

## **ANTIBACTERIAL ACTIVITY OF AWAR-AWAR LEAVES (*Ficus septica* Burm f) AGAINST *Staphylococcus aureus* ATCC 29523 AND *Escherichia coli* ATCC 35218**

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### **ABSTRACT**

This research was conducted to observe the activity of ethanol extract of Awar-awar (*Ficus septica* Burm f) leaves as antibacterial against *Staphylococcus aureus* ATCC 29523 and *Escherichia coli* ATCC 35218 based on the formation of clear zone and analysis of cell leak. The result showed that ethanol extract of awar-awar leaves performed antibacterial activity against *S. aureus* ATCC 29523 and *E. coli* ATCC 35218. Based on the clear zone diameter, that antibacterial activity was categorized as medium. The analysis of cell leak using spectrophotometer at  $\lambda$  260 nm and 280 nm did not indicate that ethanol extract of awar-awar leaves can cause cell leak on *S. aureus* ATCC 29523 and *E. coli* ATCC 35218.

### **Keywords**

*antibacterial activity, awar-awar leaves, E. coli ATCC 35218, S. aureus ATCC 29523*

### **INTRODUCTION**

Most of infectious diseases were caused by bacteria. *Staphylococcus aureus* and *Escherichia coli* are important pathogen which usually resistant to some medicines. That resistance lead to difficulties in choosing appropriate antimicrobial for therapy. As reported by Depkes (2008) that many antibiotics have been used for curing of infectious diseases, however infection problem still continue. Volk and Wheeler (1993) stated that antibiotic cure can cause resistance of bacteria, then we need new product which potential as antibacterial to solve that infection problem.

Plants contain some active chemical compounds which are potential to kill or

inhibit bacterial growth. Awar-awar (*Ficus septica* Burm F) is one of potential plants that can be used for medication. This plant can be found in Java, Madura, and Sulawesi (Steenis, 2005). Awar-awar was used as traditional medication in Bali. Awar-awar leaves can be used as medicine for skin disease, appendix disease, *bisul*, snake bite, and asthma (Didik *et al*, 2002). The roots can be used to cure some stomachaches such as dysentri and cholerae, also to cure toxin and asthma (Segatri, 1995).

Awar-awar contains some chemical compounds such as saponin, flavonoid, and tannin, also alkaloid such as tilosrebrin (hauptalkaloid), tiloforin, septisin, and antofin. The leaves and roots contain stigmasterol and  $\beta$ -sitosterol. The roots

also contain sterol and polyphenol (Didik *et al*, 2002).

Some researches have been conducted to assess active compound of plants or natural compounds to inhibit *S. aureus* and *E. coli*. Those researches are *Azadirachta indica* (mimba) seed to inhibit *S. aureus* (Ambarwati, 2007) and *Anredera cordifolia* (binahong) leaves to inhibit *S. aureus* and *Pseudomonas aeruginosa* (Khunaifi, 2010). Other investigations of *S. aureus* growth inhibition were done using *Alpinia galanga* (Yuharmen *et al*, 2002), *Piper betle* leaves (Poeloengan *et al*, 2006; Hermawan *et al*, 2007), *Plumbago zeylanica* L leaves (Poeloengan, 2009), and red betel leaves (Reveny, 2011). While *Curcuma xanthorrhiza* (Meilisa, 2008), *Artocarpus altilis* leaves (Sulistyaningsih *et al*, 2009), and *Morinda citrifolia* (Dewi, 2010) were used to inhibit *E. coli*.

Based on the above explanation, awar-awar has not been used for the investigation of *S. aureus* and *E. coli* inhibition. Some researches using awar-awar were the usage of methanol extract of awar-awar roots to inhibit *Vibrio cholera* and *E. coli* (Sukadana, 2000), also ethanol extract of awar-awar leaves againsts citotoxic activity of cancer cells (Seki *et al*, 2010).

Our preliminary test showed that awar-awar leaves juice could inhibit *S. aureus* growth at concentration 25% (clear zone diameter 0,67 mm), 50% (clear zone diameter 1,0 mm), and 75% (clear zone diameter 2,0 mm). Those results show that awar-awar leaves juice could inhibit *S. aureus* growth even the inhibition was weak. Therefore we conducted this research

using ethanol to extract the active compounds of awar-awar leaves.

**Tabel 1.** Clear zone diameter after the treatment of ethanol extract of Awar-awar leaves on *S. aureus* ATCC 29523 and *E. coli* ATCC 35218

No	Treatment		Diameter of clear zone (mm)	
			<i>S. aureus</i> ATCC 29523	<i>E. coli</i> ATCC 35218
1	Extract of Awar-awar leaves	10%	7,7	8
		20%	9,7	9
		30%	8.0	8.3

## MATERIALS AND METHODS

### 1. Ethanol extraction of awar-awar leaves powder

Extraction was proceed by maseration. Awar-awar leaves powder (200 g) was dissolved with 800 mL ethanol 96%. Sample was homogenized using shaker at 120 rpm for 24 hours. Than, sample was filtrated and the filtrat was evaporated until gel crude extract was formed.

### 2. Antibacterial activity test of awar-awar leaves extract using disc difusion method

Bacterial culture of *S. aureus* ATCC 29523 dan *E. coli* ATCC 35218 (0,1 mL each) from NB medium was separately inoculated in NA medium using spread plate method. Various ethanol extract of awar-awar leaves (10%, 20%, 30%, 40%, dan 50%) were impregnated onto 5 mm paper disk and then placed on the seeded NA medium using a

sterile forceps. Plates were incubated for 24 hours at 37°C. and the diameter of the zone of inhibition around the disk is measured to the nearest millimeter (Greenwood, 1995). Based on Davis and Stout (1971), the inhibition activity is categorized as weak inhibition if the diameter of clear zone is  $\leq 5$  mm, as medium inhibition if the diameter is around 5-10 mm, as strong inhibition if the diameter around 10-19 mm, and as very strong if the diameter is  $\geq 20$  mm.

### 3. Analysis of cell leak (Bunduki *et al*, 1995)

Bacterial suspension (10 mL) which has been grown along 24 hours was centrifuged at 3.500 rpm for 20 minutes. Filtrat was discharged and 5 mL NaCl 0,85% was added into cell sediment. Then, extract awar-awar leaves which produced smallest clear zone (concentration 10%) was added and incubated at 120 rpm, 37°C, for 24 hours. Similar bacterial suspension without extract addition was used as comparison. Next, bacteria-extract suspension was centrifuged at 3.500 rpm for 20 minutes. The optical density of supernatant then was analyzed using spectrophotometer UV-Vis at  $\lambda$  260 nm for nucleic acid analysis and at  $\lambda$  280 nm for protein analysis.

## RESULTS

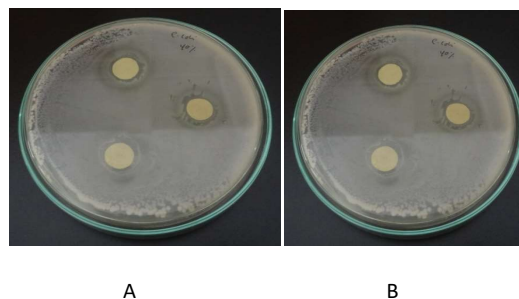
Data in **Table 1** shows that ethanol extract of awar-awar leaves with various concentration can inhibit the growth of *S. aureus* ATCC 29523 and *E. coli* ATCC 35218. Based on Davis and Stout (1971), the inhibition was categorized as medium. The images of clear zone are shown on **Fig. 1** and **2**.

Test of bacterial cell leak of *S. aureus* ATCC 29523 and *E. coli* ATCC 35218 was conducted using spectrophotometer UV-Vis

at  $\lambda$  260 nm for measurement of nucleic acid nitrogen and at  $\lambda$  280 nm for nitrogen of protein. The result is shown in **Table 2**.

## DISCUSSION

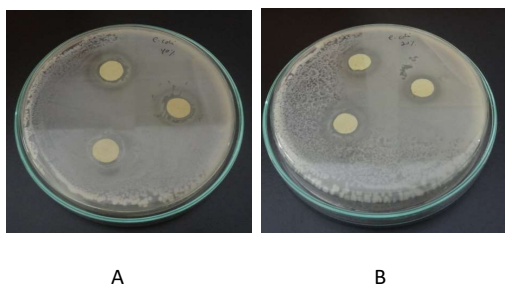
Our preliminary study showed that juice of awar-awar leaves, with aquades as the solvent, can inhibit *S. aureus* ATCC 29523 growth with 2 mm clear zone diameter. Based on Davis and Stout (1971), that inhibition activity was categorized as weak, because the clear zone was  $< 5$  mm. However, we conclude that awar-awar leaves were potential as antibacteria and can be improved by extracting it using another solvent.



**Figure 1.** The image of clear zone after the treatment of 20% (A) and 30% (B) ethanol extract of Awar-awar leaves on *S. aureus* ATCC 29523

**Tabel 2.** The result of cell leak analysis using spectrophotometer at  $\lambda$  260 nm and 280

Sample	Absorbance	
	$\lambda$ 260 nm	$\lambda$ 280 nm
<i>S. aureus</i> ATCC 29523	1,035	0,463
<i>E. coli</i> ATCC 35218	0,885	0,434
<i>S. aureus</i> ATCC 29523 + extract	undetected	undetected
<i>E. coli</i> ATCC 35218 + extract	undetected	undetected



**Figure 2.** The image of clear zone after the treatment of 20% (A) and 30% (B) ethanol extract of Awar-awar leaves on *E. coli* ATCC 35218

Ethanol was chosen as extraction solvent, because it is a polar solvent, while active compounds in awar-awar leaves are polar compounds. Therefore it can extract the active compounds effectively. Moreover, it is easy to get ethanol, ethanol is cheap, not easy to evaporate, and not affect the active compounds (Ahmad, 2006).

In this research we used *S. aureus* ATCC 29523 (Gram positive) and *E. coli* ATCC 35218 (Gram negative) to check the antibacterial spectrum of awar-awar leaves. ar-awar. Pelczar and Chan (1998) stated that a compound has wide spectrum if that compound can inhibit the growth of Gram positive and Gram negative bacteria. If the compound only inhibit the growth of Gram

positif or Gram negative bacteria, then it can be categorized as a narrow spectrum compound. Based on the result (**Table 1** and **Fig. 2**), it can be concluded that ethanol extract of awar-awar leaves has wide spectrum antibacterial compound.

Awar-awar leaves contain some secondary metabolites, such as flavonoid, saponin, and tanin. Those secondary metabolites can be extracted using ethanol as solvent. Flavonoid can form complex compound with extracellular protein. That complex compound is able to be dissolved, then can damage bacterial cell membrane and followed by the release of intracellular compounds of bacterial cell. Tanin can wrinkle bacterial cell wall or membrane, herefore it can disturb cell permeability which lead to growth disturbance. While aponin can damage cell membrane by decreasing cell wall turgor which can lead to cell wall lysis.

Usually, the diameter of clear zone will increase parallel with the increase of kxtract concentration. However, in this research we found that the celar zone diameter was decrease when the extract concentration was increased to 30%. Dewi (2010) stated that the increase of clear zone diameter not always corespond with the increase of antibacterial concentration. Antibacterial activity can be affected by the concentration of antibacterial compound, temperature, pH, microbial species, and organic compound.

The result showed that *S. aureus* ATCC 29523 and *E. coli* ATCC 35218 gave different respond to our treatment. *S. aureus* ATCC 29523 was more sensitive to the extract than *E. coli* ATCC 35218. Cell wall stucture of *S. aureus* ATCC 29523 is more simple than of *E. coli* ATCC 35218. The cell wall of *S. aureus* ATCC 29523 is composed of one thick layer, while cell wall of *E. coli* ATCC 35218 is composed of some layers. Therefore, *S. aureus* ATCC 29523 more sensitive to antibacterial compounds.

The bacterial respond to ethanol extract of awar-awar leaves was also observed by analysing the disturbance or damage of membrane cell, based on the bacterial growth medium turbidity. Bacterial cell membrane damage was measured based on the compounds which is released by the cell, those are nitrogen from nucleic acid (measured at  $\lambda$  260 nm) and nitrogen from protein (measured at  $\lambda$  280 nm).

Data in **Table 2** shows that the absorbance at  $\lambda$  260 nm and 280 nm were undetected. That undetected absorbance perhaps was caused by the intense color of awar-awar leaves extract. Therefore spectrophotometer UV-Vis could not detect the nucleic acid and protein which were released from bacterial cell.

Different result was shown by Febriyanti (2010) who observed the effect of betel leaf volatile oil fraction against Gram positive bacteria such as *Bacillus subtilis*, *S. mutan*, and *S. aureus*. While Azis (2010) observed the antibacterial mechanism of ethanol extract of white lily bulbs againts *Propionibacterium acnes*, *S. aureus*, dan *S. epidermis*. Both showed the increase of absorbance when bacteria were contacted with the extract. It can be summarized that the different result of our research with those two researches can be affected by the different of the extracted material, the kind of extract, and the strain of bacteria. Next observation using other methods is recommended to observe the antibacterial mechanism of ethanol extract of awar-awar leaves.

## CONCLUSION

1. Ethanol extract of awar-awar leaves can inhibit the growth of *Staphylococcus aureus* ATCC 29523 and *Escherichia coli* ATCC 35218. The inhibition was categorized as medium inhibition.

2. Antibacterial mechanism of ethanol extract of awar-awar leaves against *Staphylococcus aureus* ATCC 29523 and *Escherichia coli* ATCC 35218 could not be observed based on the analysis of cell leak.

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