

PRELIMINARY STUDY ON ANTIBACTERIAL ACTIVITY OF SAWO KECIK (*Manilkara kauki* (L.) Dubard) ROOTS EXTRACT

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Abstract. The roots of sawo kecik (*Manilkara kauki* (L.) Dubard) contain astringent that can be used to treat diarrhea for infants. However, the active antibacterial substance in sawo kecik roots has not been known. Therefore, the research to understand the antimicrobial activity of sawo kecik roots extract against *Escherichia coli* and *Staphylococcus aureus* was performed. Sawo kecik roots were macerated using methanol and chloroform. The result was then treated with *E. coli* and *S. aureus* with a concentration of 5 ppm and compared to negative control (solvent) and further observed and analyzed how reduced the bacterial growth with Two Ways ANOVA without interaction. The research results indicated that the sawo kecik roots methanol extract was effective to slow down the growth of *S. aureus* (0.160 ± 0.007) but ineffective against *E. coli*, whereas sawo kecik roots chloroform extract was not effective to reduce both bacteria. It can be concluded that further research is required to measure and verify the antibacterial activity of the extract using higher concentration samples and different research methodology.

Keywords: antibacteria, *Escherichia coli*, *Manilkara kauki*, roots, *Staphylococcus aureus*.

Citation

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INTRODUCTION

Antibacteria is a compound used to slow down the growth of bacteria. Antibacteria usually resides in the organism as a secondary metabolite. The common mechanism of the antibacterial compound is by damaging the cell wall, changing the membrane's permeability, disrupting protein synthesis, and obstructing the enzyme (Pelczar & Chan, 2005). The compounds that can damage the cell wall are phenol, flavonoids, and alkaloids. These phytochemical compounds have the poten-

tial to be natural antibacteria to pathogens such as *Escherichia coli* and *Staphylococcus aureus* (Septiani, 2017). *Escherichia coli* is a pathogenic bacteria in humans. This bacteria can cause indigestion and disrupt the stomach work system. While *Staphylococcus aureus* bacteria is the most common cause of infection in the world (DeLeo et al., 2010).

Sawo kecik (*Manilkara kauki* (L.) Dubard) is categorized as a Sapotaceae family spread across the Asia Pacific and Australia. In Indonesia, this plant can be found in Karimun Island, Kangean Island, Bali, Nusa

Tenggara, Buton, Sulawesi, Maluku, and Papua (Hardiyanto, 2008). The roots and bark of sawo kecil contain astringent that can be used to treat diarrhea for infants (Khare, 2007). Several phytochemicals studies on the *Manilkara* Genus have revealed several polyphenols, terpenoids, anthraquinones, and saponins (Gopalkrishnan, 2004). The research performed by Prayudhani et al. (2013) stated that the ethanol extract of sawo kecil bark (*M. kauki*) consisting of flavonoids (quercetin and apigenin) can slow down the growth of *Escherichia coli*. *Manilkara kauki* is closely related to *Manilkara hexandra*, and *Manilkara zapota*. Mahida & Mohan (2007) stated that methanol extract leaves of *Manilkara hexandra* (Roxb.) Dubard shows antibacterial activity against *Staphylococcus* and *Salmonella*. Another researcher, Barghavi et al (2013) stated that the extract of *Manilkara zapota* roots that contains alkaloids, glycoside, saponin, tannin, and carboxylic acids can slow down the growth of *Staphylococcus aureus* and *Escherichia coli*. The potential antibacterial compound is usually a secondary metabolite compound. The organ that stores the metabolite compound itself depends on the plant types. For example, in a perennial plantation, the highest secondary metabolite is found in bulbs, roots, rhizomes, and the bark of roots and stems (Pagare et al., 2015). Over the past decade, research on antibacterial properties of *Manilkara kauki* has focused on the leaves, stem, or stem bark. Until now, the research about antibacterial activity of sawo kecil (*Manilkara kauki*) roots has not yet been known. Therefore, the goal of this preliminary study was to assess the antibacterial activity of sawo kecil roots extract against *E. coli* and *S. aureus* bacteria.

MATERIALS AND METHODS

All the experiments were conducted from July until September 2018. The variation of the solvent used is the independent variable, while the diameter inhibition zone of *Escherichia coli* and *Staphylococcus aureus* growth is a dependent variable. The control variables were incubation temperature, length of incubation time, age, and suspension density of bacteria. The sawo kecil (*Manilkara kauki* (L.) Dubard) roots were obtained from Universitas Negeri Malang, seedling sub-section. *Escherichia coli* and *Staphylococcus aureus* bacteria were obtained from Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Science, Universitas Negeri Malang. *Escherichia coli* and *Staphylococcus aureus* culture aged 18-24 hours on Mueller Hinton Agar disc media.

Preparation of Extract

The sawo kecil roots were taken from a three-month-old plant sample to be washed and cut into small pieces. The pieces were then dried using an oven for three days and ground to powder. The resulting powder was weighed 10 grams. The simplisia of sawo kecil roots was then macerated for 1×24 hours in a methanol and chloroform solution with a simplisia to solution ratio of 1:3. The next step was filtration and evaporation for 3×24 hours until macerate was formed (Mustarichie et al., 2011). The inhibitory potential is considered good if it shows activity at a concentration under 100 µg/ml or 100 ppm (Silva et al., 2015; Aristyawan et al., 2017), thus the formed macerate was collected and diluted in the solution at a concentration of 5 ppm, the lowest possible concentration.

Suspension of Bacteria

The suspension of *Escherichia coli* and *Staphylococcus aureus* were performed in Nutrient Broth media and cultivated at 37°C for 24 hours. The suspension used was the one with the same muddiness to standard 0.5 McFarland which equal to a bacteria concentration of $1,5 \times 10^8$ CFU/ml. *E. coli* and *S. aureus* were then smeared evenly to the surface of the Mueller Hinton Agar media cup using a sterilized cotton swab (Hudzicki, 2009; Jorgensen & Turnidge, 2015).

Paper Discs Placement

Paper discs containing sawo kecik roots methanol extract with the concentration of 5 ppm, methanol solvent (negative control). Each disc is performed twice. The paper disc placement was also performed for chloroform extract. The petri dish was then incubated for 24 hours at 37°C temperature.

Data Analysis

The data of diameter inhibition zone were analyzed for normality and homogeneity using Shapiro-Wilk and Bartlett, then two-way ANOVA without interaction tested with assistance of Microsoft Excel.

RESULTS AND DISCUSSION

Antibacterial Activity of Methanol Extract from Sawo Kecik Roots

It was discovered that sawo kecik roots methanol extract 5 ppm showed a bigger zone of inhibition to *S. aureus* compared to *E. coli* (Table 1). However, compared between methanol extract and methanol solvent to *E. coli*, the result showed that methanol extract did not have an antibacterial effect. Methanol is one of the polar solvents. Pingale & Dash (2015) stated that the screening result of *Manilkara zapota* roots extracted using methanol produced alkaloids, saponins, flavonoids, and tannins, as well as phenolic compounds. With this, it can be assumed that using the same solvent (methanol) for *Manilkara kauki* roots may produce similar results in *Manilkara zapota* roots. Sawo kecik is rough extract and contains several other compounds that can affect the ability of secondary metabolites to slow down bacterial growth. Results of the inhibition zone of methanol extract from sawo kecik roots against *E. coli* and *S. aureus* shown in Figure 1.

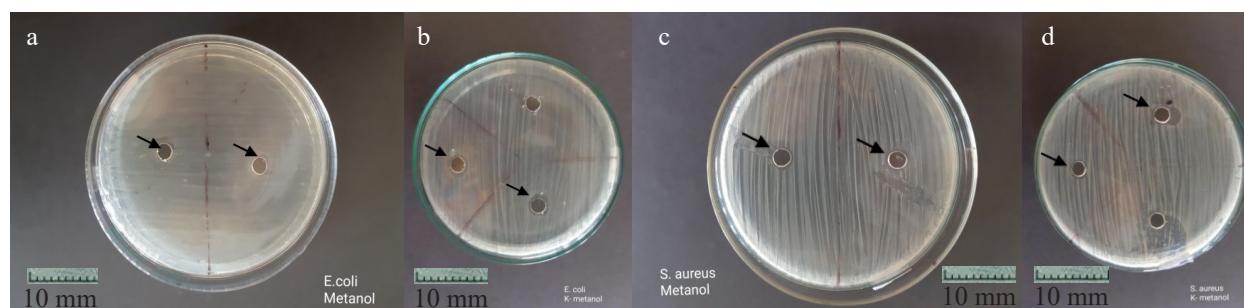


Figure 1. Results of the inhibition zone of methanol extract from sawo kecik roots against *E. coli* and *S. aureus* (a. methanol extract-*E. coli*, b. methanol solvent-*E. coli*, c. methanol extract-*S. aureus*, d. methanol solvent-*S. aureus*)

Table 1. Results of antibacterial activity of methanol extract from sawo kecik roots against *E. coli* and *S. aureus*

Treatment	Zone of Inhibition (mm)	
	<i>E. coli</i>	<i>S. aureus</i>
Methanol extract	0.155 ± 0.092	0.160 ± 0.007
Methanol solvent (negative control)	0.200 ± 0.063	0.145 ± 0.007

Pelczar & Chan (2005) stated that concentration level is one of the factors that affect antibacterial activity. Based on the research by Prayudhani (2013), the bark ethanol extract of *M. kauki* with a concentration of 55% was found to be the most effective combination to slow down the growth of *E. coli* with the average inhibition zone diameter of 12.3 mm (51% toward Levofloxacin). Therefore, there is a need to perform another research about the active substance in sawo kecik roots using methanol solvent and add more variables of concentration to determine its antibacterial activity.

Sawo kecik roots methanol extract treatment to *S. aureus* (0.160 mm) indicated a higher inhibition zone compared to *E. coli* (0.155 mm). Ekalina et al. (2017) stated that *M. zapota* methanol extract was effective as antimicrobial to several positive and negative gram bacteria species. *S. aureus* is a positive gram bacteria. Structurally, this bacteria has a thicker peptidoglycan structure, rather lipid, and its cell walls contain polysaccharides (teikoic acid). Teikoic acid is a polymer that dissolves in water and functions as positive ion transport in and out of cells (Alni et al., 2011 in Septiani et al., 2017). Due to this dissolves in water characteristic, it indicates that positive gram bacterial cell wall has a polar characteristic, thus easy to penetrate the peptidoglycan layer and cause higher inhibition zone in positive gram bacteria compared to negative gram bacteria.

Unlike *S. aureus*, *E. coli* is a negative gram bacteria. Structurally, the cell walls of negative gram bacteria are more complex

compared to positive gram bacteria that resistant toward antibacteria. The cell walls consist of three layers, lipoprotein (outer layer), lipopolysaccharide (middle layer), and peptidoglycan (inner layer). Lipopolysaccharide plays a role to prevent bioactive antibacterial material to enter. Peptidoglycan contains a high amount of lipid. The outer layer of negative gram bacteria cell wall consists of phospholipid and several proteins (or also called auto layer) (Brook et al., 2013; Helmiyati & Nurrahman, 2010 in Septiani, 2015). Therefore, it is a polar compound that cannot denature the cell walls of negative gram bacteria and slow down their growth.

Antibacterial Activity of Chloroform Extract from Sawo Kecik Roots

It was discovered that sawo kecik roots chloroform extract 5 ppm could not act as an antibacterial compound compared to the zone of inhibition using chloroform solvent (Table 2). Pingale & Dash (2015) stated that *Manilkara zapota* roots chloroform extract contains steroids and sterols, triterpenoids, and alkaloids. With this, it can be assumed that by using the same solvent (chloroform), the compounds obtained from the result of *Manilkara kauki* roots extract may similar to *Manilkara zapota* roots. Results of the inhibition zone of chloroform extract from sawo kecik roots against *E. coli* and *S. aureus* are shown in Figure 2.

The chloroform extract treatment of sawo kecik roots did not show any antibacterial effect compared to chloroform solvent. Eloff (2019) stated that non-polar solvent is

not effective to be used to observe antibacterial activity using agar medium. The agar medium method is better to be used for a single compound with known polarity. Due to the plant extract contained multiple antimicro-

bial compounds with different polarities, this method was not effective. This result indicated that other researches method is required to observe antibacterial activity using non-polar solvent.

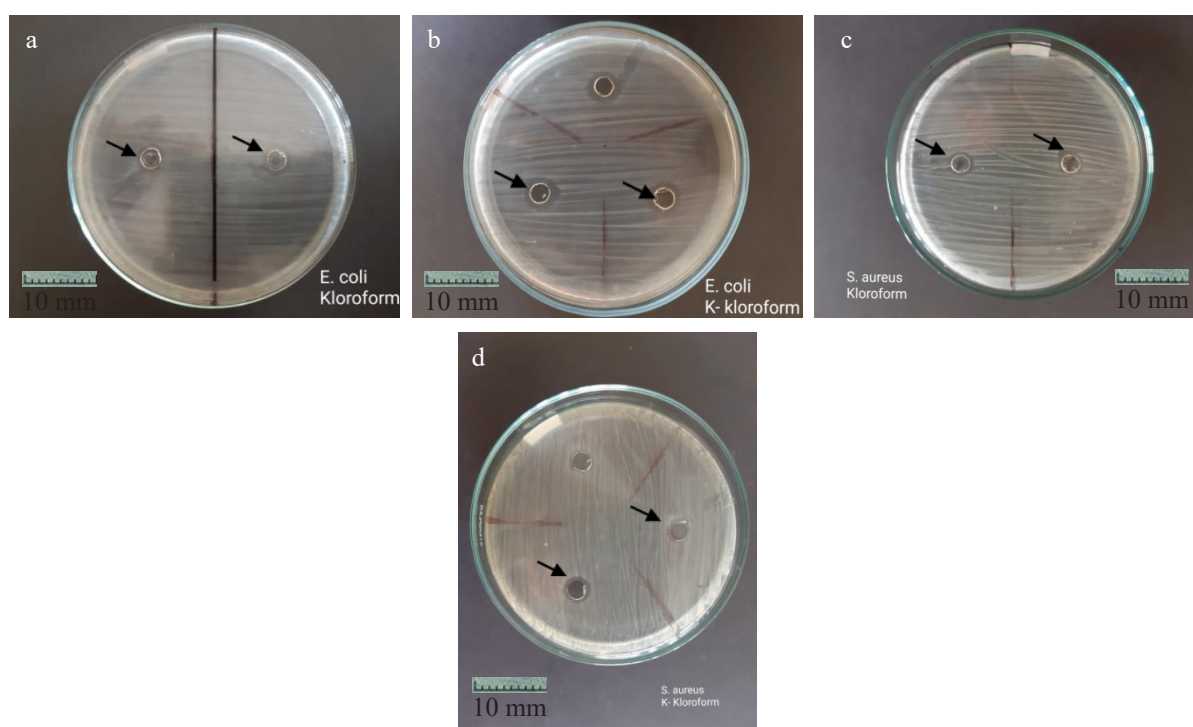


Figure 2. Results of the inhibition zone of chloroform extract from sawo kecik roots against *E. coli* and *S. aureus* (a. chloroform extract-*E. coli*, b. chloroform solvent-*E. coli*, c. chloroform extract-*S. aureus*, d. chloroform solvent-*S. aureus*)

Table 2. Results of antibacterial activity of chloroform extract from sawo kecik roots against *E. coli* and *S. aureus*

Treatment	Zone of Inhibition (mm)	
	<i>E. coli</i>	<i>S. aureus</i>
Chloroform extract	0.253 ± 0.138	0.145 ± 0.042
Chloroform solvent (negative control)	0.425 ± 0.035	0.208 ± 0.060

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