# ANTIBACTERIAL ACTIVITY OF METHANOLIC EXTRACT OF *Plumeria acuminata* STEMBARK AGAINST *Escherichia coli* AND *Staphylococcus aureus*

# *Aktivitas Antibakteri Ekstrak Metanol Kulit Batang Kamboja (Plumeria acuminata) Terhadap Escherichia coli dan Staphylococcus aureus*

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***ABSTRAK***

*Penyakit infeksi merupakan salah satu masalah kesehatan di Indonesia. Hal ini disebabkan oleh bakteri seperti Escherichia coli dan Staphylococcus aureus. Salah satu tanaman berpotensi sebagai antibakteri adalah Plumeria acuminata (Kamboja). Tujuan dari penelitian ini adalah untuk mengidentifikasi kandungan fitokimia dan untuk mengetahui aktivitas antibakteri terhadap E. coli dan S. aureus dari ekstrak metanol kulit batang P. acuminata (MEPAS). Kulit batang P. acuminata dimaserasi dengan metanol selama 3x24 jam dan kemudian diidentifikasi kandungan fitokimia menggunakan kromatografi lapis tipis. Uji antibakteri dilakukan dengan metode difusi Kirby-Bauer menggunakan empat kelompok perlakuan, kontrol negatif (media) dan sefotaksim sebagai kontrol positif. Zona hambat dianalisis dengan analisis probit untuk mendapatkan nilai IC50. Hasil penelitian menunjukkan bahwa MEPAS terdapat alkaloid, terpenoid, flavonoid, dan saponin. Nilai IC50 MEPAS terhadap E. coli dan S. aureus adalah 2.325 ppm dan 1.800 ppm. Pada konsentrasi yang sama, kontrol positif menunjukkan lebih aktif daripada ekstrak.*

***Kata Kunci:*** *Plumeria acuminata, antibakteri, Escherichia coli, Staphylococcus aureus.*

**ABSTRACT**

Infectious disease is one of health problem in Indonesia. It is caused by bacteria such as *Escherichia coli* and *Staphylococcus aureus.* One of potential plant as antibacterial is *Plumeria acuminata* (Kamboja). The aim of this study is to identify the phytochemical contents and to examine antibacterial activity against *E. coli* and *S. aureus* from methanolic extract of *P. acuminata stembark* (MEPAS). The stembark of *P. acuminata* was macerated by methanol for 3x24 hours and then identified for phytochemical compound by Thin Layer Chromatography. Antibacterial assay was done by Kirby-Bauer method using four treatment groups, negative control (media), and cefotaxime as positive control. The inhibition zone was analyzed by probit analysis to obtain the IC50 value. The results showed that MEPAS contained alkaloid, terpenoid, flavonoid, and saponin. The IC50 value of MEPAS against *E.coli* and *S. aureus* was 2,325 ppm and 1,800 ppm, respectively. In the same concentration, the positive control showed more active than the extract.

**Keywords:** *Plumeria acuminata, antibacterial, Escherichia coli, Staphylococcus aureus.*

## INTRODUCTION

Food and water contamination by bacteria such as *Escherichia coli* and *Staphylococcus aureus* can cause infectious diarrhea. Diarrhea is an endemic disease in Indonesia and potentially as extraordinary events which is often cause of death. The basic health research 2007 conducted in Indonesia reported that diarrhea is the first cause of death in neonatus (31.4%) and infants (25.2%), whereas in all age groups is the fourth cause of death (13.2%) (Balitbangkes, 2008). The need of antibiotics for infection therapy is still relatively high, but on the other side appears pathogenic microorganisms that are resistant to antibiotics. Therefore, we need to explore new antibiotics, especially from natural product as an alternative therapy for infectious diseases.

Kamboja (*Plumeria acuminata*) have been used to treat rheumatism, ulcers, sores, and swelling from many parts of plant such as flowers, leaves, exudates, and also stembark. The chemical constituents of *P. acuminata* are fuvoplumierin, geraniol, farnesol, sitronellol, linallol, alkaloid, saponin, flavonoid, bitter substances, and resin (Hariana, 2008). Reportedly, *P. acuminata* bark extract has antifungal activity against *Aspergillus* and *Candida* species that caused otomycosis (Villanueva *et al*., 2008; Boncalon *et al*., 2009). Priwanda (2006) showed that chloroform extract of *P. acuminata* stembark has antiangiogenic properties. Prihandono (1996) also reported that petroleum ether, chloroform, and methanol extracts of *P. acuminata* flower exhibited antibacterial activity against *E. coli* and *S. aureus*. In the present study, methanolic extract of *P. acuminata* stembark (MEPAS) was used as a sample to be identified the phytochemical compounds and was examined its antibacterial activity against *E. coli* and *S. aureus*.

## MATERIALS AND METHOD

### Materials

Kamboja (*P. acuminata*) stembark was collected from Kaliputih, Purwokerto, Indonesia. Nutrient agar and nutrient broth as media, *E. coli* and *S. aureus*, silica gel F254, Dragendorff and Vanillin-sulfuric acid reagent, cefotaxime, methanol, chloroform, ethyl acetate, n-hexane, n-butanol, and glacial acetic acid.

### Methods

*Preparation of MEPAS*

Kamboja stembark powders as much as 400 g was macerated with methanol (1:5) for 3x24 hours, filtered, and the filtrates were evaporated, then concentrated on waterbath until getting solvent-free and viscous extract.

*Phytochemical identification of MEPAS*

All samples were spotted on silica gel $F_{254}$ plate and developed in mobile phase then the samples were sprayed with specific reagents (Table 1). These spots were observed under $UV_{254}$ and $UV_{366}$ lights, then its hRf values were determined.

Table 1. TLC system on phytochemical identification of MEPAS

| Secondary Metabolites | Mobile Phase (ratio) | Spraying reagents |
|---|---|---|
| Terpenoid (Harborne, 1987) | n-hexane: ethyl acetate (3:2) | Vanillin-sulfuric acid, heat at 100°C until coloration appear |
| Alkaloid (Wagner *et al*., 1996) | n-butanol: glacial acetic acid: water (4:1:1) | Dragendorff |
| Flavonoid (Harborne, 1987) | n-butanol: glacial acetic acid: water (3:1:1) | steamed by ammonia |
| Saponin (Wagner *et al*., 1996) | chloroform: methanol (7:3) | Vanillin-sulfuric acid, heat at 100°C until coloration appear |

*Antibacterial assay by Kirby-Bauer method*

Each extract was diluted in distilled water to obtain concentrations of 1,000; 2.000; and 3.000 ppm. Controls used were media+bacteria (negative control) and media+antibiotic+bacteria (positive control). Nutrient broth and bacterial suspension (1.5 x 108 CFU/mL) were added to reach a total of 100 µL. Incubation was taken place at 37°C for 24 hours. Experiments were done in triplicate and then were measured the inhibition zones against *E. coli* and *S. aureus*.

*Data analysis*

The growth inhibition of MEPAS against *E. coli* and *S. aureus* was calculated with the following equation (Ishikawa *et al*., 2001).

$$I = \frac{(d2 - d1)}{d1} x\ 100\%$$

Explanation:
I : growth inhibition (%)
d1 : diameter of paper disc (6 mm)
d2 : diameter of clear zone (mm)

Furthermore, it was made the linear regression equation y = a + bx (x as the concentration and y is the probit number), then being determined the IC50 value (Inhibitory Concentration) by probit analysis (Mursyidi, 1985).

## RESULTS AND DISCUSSION

Methanolic extract of kamboja (*P. acuminata*) stembark has dark brown colour and 14.1% of rendemen.

Methanol is used as a solvent because it can dissolve most of the secondary metabolite groups and the most frequently used in the natural product isolation (Darwis, 2000). The TLC profile showed that phytochemical contents of MEPAS were flavonoid (hRf 43), terpenoid (hRf 50), saponin (hRf 51), and alkaloid (hRf 70) (Table 2). A tube test by mixing MEPAS with distilled water and then shaken, the result showed a stable persistent froth that confirm the presence of saponin in MEPAS.

Table 2. The phytochemical contents of MEPAS by TLC method

| hRf | Spots appearance* | | | Compound groups | Reference |
|---|---|---|---|---|---|
| | UV254 | UV366 | Visible | | |
| 70 | - | Blue | Brown | Alkaloid | Wagner et al. (1996) |
| 50 | - | Blue light | Turquoise | Terpenoid | Harborne (1987) |
| 43 | - | Yellow light | Yellow | Flavonoid | Harborne (1987) |
| 51 | - | - | Blue-violet | Saponin | Wagner et al. (1996) |

*after being sprayed with specific reagent.

Methanolic extract of *P. acuminata* stembark showed antibacterial activity against *E. coli* and *S. aureus* at concentrations of 1.000; 2.000; 3.000; and 4.000 ppm based on the inhibition zone (Table 3). The higher concentration of the extract, the higher percentage of growth inhibition which indicated that antibacterial effect of MEPAS was dose-dependent (Figure 1). MEPAS at the concentration ranging between 250 and 1,000 ppm showed inhibitory activity against all tested bacteria, including *E. coli* and *S. aureus* (Gupta *et al.*, 2008).

Based on the probit analysis, the IC50 value of MEPAS against *E. coli* was 2325 ppm and *S. aureus* was 1800 ppm (Figure 1). Antibacterial activity of methanolic extract against *S. aureus* was greater than that of *E. coli*. Gupta et al. (2008) reported that methanolic extract of *P. acuminata* Ait. leaves was more sensitive to Gram positive than Gram negative bacteria. This could be caused by differences in extracts penetration through the bacteria cell wall. Both strains of these bacteria have different cell wall composition.

Table 3. The result of inhibition zone in all samples against *E. coli* and *S. aureus*

| **Samples** | **Diameter of inhibition zone ± SE (mm, n=3)** | |
|---|---|---|
| | ***E. coli*** | ***S. aureus*** |
| Negative control (media) | ND | ND |
| MEPAS 1000 ppm | 7,33 ± 0,33* | 7,67 ± 0,33* |
| MEPAS 2000 ppm | 8,33 ± 0,33* | 9,00 ± 0,00* |
| MEPAS 3000 ppm | 9,33 ± 0,33* | 10,00 ± 0,58* |
| MEPAS 4000 ppm | 10,67 ± 0,33* | 11,67 ± 0,33* |
| Positive control (Cefotaxim) | 19,00 ± 0,00 | 18,00 ± 0,00 |

Note: MEPAS = Methanolic extract of P. acuminata stembark, ND = Not detected, ø paper disc = 6 m
* : significantly difference compared to positive control (LSD test, p<0.05)

*Staphylococcus aureus* is a Gram positive bacteria group that has a simple structure with peptidoglycan layer more than the lipid layer, whereas the cell wall structure of *E. coli* is relatively complex. Cell wall of Gram negative bacteria composed of three layers, that is lipoprotein (outer), lipopolysaccharide (middle), and peptidoglycan (inner) (Hugo and Russell, 1998).

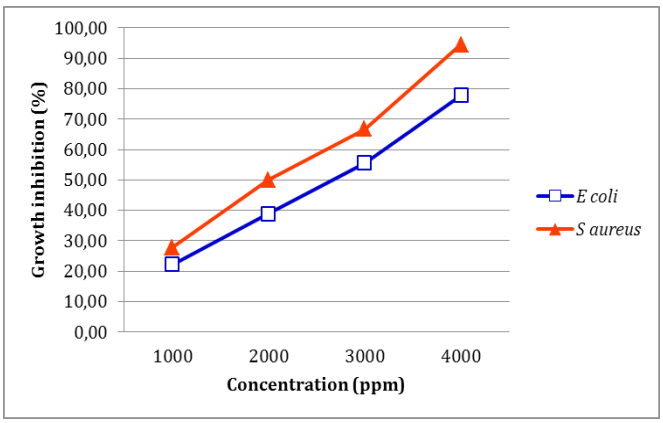


Figure 1. Growth inhibition activity of methanolic extract of P. acuminata stembark against *E. coli* (blue) and *S. aureus* (red)

The IC50 value of MEPAS was used to determine the concentration of the cefotaxime antibiotic as a positive control. Cefotaxime was the third-generation cephalosporin who has a broad spectrum, that is active on a wide range of Gram positive and Gram negative bacterial strains, especially aminoglycosides resistant strain. Mechanism of action of cefotaxime through inhibition of bacteria cell wall synthesis by binding to one or more penicillin binding proteins (PBPs) which will inhibit the transpeptidase synthesis on peptidoglycan layer of bacteria cell wall (Bijie *et al*., 2005). MEPAS able to inhibit the growth of *E. coli* and *S. aureus* bacteria, but its antibacterial activity was lower than cefotaxime. It could be caused by crude extract and there are ballast substances that can inactivate the antimicrobial substance that decreases its effectiveness (Pelczar and Chan, 2005).

Methanolic extract of *P. acuminata* stembark has been shown to contain alkaloid, terpenoid, flavonoid, saponin that is potential as an antibacterial with a different mechanism of action. Alkaloid has antibacterial activity through the inhibition mechanism by interfering with components of bacterial peptidoglycan in the cell so that the cell wall layers are not fully formed and caused the death of these cells (Robinson, 1995). Terpenoid were able to inhibit the transduction of a growth factor into cells so that cell proliferation is hampered due to the formation of the cell surface receptor agonist (Fatoni *et al*., 2005). The mechanism of inhibition of bacteria by saponin is through the incorporation of saponin which is polar group with a phospholipid layer that also is polar so can damage the permeability of bacteria cell membrane (Lay and Hastowo, 1995).

## CONCLUSION

Methanolic extract of *P. acuminata* stembark (MEPAS) contains alkaloid, terpenoid, flavonoid, and saponin. The IC50 value, a parameter of extract potency, of MEPAS against *E. coli* was 2.325 ppm and *S. aureus* was 1.800 ppm.

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