# ANTIBACTERIAL EFFECT OF *MUNTINGIA CALABURA L.* LEAF EXTRACT TO *PSEUDOMONAS AERUGINOSA*

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## ABSTRACT

**Background:** *Pseudomonas aeruginosa* is an opportunistic pathogen which can cause infection in every part of the human body lacking normal defense. This bacteria is one of the leading cause of nosocomial infection in Indonesia. Treating infection caused by *Pseudomonas aeruginosa* is now more challenging due to its resistance to many classes of antibiotics. On the other hand, *Muntingia calabura L*. is a genus of plants in the family *Elaeocarpaceae*, which is spread widely throughout South East Asia, including Indonesia. Some of the researches in the past show antibacterial properties of these plants.
**Aim:** to determine the antibacterial effect of *Muntingia calabura L.* Leaf extract to *Pseudomonas aeruginosa*.
**Method:** This research is an experimental study using *in vitro* technique and non-equivalent control group design. The method used in this study was microdilution in 96-well microplate and concentrations of *Muntinga calabura L*. Leaf extract used in this study were 50-800mg/mL. Minimum Inhibitory Concentration (MIC) was measured by a spectrophotometer, while Minimum Bactericidal Concentration (MBC) was observed by direct streaking to the agar plate.
**Result:** MIC value was found at the concentration range of 400-800 mg/mL, while MBC was found at concentration 800 mg/mL.
**Conclusion:** There is a potential bactericidal effect of *Muntingia calabura L.* To *Pseudomonas aeruginosa*.

**Keywords:** *Muntingia calabura L*., antibacterial, *Pseudomonas aeruginosa*.

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## INTRODUCTION

*Pseudomonas aeruginosa* is a bacterium that causes many nosocomial infections. The prevalence surveys conducted by the World Health Organization (WHO) in 55 hospitals from 14 countries show an average of 8.7% of hospital patients have nosocomial infections[1]. The highest prevalence of nosocomial infections is in the Middle East (11.8%) and Southeast Asia (10%)[2]. Moreover, the prevalence data from 10 educational public hospitals in Indonesia reported that the incidence of nosocomial infections was 6-16% with an average of 9.8%[3]. Based on the research on October to December 2011 at the Midwifery Inpatient Hospital Abdul Muluk Bandar Lampung obtained the bacteria that cause infections, namely *Pseudomonas sp.* (25%), Escherichia coli (19.44%), *Klebsiella sp.* (16.67%), *Staphylococcus epidermidis* (13.89%), *Staphylococcus aureus* (8.32%), and *Enterobacter sp*. (5.56%)[4].

*Pseudomonas aeruginosa* becomes more difficult because of the resistance to various antibiotic choices. The resistance of various types of antibiotics has increased due to the widespread and irrational use of antibiotics. Multi-Drug Resistant (MDR) of *Pseudomonas aeruginosa* is resistant to at least three classes of antibiotics namely β-lactam, carbapenem, aminoglycoside, and fluoroquinolone[5,6]. The research was conducted at Arifin Achmad Hospital in Indonesia throughout 2015 reported on the prevalence of MDR of *Pseudomonas aeruginosa* is 45.5%[7].

*Muntigia calabura L.* or kersen is a species of fruit plant that belongs to the family *Elaeocarpaceae* and is easily found in Southeast Asia including Indonesia. Furthermore, the phytochemical analysis results on how kersen leaf's extracts (*Muntingia calabua L.*) contain flavonoids, saponins, and tannins that contain substances that have antibacterial activity[8, 9]. This study discusses the antibacterial effect of the leaf's extract (*Muntingia calabua L.*) on *Psedumonas aeruginosa*.

## METHOD

This study used an in vitro experimental study with an unequal control group design. The study was conducted in three places, namely the Central of Health Laboratory on Surabaya, the Research Laboratory of the Faculty of Pharmacy in Widya Mandala Catholic University on Surabaya, and the Microbiology Laboratory of the Faculty of Medicine in Widya Mandala Catholic University on Surabaya. The sampling technique in this study was purposive

sampling in one *Pseudomonas aeruginosa* colony. The sample used was the bacterium *Pseudomonas aeruginosa* ATCC 27853 obtained from the Central of Health Laboratory on Surabaya.

Kersen leaf's extract (*Muntingia calabura L.*) is made in Materia Medica, Batu, East Java by maceration method using 95% ethanol solvent which is soaked for 24 hours, then evaporated using a rotary evaporator.

This study used 5 concentrations of kersen leaf extract (Muntingia calabura L.), which are 50 mg/mL, 100 mg/mL, 200 mg/mL, 400 mg/mL, and 800 mg/mL. Minimal Inhibitory Concentration (MIC) value was obtained by the microdilution method at 96 wells, then measured the optical density (OD) value by using a spectrophotometer. The MBC value was determined by streaking directly to the agar plate. Microdilution test was carried out by inserting 100 µL of Mueller Hinton Broth (MHB) media, 100% of kersen leaf (*Muntingia calabura L.*) extract and diluted in a row. After that, a solution containing *Pseudomonas aeruginosa* was added with 0.5 McFarland as much as 100 µL on the microplate and incubated in an incubator at 370C for 24 hours, then measured the absorbance / OD values with a spectrophotometer. The MBC test was done by etching incubation results from micro plates on Mueller Hinton, then incubated at 370C for 24 hours.

**RESULT**

Based on the graph, a 400 mg / mL concentration is needed for bacterial growth of 90.7% and at a concentration of 800 mg/mL is needed for bacterial growth of 100%. The conclusion that can be drawn from the graph is the MIC is estimated in the concentration range of 400-800 mg/mL.

*Figure 1. Graph Obstacles' Percentage*

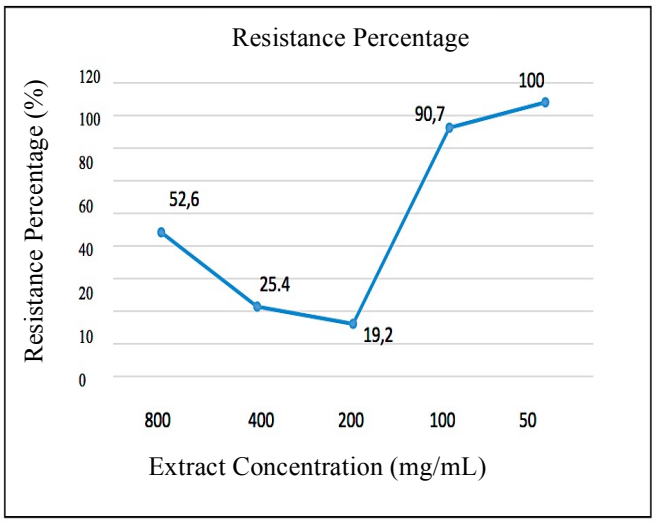
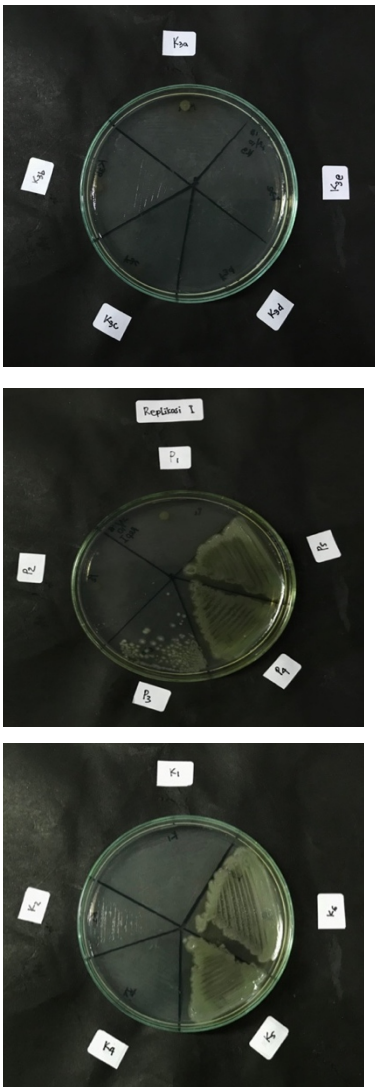


*Figure 2. The results of streaking agar*



## DISCUSSION

### 6.1 Kersen Leaf's Characteristics (*Muntingia calabura L.*)

This research uses extracts of kersen leaf (*Muntingia calabura L.*) obtained from Materia Medika, this extract was made by the maceration method and the solvent used was 95% ethanol. According to the research of William Patrick Cruiz Buhian et al., The results of the phytochemical analysis of ethanol extracts from leaves and stems of kersen (Muntingia calabura L.). The selection was based on the nature of the combined polarity that wants to withdraw.

This research tries to obtain secondary metabolite compounds, namely saponins, tannins and flavonoids which are discussed in the antibacterial process. These compounds are polar which means the solvent used is polar as well so this research uses 95% ethanol solvent. The research conducted by Z.A. Zakaria and colleagues, tested the antibacterial effect of kersen leaf extract (*Muntingia calabura L.*) in ethanol, methanol and chloroform solvents on various types of bacteria. The results showed that ethanol extract showed the highest antibacterial activity against *Corynebacterium diphtheria*, *Proteus vulgaris*, and *Staphylococcus epidermidis* compared to methanol and chloroform extracts[10].

### 6.2 Bacterium's Characteristics

The bacterium used in this study was *Pseudomonas aeruginosa* ATCC 27853 from the Central of Health Laboratory on Surabaya. Therefore, a series of biochemical tests and Gram staining were carried out to identify Pseudomonas aeruginosa and to ensure for no other bacterial contamination. The results obtained in Gram staining are red gram-negative of rod bacteria. Biochemical test results obtained negative

glucose test results, KIA alkali/alkali test, positive Simon Citrate test, indole test, MR, negative VP, positive oxidase test, and positive motility test. The results of bacterial culture showed that the colonies were round, flat, and produced a greenish color. All the results prove that the bacterium grows is *Pseudomonas aeruginosa* without contamination.

**6.3 The Discussion of MIC Test**

The MIC test results in the form of a graph of the percentage of obstacles produced by the spectrophotometer showed that the MIC of kersen leaf extract (Muntingia calabura L.) against *Pseudomonas aeruginosa* lies in the concentration range of 400-800mg/mL. This is indicated by the percentage inhibition concentration of 400mg/mL of 90.7% and at a concentration of 800mg/mL of 100%. This result suits the MIC criteria because bacterial growth inhibition is more than 90%[11].

According to the research conducted by Akhmad Yusuf Sulaiman and colleagues, an antibacterial effect of kersen leaf extract was tested against Streptococcus viridans. The antibacterial test was carried out using the disk diffusion method with a concentration of 12.5%, 25%, 50%, and 75% from the 30g extract of 100% concentration. The results showed that the kersen leaf extract at a concentration of 75% had the greatest ability to inhibit the growth of Streptococcus viridans. This study also concluded that the greater the extract, the greater the antibacterial properties.

**6.4 The Discussion of MBC Test**

The results of MBC test of kersen leaf extract (*Muntingia calabura L.*) against Pseudomonas aeruginosa is a concentration of 800mg / mL. This is evident from the picture in figure 2 the concentration of 800mg / mL did not show bacterial growth.

According to prior research by Dellyna Feronica Manik and colleagues, the results of MBC test of kersen leaf extract (*Muntingia calabura L.*) against Staphylococcus aureus were found at a concentration of 1.25 mg/mL[12]. The discrepancy with MBC values found in previous studies and current research can be done because the bacteria used in previous studies are gram-positive bacteria while the bacteria used in this study are gram-negative. Gram-negative bacteria have components of the outer membrane layer of lipopolysaccharide which play an important role in the function of the barrier. The outer membrane of Gram-negative bacterial cells works as a protective barrier that enters toxic molecules into the cell and in addition to the layer that stabilizes the cell so it is more difficult to be penetrated by

antibacterial compounds[13]. In addition, preliminary research using different extraction methods and solvents can produce active levels contained in the extract and affect the results obtained.

## CONCLUSION

The MIC value of kersen leaf extract (*Muntingia calabura L.*) against *Pseudomonas aeruginosa* lies in the concentration range of 400-800 mg/mL and MBC value of kersen leaf extract (*Muntingia calabura L.*) against *Pseudomonas aeruginosa* lies in a concentration of 800 mg / mL. All in all, kersen leaf extract (*Muntingia calabura L.*) has bactericidal potential against *Pseudomonas aeruginosa*.

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## REFERENCES

1. Aditi, S. Pengetahuan Dan Sikap Mahasiswa Akper Terhadap Pencegahan Infeksi Nosokomial Flebitis. *Students E-Journals* 1–14 (2014).
2. Preface, I. Nosocomial Infections Surveillance System. 42–48 (2008).
3. Kurniawati, A. F., Satyabakti, P. & Arbianti, N. Perbedaan Risiko Multidrug Resistance Organism (MDROS) Menurut Faktor Risiko dan Kepatuhan Hand Hygiene. *J. Berk. Epidemiol.* **3,** 277–289 (2015).
4. Andy Samuel ; Prof. DR. dr. Efrida Warganegara, M.Kes, S. Pola Resistensi Antibiotik terhadap Isolat Bakteri Aerob Penyebab Infeksi Luka Operasi di Ruang Rawat Inap Bagian Bedah dan Kebidanan Rsud Dr. H. Abdul Moeloek Bandar Lampung. 21–35 (2013).
5. Japoni, A., Farshad, S. & Alborzi, a. Pseudomonas aeruginosa: Burn infection, treatment and antibacterial resistance. *Iran. Red Crescent Med. J.* **11,** 244–253 (2009).
6. Brooks, G. F., Jawetz, E., Melnick, J. L., & A. *Jawetz, Melnick & Adelberg's medical microbiology (26th edition.)*. (London: McGraw-Hill Medical, 2013).
7. Dewi Anggraini, Utari Gusti Yulindra, Maya Savira, Fauzia Andrini Djojosugito, N. H. Prevalensi dan Pola Sensitivitas Antimikrob Multidrug Resistant Pseudomonas aeruginosa di RSUD Arifin Achmad Prevalence and Antimicrobial Susceptibility Profile of Multidrug Resistant Pseudomonas aeruginosa in Arifin Achmad General Hospital. **50,** 6–12 (2018).
8. K., L. T. *Edible Medicinal And Non Medicinal Plants: Volume 3, Fruits*. (Springer Dordectht Heidelberg London New York, 2013). doi:10.1007/978-94-007-2534-8
9. Salleh, M. Z. *et al.* Muntingia calabura : A review of its traditional uses , chemical properties , and pharmacological observations Muntingia calabura : A review of its traditional uses , chemical properties , and pharmacological observations. **0209,** (2014).
10. Z.A.Zakaria, C.A. Fatimah, A.M. Mat

Jais, H. Zaiton, E.F.P. Henie, M.R. Sulaiman, M.N Somchit, M. T. and D. K. The in vitro Antibacterial Activity of Muntingia calabura Extracts. *Int. J. Pharmacol.* (2006).

11. Amyes Sebastian, Rex S Miles, Christopher J Thompson, G. T. *Antimicrobial Chemotherapy Pocketbook*. (1996).
12. Manik, D. F., T, H. & H, A. Dengan Aktivitas Antibakteri Ekstrak Etanol Dan Fraksi-Fraksi Daun Kersen. 1–11 (2014).
13. Ratnasari, M. Uji Aktivitas Antibakteri Ekstrak Daun Kersen (Muntingia calabura L.) Dalam Bentuk Sediaan Gel Terhadap Staphylococcus aureus dan Escherichia coli. (2017).