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# Antibacterial activity and phytochemical screening of Kesambi (Sapindaceae) against Eschericia coli and Staphylococcus aureus

Boima Situmeang<sup>1\*</sup>, Agus Malik Ibrahim<sup>1</sup>, Nurhayati Bialangi<sup>2</sup>, Weny J.A Musa<sup>2</sup>, Saronom Silaban<sup>3</sup>

<sup>1</sup>Department of Chemistry, Sekolah Tinggi Analis Kimia Cilegon, Cilegon, Indonesia <sup>2</sup>Department of Chemistry, Faculty of Mathematic and Natural Science, Gorontalo State University, Indonesia <sup>3</sup>Department of Chemistry, Faculty of Mathematic and Natural Science, Universitas Negeri Medan, Medan 20221, Indonesia

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#### Abstract:

Kesambi plant (*Schleichera oleosa*) is a medicinal plant belongs to family *Sapindaceae*, commonly found in tropical and subtropical regions of the world. In Indonesia, kesambi plants can be found in Java and Bali island. The purpose of this study is to extraction and antibacterial activity test of steam bark extract of kesambi against *Escherichia coli* and *Staphylococcus aureus*. Extraction was done by maceration method using *n*-hexane, ethyl acetate and methanol. Antibacerial test using Kirby bauer method by measure inhibition zone. Phytochemical screening shown that *n*-hexane extract contain triterpenes and steroid compounds. Ethyl acetate extract contain triterpenes, flavonoid, steroid, and fenolic compounds. Methanol extract contain flavonoid, fenolic and triterpenes compounds. Inhibition zone of *n*-hexane, ethyl acetate and methanol extracts against *E. coli* and *S. aureus* at concentration of 5000 µg/mL showed 4.4; 7.9; 4.6 mm and 4.7; 8.9; 4.5 respectively, while amoxicillin and ciprofloxacin as positive control at concentration of 100 µg/mL showed 6.9 and 7.1 mm respectively. Kesambi extract have potential as antibacterial against *E. coli* and *S. aureus*.

#### Keywords:

Kesambi; antibacterial; Eschericia coli; Staphylococus aureus

#### Introduction

Infectious disease and parasites are one of the major diseases in the world. According to World Health Organization (WHO) data in 2011, infectious and paracites diseases are the third leading cause of death in the world. In Indonesia, infection deseases is one of the major caused of death for children and still a big health problem in all levels of society (Singh & Pandeya, 2011). Infectious disease of the skin tissue that commonly affects the public caused by various bacteria (Mustaffa et al. 2011). Bacteria cause the most common skin disease and infection is Staphylococcus aureus and Escherichia coli. One of the palnt that have potential to treat various deseases is Kesambi. Schleichera oleosa (Kesambi) is well known as medicinal plant (Dhego et al. 2017). Kesambi is one of plants that have the potential to treat various deasease. Kesambi plant

\* Corresponding author.

boima@stakc.ac.id

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or kusum widely spread in Asia such as India, Nepal, Malaysia and Indonesia (Anuragi & Mishra, 2017). In Indonesia, this plant can be found in Java and Bali island especially in Cilegon region (Ghosh et al. 2011; Situmeang et al. 2016). The presence of various types of phytochemicals such as tannins, alkaloids, glycosides, volatile oils etc. showed that the plant possesses considerable medicinal importance (Bachli, 2007;). The storage organs of the plant contains these active principles. Kesambi is an important multipurpose ethnomedicinal plant with basic and applied importance to civil as well as tribal society.

Natural products have been a source of medicinal agents and traditional medicine system that have been used for thousand of years in countries. World Health Organization also has been estimated that approximately 80 % of the world's inhabitans rely mainly on traditional medicines for their primary health care (Singh & Pandeya, 2011). As the plant is being used extensively in Indonesia as an herbal medicine, it is necessary to have knowledge of antibacterial activity against *E. coli* and *S. aureus* of the plant of our native species.

# Materials and Methods

#### Material and instrumentation

The research specimen is kesambi steam bark collected from Cimerak vilage, Cilegon district, Banten province, Indonesia in march 2019. The chemicals used in this research were ethyl acetate, *n*-hexane, methanol, distilled water, alcohol 70%, ciprofloxacin 100 ppm, amoxycilin 100 ppm, bacto agar, and Nutrien broth media. Instrument were used laminar air flow, memmert incubator, hirayama HVE-50 autoclave, Erlenmeyer, paper disc, micro plates, micro tubes, micro pipets and evaporator.

## Extraction

Dried kesambi steam bark (2 kg) were soaked in 5 L *n*-hexane, ethyl acetate and methanol solvent for 2 days respectively. The shole mixture was then filtered through filter paper and the filtrate was then evaporated under reduce pressure at 50 °C using a Buchi rotary evaporator. All the extracts were filtered through a cotton plug followed by Whatman filter paper and then concentrated by using a rotary vacum evaporator to provide of n-hexana, ethyl acetate, and of methanol crude extract. All extract were test phytochemical screening (Marlinda et al. 2012; Situmeang et al. 2016).

#### Antibacterial activity test

Selected bacteria (E. coli and S. aureus) was cultured for 24 h at 37 °C under aerobic condition on nutrient broth media. Afterward, the bacteria were suspended in a 0.8% Nutrion broth solution (w/v). The turbidity of suspension of bacteria was corrected to the 0.5 Mc Farland standard (1.2  $\times$  10<sup>8</sup> bacterial cells/mL). Agar plate was inoculated with 200 µL bacterial suspension. 15 µL of the sample with concentrations of 5000 µg/ were dripped on paper disc on agar media and incubated for 24 h at 37 °C under aerobic condition. The presence of clear zones around the paper disc was indicated that the samples has antibacterial activity. The assays were also carried out to the negative control (methanol 96%) and standard antibiotic amoxicillin and ciprofloxacin (positive control) with concentration 100 µg/L. The inhibition zone of the sample was determined by measuring the diameter of the clear zone around the paper disc. The assays were conducted in two repetitions (Roslizawaty et al. 2013; Dhego et al. 2017). The research flow chart shown in Fig 1.



Fig 1. Research flow chart

## Results

The steam bark of kesambi was dried and successively extracted with *n*-hexane, ethyl acetate and methanol solvent. All extract were phytochemical screening test using several reagent. Phytochemical screening shown thant *n*hexane extract contain triterpenes and steroid compounds. Ethyl acetate extract contain triterpenes, flavonoid, steroid. and fenolic compounds. Methanol extract contain flavonoid, fenolic and triterpenes compounds. The result of *n*-hexane extract were 5 g, ethyl acetate 17 g, and methanol 297 g. the result of antibacterial acivity test shown in Table 1.

Antibacterial activity test shown ethyl acetate extract has the high inhibition zone against E. coli 2). Amoxicillin and *S*. aureus (Fig and ciprofloxacin positive control with as concentration of 100 µg/mL showed high inhibition zone. Amoxicillin at concentration 100 ppm gives 6.9 ppm and ciprofloxacin gives 7.1 mm (Fig 3).

#### Discussion

The resulted of extraction shown that methanol extract have the much quantity with red colour compared with *n*-hexana and ethyl acetate extract. This result indicated that kesambi extract contain more polar chemical compounds that nonpolar. The result of phytochemical screening shown that kesambi steam bark extract positive contain triterpenoid in all solvent phases. Positive flavonoids and fenolic compounds in ethyl acetate and methanol extract, while steroids positive in *n*hexana and ethyl acetate. This is concictent according to *Schleichera oleosa* steam bark reported by Srinivas & Baboo (2013) and Dhego et al. (2017).

Antibacterial activity test shown ethyl acetate extract has the high inhibition zone against *E. coli* and *S. aureus*. This is correlated that ethyl acetate extract contains much chemical compounds than n-hexana and methanol extract (Ajileye et al. 2015). *E. coli* and *S. aureus* are aerobic bacteria. This is the first reported antibacterial activity test from kesambi steam bark extract collected from Cimerak village, Indonesia.

Amoxicillin and ciprofloxacin as positive control with concentration of 100  $\mu$ g/mL showed high inhibition zone. Amoxicillin choosed as positive control because amoxicillin is one of several antibiotic which used by people to treat diarrhea desease (Edziri et al. 2012; Favela-Hernández et al. 2012; Febrina et al. 2017; Simorangkir et al. 2017). Methanol as negative control not shown inhibition zone. Its means that bacteria *E. coli* and *S. aureus* not gives effect or inhibition zone.

## Table 1

Antibacterial activity test of steam bark kesambi extract

Sample	Concentration µg/L	Inhibition zone (mm)	
		E. coli	S. aureus
<i>n</i> -hexane	5000	4.4	4.7
Ethyl acetate	5000	7.9	8.9
Methanol	5000	4.3	4.5
Amoxicillin	100	6.9	nt
Ciprofloxacin	100	nt	7.1
Methanol	96%	0	0



#### Fig 2. Diagram of antibacterial activity



Fig 3. Extract, positive control and negative control

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# Conclusion

Phytochemical screening shown thant *n*-hexane contain triterpenes and extract steroid compounds. Ethyl acetate contain extract triterpenes, flavonoid, steroid, and fenolic compounds. Methanol extract contain flavonoid, fenolic and triterpenes compounds. Antibacterial activity test shown that ethyl acetate and methanol extract have higher inhibition zone value. Inhibition zone of *n*-hexane, ethyl acetate and methanol extracts against E. coli and S. aureus at concentration of 5000 µg/mL showed 4.4; 7.9; 4.6 mm and 4.7; 8.9; 4.5 respectively, while amoxicillin and ciprofloxacin as positive control at concentration of 100 µg/mL showed 6.9 and 7.1 mm respectively.

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