Antibacterial Activity of Ketapang *(Terminalia cattapa L.)* Leaf Extract Against *Staphylococcus aureus* and *Pseudomonas aeruginosa* Isolates of Diabetic Wounds

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Abstract. This research aims at measuring antibacterial activity differences of ethanolic extract of ketapang leaf in the concentrations of 10 mg/mL, 25 mg/mL, 50 mg/mL, 75 mg/mL, and 100 mg/mL against *S. aureus* ATCC 6538, *Staphylococcus aureus Methycilin Resistance* (MRSA), and *P. aeruginosa* isolates of diabetic wounds. The ethanolic extraction of ketapang leaf was made using a maceration method. Antibacterial activity test was conducted based on the use of agar well diffusion assay method with Mueller Hinton Agar media, while the Minimum Inhibitory Concentration (MIC) and Minimum bactericidal concentration (MBC) were conducted using Mueller Hinton Broth media via microdilution method. The antibacterial activity was calculated based on MIC and MBC. The ketapang leaf dry extract with ethanol solvent resulted in paste as much as 13.6%. The higher the concentration of ketapang leaf extract, the higher its inhibiting power to those three bacterial types (*S. aureus* ATCC 6538, MRSA and *P. aeruginosa*). The highest inhibiting power of ketapang leaf extract was to MRSA (21.6 ± 0.8944) mm, while the smallest was to *P. aeruginosa* (16.0 ± 0.7071) mm. The MIC and MBC values of *P. aeruginosa* were 6.25 mg/mL, while those of *S.aureus* ATCC 6538 and MRSA were 50 mg/mL. The results of this research can provide information related to the ketapang leaf extract with ethanol solvent, while both Gram-positive and Gram-negative bacteria can be used as the antibacteria.

Keywords: Terminalia cattapa L · antibacterial activity · Staphylococcus aureus · Pseudomonas aeruginosa · MRSA

1 Introduction

Staphylococcus aureus and *Pseudomonas aeruginosa* are bacteria which have mostly infected the chronical wound of DM sufferers [1]. The infection then develops and causes the *Deabetic foot Infection* (DFI), which should eventually be amputated [2, 3].

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The effects of DFI include neuropathy and ischemia on local and systemic inflammatory responses, thus, the DFI preliminary diagnose is not easy, due to the improper antibiotic administrations. The improper and excessive antibiotic administrations result in bacterial resistance and trigger the multi-drug resistance (MDR) [1, 4].

Plant is a good source of antibacterial compounds since containing a variety and diversity of chemical compound structures [5]. *Terminalia cattapa L,* also known as Ketapang in Indonesia, is one medicinal plant found in tropical and subtropical countries and beneficial as medicine, such as antidiabetic. *Terminalia catappa* L. belonging to the family *Combretaceae. T. catappa* is used primarily as an ornamental, shading, and salt-tolerant street tree, the leaves provide food for the Tasar silkworm, and the seeds are edible like almonds with similar oils [6]. The Extract from the brown leaves of *T. catappa* L. has been reported to attenuate the growth of *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853) [7].

This research aims at measuring the differences of antibacterial activity from Ketapang leaf extract with various ethanol solvents to the growth of *S. aureus* ATCC 6538, *Staphylococcus aureus Methycilin Resistance* (MRSA), and *P. aeruginosa* isolate in the wound of DM sufferers. Ethanol is a universal solvent which can pull both polar and non-polar compounds, thus, some active compounds contained in the plants which have both polar and non-polar characters were also pulled [8].

2 Materials and Method

2.1 Ketapang Leaf

The Ketapang leaves used were the fresh falling ones with brownish yellow color obtained from the parks of Universitas Muhammadiyah Semarang, Kedungmundu Semarang, Indonesia. Ketapang leaves were cleanly washed using the running water

Fig. 1. Ketapang leaves *(Terminalia cattapa L.*).

and then rinse using the distilled water. The leaves were then set aside to free from water, sliced in small size, and dried in the dying cabinet at the temperature of 50 ºC for 24 [5] hours until the constant dry weight was obtained. The dried Ketapang leaves were then smoothed using blender and resulted in powder, stored in a sterile place up to the time the powder was then used (Fig. 1).

2.2 Ketapang Leaf Extract

Ketapang leaf extract was made using maceration method with ethanol solvent. 150 g of ketapang leaf powder was immersed in 450 mL of ethanol solvent 96%, set aside for 3 × 24 h at room temperature to avoid direct sunlight and shake. The solvent was regularly replaced every 24 h [9], the Ketapang leaf extract solution was then filtered using Whatman filter paper No.1. The ketapang leaf extract filtrate was concentrated with the rotary evaporator at the temperature of 50 ºC. The obtained concentrated extract was used for the inhibition power test.

2.3 Preparation for Bacterial Culture Test

The bacterial test of *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus Methycilin Resistance* (MRSA) and *Pseudomonas aeruginosa* isolates on the diabetatic wound was identified using Vitek®MS (bioM´erieux, Marcy l'Etoile, France). The bacteria were sub-cultured with BAP(OXOID) media using the sheep blood of 5%, incubated for 24 h at the temparature of 35 ± 2 ºC. The bacterial colony was then suspended into the MHB media until the turbidity was equal to the McFarland standard of 0.5 (5×10^8 CFU/mL).

2.4 Antibacterial Activity Test of Ketapang Leaf Extract

The antibacterial activity test using agar well diffusion assay [10] was conducted by 100 µL bacterial culture equal to the McFarland standard of 0.5 innoculated on the surface of Muller Hilton Agar (MHA/OXOID) media through the dense streak using the sterilized ocular inoculation. After set aside for 10 min, starting from the initial inoculation, then a well was made in each agar plate using the sterilized steel Cork Borer (diameter 0.6 cm). 5 wells were made in each petry disk and then each well was added with 100 µL ketapang leaf extract in accordance with the required concentrations (10 mg/mL, 25 mg/mL, 50 mg/mL, 75 mg/mL, and 100 mg/mL). After incubated at the temperature of 35 ± 2 °C for 24 h, the antibacterial activities from the Ketapang leaf extract were determined by measuring the bacterial growth inhibiting zone using the vernier calipers.

2.5 Minimum Inhibitory Concentration (MIC)

MICs were determined with the broth micro-dilution method using 96-well microplates (CLSI, 2020). 12 wells were prepared and each well was then added with 100 µL of MHB media. The first well was added with 100 µL of ethanol extract from the Ketapang leaves (100 mg/mL), and further homogenous. 100 µL of mixture contained in the first well was then put into the second well, homogenous, and then 100 µL of mixture from

the second well was put into the third well and so forth up to the eleventh well. 100 μL of bacterial suspension equal to the McFarland standard of 0.5 was added into the twelfth well and then used as the positive control. Incubation was then preformed for 24 h at the temperature of 37 °C. Each treatment was repeated twice. MIC is the lowest concentration inhibiting the bacterial growth [11].

2.6 Minimum Bactericidal Concentration (MBC)

10 μL of bacterial suspension from each well during the MIC test was sub-cultured using the streak method on the BAP media containing 5% of sheep blood [12] and then incubated at the temperature of 37 °C. MBC is the lowest concentration of Ketapang leaf extract which has the ability to kill bacteria shown by the absence of bacterial colony growth on the BAP media.

3 Results and Discussion

3.1 Ketapang Leaf Extract

The results of Ketapang leaf extract made with the maceration method using the ethanol solvent 96% obtained the pasta of 13.6%. The amount of the obtained pasta depended on the solvent type and number of the extracted materials [13]. The ethanol extract of Ketapang leaves contains flavonoids, quinone, phenolics, triterpenoids, and tannins, yet does not contain alkaloids, steroids, and saponin[7].

3.2 Antibacterial Activity Test Results of Ketapang Leaf Extract

The antibacterial activity test results of Ketapang Leaf Extract with three solvent types (ethanol, methanol, and ethyl acetate) on the concentrations of 10 mg/mL, 25 mg/mL, 50 mg/mL, 75 mg/mL and 100 mg/mL to the growth of *S. aureus* ATCC 6538, MRSA, and *P. aeruginosa* isolates from the wound of DM sufferers using agar well diffusion assay was shown in Table 1 and Fig. 2. Distilled water was used as the negative control, the *S. aureus* ATCC 6538 as the positive control, Vancomycin antibiotic as the MRSA, and Amikacin as *P. aeruginosa.*

The ethanol extract of Ketapang leaf showed the inhibiting zone starting from the diameter of 11.2 mm to 21.6 mm to the bacteria of *S. aureus* and *P. aeruginosa.* Ethanol is a solvent commonly used for extraction, and its extraction result is commonly more potential than the other solvent [5]. This result was in accordance with the repot of research conducted by [14]. The ethanol extract of *Pipper betle* leaf showed having the inhibiting zone to MRSA, Esbl-*Enterobacteriaceae* and non- *Enterobacteriaceae.*

Increasing the extract concentration also increased the inhibition zone diameters for the third bacteria. *Pseudomonas aeruginosa* was more resistant to high extract concentration than *S. aureus* ATCC 6538 and MRSA. The results of this research were in line with those of research conducted by [7], mentioning that the higher the concentration of Ketapang leaf extract, the bigger the inhibiting zome diameter to both *S. aureus* and *P. aeruginosa*, while the inhibiting zone diameter to *P. aeruginosa* was smaller than to

Table 1. The Inhibiting Zone Diameter of Ketapang Leaf Extract with ethanol solvent to *S. aureus* ATCC 6538, *S. aureus* (MRSA) and *P. aeruginosa.*

Extract	Concentration	Inhibition zone diameter (mm)		
		P. aeruginosa	*S. aureus* MRSA	*S. aureus* ATCC 6538
Ketapang Leaf	10 mg/mL	11,2 ± 0,7656	14,2 ± 1,0954	12,8 ± 1,0954
	25 mg/mL	12,2 ± 0,8366	16,0 ± 0,7071	13,8 ± 1,0954
	50 mg/mL	13,2 ± 0,8366	17,6 ± 0,8944	15,4 ± 0,8944
	75 mg/mL	14,4 ± 0,8944	19,6 ± 0,8944	17,0 ± 0,7071
	100 mg/mL	16,0 ± 0,7071	21,6 ± 0,8944	18,2 ± 0,4472
Vancomycin	30 µg	-	21	21
Amikasin	30 µg	22	-	-
distilled water	-	0	0	0

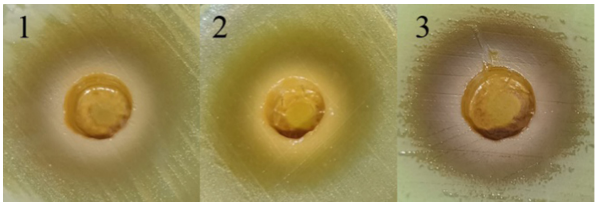

Fig. 2. Inhibiting zone of Ketapang leaf extract with ethanol solvent to: 1) *S. aureus* ATCC 6538, 2) MRSA, and 3) *P. aeruginosa.*

S. aureus. This research was also in line with that conducted by [15], mentioning that the higher the ethanol concetration extract of avocade, the higher the inhibiting power to the growth of *P. aeruginosa.*

The extracts from the genus *Terminalia* sp. Plants are rich with phytochemicals, such as terpenes, flavonoids, and phenolic acids. These molecules are related to the antibacterial, antioxidant, anti-inflammatory, antifungal, antiviral, anti-parasitic, anti-diabetic, and anti-cancer activity of *Terminalia* plants. [7].

3.3 Results of MIC and MBC Tests

The antibacterial activity of ethanol extract from Ketapang leaves was examined in vitro using the microdilution method to two species of gram-positrive bacteria of *S. aureus* ATCC 6538 and MRSA as well as the gram-negative bacteria of *P. aeruginosa.* The results were shown in Table 2 and Fig. 3. Furthermore, subcultures were conducted on the BAP media of all wells. The results were shown on Fig. 4 and Fig. 5.

The results of MIC and MBC tests from three bacterial species showed the value of 6.25–50 mg/mL. The MIC and MBC values of *P. aeruginosa* were 6.25 mg/mL considered the lowest when compared to two species of *S. aureus* ATCC 6538 and MRSA. The research results showed that Ketapang leaf extract equipped with ethanol

Table 2. MBC of ethanol extract from Ketapang leaves against *S. aureus* ATCC 6538, MRSA and *P. aeruginosa*

Concentration mg/mL	MBC		
	S. aureus ATCC6538	*S.aureus* MRSA	*P. aeruginosa*
50.000	50.0	50.0	-
25.000	+	+	-
12.500	+	+	-
6.250	+	+	6.25
3.125	+	+	+
1.562	+	+	+
0.781	+	+	+
0.390	+	+	+
0.195	+	+	+
0.097	+	+	+
0.048	+	+	+
Vancomycin	+	+	-
Amikasin	-	-	+
distilled water	-	-	-

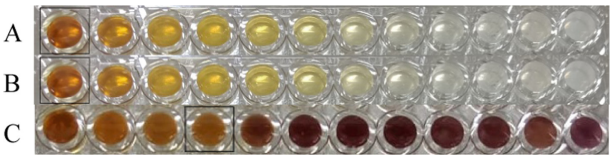

Fig. 3. MIC Values of Ketapang Leaf Extract with ethanol solvent to: A) *S. aureus* ATCC 6538, B) MRSA, C) *P. aeruginosa.*

solvent showed the antibacterial activity to the wound-causing bacteria of MRSA and *P.aeruginosa* isolate.

The antibacterial activity of the plant related to the phytochemical compound has the function to protect the plant from infection. The phytochermical compound commonly found in plant is terpenoid, alkaloid and flavonoid [16]. Although identification of active compound group from Ketapang leaf was conducted in this research, according to (Allyn, *et al.*, 2018), the extract obtained from the *Terminalia* sp. Plant genus is rich with phytochemicals, such as terpenes, flavonoids, and phenolic acids. Those three compounds were proven having the antibacterial activity [17]. Those phytochemical compounds can inhibit the bacteria by damaging the cell wall [18]. Ketapang leaf was proven potential as the antibacterial agent, especially to the wound causing bacteria of MDR isolate. This

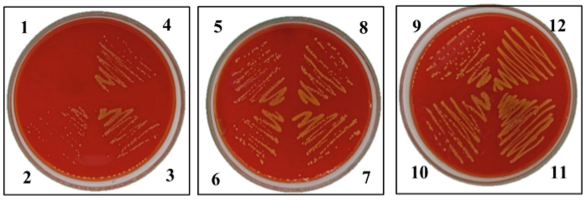

Fig. 4. MBC Values of Ketapang Leaf Extract with ethanol solvent to S. aureus ATCC 6538 and MRSA at 50 mg/Ml.

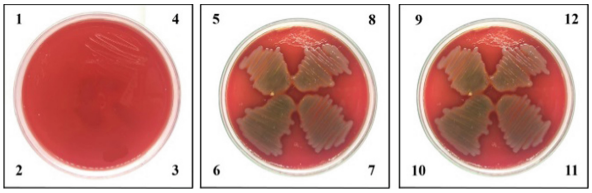

Fig. 5. MBC Values of Ketapang Leaf Extract with ethanol solvent to *P.aeruginosa* at 6.250 mg/mL.

research can provide new information related to the benefit of ketapeng leaf as one of natural sources as antibacterial agent to the wound-causing MDR bacteria.

Ketapeng leaf is proven potential as an antibacterial agent, especially to the wound-causing MDR bacterial isolate. The highest inhibiting power of ketapang leaf extract was to MRSA (21.6 ± 0.8944) mm, while the smallest was to *P. aeruginosa* (16.0 ± 0.7071) mm. The MIC and MBC values of *P. aeruginosa* were 6.25 mg/mL, while those of *S.aureus* ATCC 6538 and MRSA were 50 mg/mL. The results of this research can provide information related to the ketapang leaf extract with ethanol solvent, while both Gram-positive and Gram-negative bacteria can be used as the antibacteria.

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