

## Minimum inhibitory concentration determination of *Bambusa vulgaris* leaves extract against skin and digestive diseases bacteria

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### Abstract

Skin infections caused by *Staphylococcus aureus* and *Staphylococcus epidermidis* and gastrointestinal infection caused by *Bacillus subtilis* and *Bacillus cereus* are commonly happened in developing countries such as Indonesia. Ethanol extract of *Bambusa vulgaris* leaves contained metabolite compounds that potential to have antibacterial activity. The aim of this study was to determine minimum inhibitory concentration (MIC) value of ethanol extract of *Bambusa vulgaris* leaves against those four bacteria. The research was conducted by Kirby-Bauer disc diffusion method. The ethanol extract of *B. vulgaris* leaves concentrations were 1.25, 2.5, 5, 10, 20, 40 and 80 mg/mL. The results showed that ethanol extract of *B. vulgaris* leaves could inhibit the growth of all four bacteria with MIC value against *S. aureus* and *B. subtilis* was 20 mg/mL, with inhibition zone diameter of  $7.33 \pm 0.416$  mm and  $6.32 \pm 0.057$  mm respectively. While the MIC value against *S. epidermidis* and *B. cereus* was 10 mg/mL, with inhibitory zone diameter obtained respectively  $7.33 \pm 0.416$  mm and  $6.07 \pm 0.029$  mm. The conclusion of this research was that ethanol extract of *B. vulgaris* leaves could use for skin infection caused by *S. aureus*, *B. subtilis*, and for gastrointestinal infection caused by *S. epidermidis* and *B. cereus*.

**Keywords:** *Bambusa vulgaris* leaves, antibacterial, disc diffusion, MIC (Minimum Inhibitory Concentration)

### Introduction

Common infections in developing countries, especially tropical areas such as Indonesia, are skin and digestive infections. These infection, mainly caused by poor sanitary conditions (Nugerahdita, 2009). The prevalence of skin diseases in Indonesia in 2012 was 8.46% and increased to 9% in 2013 (Depkes RI, 2013). Meanwhile, according to WHO, diarrhea is one of the leading causes of mortality in developing countries. The incidence of diarrhea in children each year is estimated to reach 2.5 billion and globally each year the disease causes children death by 1.6 million (Hannif, Mulyani, & Kuscithawati, 2011). *Staphylococcus aureus* and *Staphylococcus epidermidis* are Gram positive bacteria that can cause skin infections such as acne, ulcers, and burn infections (Widyasanti, Hajar, & Rohdiana, 2015). Whereas *Bacillus*

*subtilis* and *Bacillus cereus* are Gram positive bacteria that cause digestive infections such as diarrhea and food poisoning due to contamination of food (Meindl & Chopra, 2007; Rahmaningsih, Wilis & Mulyana, 2012).

One of herbal that can be used as antibacterial is *Bambusa vulgaris*. According to Onajobi *et al* (2015), *Bambusa vulgaris* can inhibit *S. aureus* with MIC value of 10.0 mg/mL for hexane extract, 7.0 mg/mL for methanol extract, and 5.0 mg/mL for ethyl acetate extract (Onajobi, Agbaje, & Alaba, 2015). Acetone extract of *B. vulgaris* proved to have antibacterial activity against *S. epidermidis* bacteria with inhibitory zone diameter of 17.67 mm (Ambika & Rajagopal, 2017). Research on *B. subtilis* using chloroform, hexane, and ethyl acetate extract of *B. arundinaceae* showed MIC values of 10.7, 5.62 and 3.02 mg/mL (Zubair

et al., 2013). Chloroform, hexane and ethyl acetate extract of *B. vulgaris* showed that it has MIC value of 2.5, 1.25 and 5 mg/mL against *B. cereus* respectively (Owolabi & Lajide, 2015).

Literature study about ethanol extract of *B. vulgaris* antibacterial activity has not found yet. Based on Annafiatuzakiah research, the phytochemical content of ethanol extract of *B. vulgaris* leaves are flavonoids, polyphenols, saponins, and triterpenoids (Annafiatuzakiah, 2017). Flavonoid, phenol, terpenoid and saponin compounds are secondary metabolites that known to have antibacterial activity (Septiani, Dewi, & Wijayanti, 2017)(Akbar, Budiarti, & Edyson, 2016).

Based on that background, the researcher is interested to test the antibacterial activity of ethanol extract of *B. vulgaris* leaves to several Gram positive bacteria namely *S. aureus*, *S. epidermidis*, *B. subtilis*, and *B. cereus*.

## Method

**Agar Media Preparation.** A total 38 grams of MHA were dissolved in 1 L of sterile aquadest and then heated until all dissolved. Then the solution was poured into the Erlenmeyer in hot conditions and the pH was checked. The solution was sterilized in the autoclave at 121°C for 15 minutes (Zimbro, Power, Miller, Wilson, & Johnson, 2009).

**Inoculum Preparation.** Pure bacterial culture was aseptically etched on MHA media using an ose needle, then incubated at 37 ° C for 24 hours (Sumadi, 2011). Colonies of *S. aureus*, *S. epidermidis*, *B. subtilis* and *B. cereus* were taken using sterile ose needles and suspended in 10 mL of 0.9% NaCl solution, then incubated at 37°C to a certain turbidity equivalent to McFarland 0.5 standard solution (growth rate of  $1 \times 10^8$  bacterial cells / mL) (Nuria, Faizatun, & Sumantri, 2009; Ayyagari, Gandhi, & Sushma, 2009). After getting the same turbidity, the suspension was used as a test bacterium.

**MIC Determination.** The method used in this study is the disc diffusion method (Kirby-Bauer test). Suspension of the test bacteria was dropped into an MHA petri dish and scratched using a cotton bud, then rotated several times. Paper discs were prepared and dipped in various concentrations of ethanol extract of *B. vulgaris* leaves. The concentration variations used were 1.25, 2.5, 5, 10, 20, 40 and 80 mg/mL. The disc is placed on the surface of the media. Then the media was incubated at 37°C for 24 hours. The diameter of the inhibition zone formed is measured using calipers in millimeters (mm). The test is carried out with three replications for each bacterium.

## Results and Discussion

The MIC determination of the ethanol extract of *Bambusa vulgaris* leaves was carried out by the Kirby-Bauer disk diffusion method with the aim of knowing the smallest concentration required for the extract to produce the diameter of the bacterial inhibition zone. The disk diffusion method is one of the methods recommended by CLSI for determining MIC values. The inhibition zone is characterized by the formation of a clear zone around the disc in a bacterial medium which is inoculated on the surface and incubated (Astutiningrum, 2016). Inhibition of bacterial growth is caused by the interaction of active compounds in plant extracts through the diffusion of antimicrobial agents with bacteria.

The mechanism of phenol as an antibacterial is by denaturing proteins and disrupting the function of cell membranes, so that the cells become lysis (Brooks, Carroll, Butel, Morse, & Mietzner, 2007). In addition, phenol can also work by inhibiting bacterial cell wall synthesis by poisoning the protoplasm and breaking the peptidoglycan bond (Naidu & Clemens, n.d.).

Table 1. Results of Inhibitory Zone Diameter

Bacteria	Conc. (mg/mL)	Mean (mm) ± SD*	Bacteria	Conc. (mg/mL)	Mean (mm) ± SD*
<i>S. aureus</i>	80	7,47 ± 0,465	<i>B. subtilis</i>	80	6,95 ± 0,132
	40	6,53 ± 0,586		40	6,73 ± 0,153
	<b>20</b>	<b>6,12 ± 0,076</b>		<b>20</b>	<b>6,28 ± 0,076</b>
	(+)	16,92 ± 0,782		(+)	18,32 ± 0,321
	(-)	-		(-)	-
<i>S. epid</i>	80	9,43 ± 0,451	<i>B. cereus</i>	80	7,18 ± 0,293
	40	8,47 ± 0,252		40	6,78 ± 0,369
	20	8,12 ± 0,388		20	6,18 ± 0,076
	<b>10</b>	<b>7,33 ± 0,416</b>		<b>10</b>	<b>6,12 ± 0,076</b>
	(+)	19,62 ± 0,765		(+)	16,23 ± 1,435
(-)	-	(-)	-		

\* = three times replication

Flavonoid compounds work as antibacterial by destroying the bacterial cell membrane that form complex compounds with extracellular proteins so that bacterial cell membranes are damaged and followed by uncontrolled entry of water into the bacterial cell. This entry of water into bacterial cells causes swelling and eventually membrane bacterial cell rupture (Black & Jacobs, 1997). Flavonoid compounds can also cause inhibition of DNA and RNA synthesis in bacteria and affect the activity of DNA gyrase by forming hydrogen bonds in the regulation of nucleic acid base (Wu, Zang, He, Pan, & Xu, 2013). Saponin works as an antibacterial, because it can cause damage to microbial cell membranes through the formation of hydrogen bonds that form complex compounds with cell membranes. Such complex bonds will interfere with cell wall permeability, which leads to the release of cell contents and leads to cell death (Mayanti, Julaeha, & Putri A, 2016). In addition, saponin can also damage porine, thereby reducing the permeability of the bacterial cell wall which will cause the bacterial cells to run out of nutrients so that bacterial growth is inhibited or dead (Sari, Djannah, & Nurani, 2010). The mechanism of triterpenoid compounds as antibacterial generally occurs through the destruction of bacterial cell membranes due to triterpenoid that tend to be lipophilic. Besides terpenoids can also lyse the bacterial cell wall by reacting with the porine in the outer walls of bacterial cells (Wu et al., 2013).

Bacterial endurance to antibacterial compounds is closely related to the structure of cell walls where Gram-positive bacteria have cell wall structures with more peptidoglycan, less lipids and polysaccharides (teichoic acid) (Sudewi & Lolo, 2016). Peptidoglycan has polar properties that are easily penetrated by polar antibacterial compounds (Pangestuti, Sumardianto, & Amalia, 2017). Acetic acid is water-soluble polymer and functions as a transport of positive ions. Due to the water solubility characteristic of the Gram-positive bacterial cell wall, the flavonoid compound (Simaremare, 2014), phenol (Robinson, 1995) and saponin (Simaremare, 2014) in bamboo leaf ethanol extract are compounds that tend to be more soluble in the polar solvent making it easier to penetrate the cell wall of Gram-positive bacteria that is polar.

### Conclusion

Based on the results obtained in this study, it can be concluded that the minimum inhibitory concentration value of ethanol extract of *Bambusa vulgaris* leaves against *Staphylococcus aureus* and *Bacillus subtilis* is 20 mg/mL. While the minimum inhibitory concentration of ethanol extract of *Bambusa vulgaris* against *Staphylococcus epidermidis* and *Bacillus cereus* is 10 mg/mL.

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