterial activity while large inhibitory zones indicate greater antibacterial activity.² This is because secondary metabolites in white pomegranate leaves contain saponins, phenols, and tannins that are antibacterial^{1,16,14,12}. Antibiotic compounds contained in white pomegranate leaf extract can inhibit the growth of MRSA.

Suggested three categories of extract antibacterial activity,¹⁵ antibacterial activity of white pomegranate leaf extract against MRSA bacteria is included in the medium category. The increase in concentration is directly proportional to the diameter of the formed inhibition zone. Hardana explained that the factors influencing the diameter of inhibitory zones are the diffusion rate of antibacterial substances, the degree of bacterial sensitivity, and the speed of bacterial growth.⁵ The diffusion of solutes from higher concentrations is faster than lower concentrations. Extracts with higher concentrations spread faster and more widely in agar media.¹⁵ This results in the growth of bacteria that are around so that is passed by the extract are inhibited.³

Metabolite compounds found in pomegranate leaves such as alkaloids and tannins are considered antibacterial.^{2,8,10,18,19} According to Saaed⁹ the alkaloid compounds found in pomegranate leaves are 2-(2-propenyl) piperidine. Alkaline groups in alkaloids react with amino acid groups in cells that cause changes in the structure and composition of amino acids so that the cell is damaged.^{9,20,21}

Flavonoids in pomegranates can inhibit bacterial growth by inhibiting the process of DNA gyrase in bacteria based on antibacterial activity by damaging cell membranes that cause intracellular leakage.^{8,21} Quercetin (a flavonoid in pomegranate) can kill bacteria by increasing the permeability of membranes and damaging bacteria inside membrane potential, causing bacterial ATP production to be disrupted, disrupting membrane transport and movement of bacteria.¹² The content of polyphenols in pomegranates can kill bacteria by denaturing enzymes, but it can also be attached to substrates such as minerals, vitamins, carbohydrates so that bacteria cannot be used for their metabolism.¹³ Polyphenols can also be absorbed in cell walls disrupting the structure and function of cell membranes.^{17,10,21}

White pomegranate leaf extract after being tested with *Escherichia coli* strain Extended-Spectrum Beta-Lactamase (ESBL) showed no inhibitory or low level of antibacterial sensitivity. ESBL bacteria are Gram-negative bacteria tend to be more resistant to antibacterial, because the cell wall of Gram-negative bacteria is more complex where it consists of 3 components located outside the peptidoglycan layer, namely lipoprotein, outer membrane, and lipopolysaccharide.^{12,15} The lipoprotein layer is useful for stabilizing the outer membrane and attaching it to the peptidoglycan layer. The outer membrane has a double-layered structure in which larger antibiotic

molecules are relatively slow when penetrating the outer membrane so that Gram-negative bacteria are relatively more resistant to antibiotics.^{12,16}

4. CONCLUSION

White pomegranate leaf extract can inhibit the growth of the MRSA strain (Methicillin-Resistant *Staphylococcus aureus*) *Staphylococcus aureus* bacteria with the smallest concentration of 30% (10.00 ± 0.0). White pomegranate leaf extract cannot inhibit the growth of *Escherichia coli*, with Extended-Spectrum Beta-Lactamase (ESBL).

DISCLOSURE STATEMENT

The authors reported no potential conflict of interest.

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