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Research Article

Comparison of Methanolic Extract of Piper Betle to Amikacin against the Growth of Pseudomonas aeruginosa

Efek Ekstrak Metanolik Daun Sirih Hijau Memiliki Efek yang Lebih Baik dalam Menghambat Pertumbuhan Koloni *Pseudomonas aeruginosa* Dibandingkan Antibiotik Amikacin

Azalia Ayu Rysaputri[1], Hery Susilo[2], Dwi Yuni Nur Hidayati[3], Irene Ratridewi[4]

[1]Medical Study Program Faculty of Medicine Universitas Brawijaya Malang

[2]Department of Oncological Surgery Faculty of Medicine Universitas Brawijaya Malang

[3]Department of Microbiology Faculty of Medicine Universitas Brawijaya Malang

[4]Department of Pediatric Faculty of Medicine Universitas Brawijaya Malang

ABSTRACT

Pseudomonas aeruginosa is one of the most common pathogens that cause Healthcare-Associated Infections (HAIs). A previous study stated that Piper betle L. extract has antibacterial activity against certain bacteria, including Pseudomonas aeruginosa. This study has the objective of comparing antibacterial activity of the methanolic extract of Piper betle L. and amikacin on the growth of Pseudomonas aeruginosa. This study used the tube dilution method with a sample of Pseudomonas aeruginosa from Microbiology Laboratory Dr. Saiful Anwar General Hospital, Malang. The results of this study showed that the value of MIC and MBC from the methanolic extract of the Piper betle L. treatment was 4800µg/mL. Meanwhile, the amikacin treatment resulted in 0.4µg/mL for MIC and 0.45µg/mL for MBC. From linear regression, it was found that the methanolic extract of Piper betle L. had a β-coefficient value closer to -1 compared to amikacin. Therefore, it can be concluded that the methanolic extract of Piper betle L. has a greater inhibiting effect on the growth of Pseudomonas aeruginosa.

Keywords: *Amikacin, antibacterial, Piper betle L., Pseudomonas aeruginosa*

ABSTRAK

Pseudomonas aeruginosa adalah salah satu bakteri patogen tersering penyebab infeksi rumah sakit. Penelitian terdahulu menyebutkan bahwa ekstrak daun sirih hijau (*Piper betle*) memiliki efek antibakteri pada beberapa bakteri termasuk *Pseudomonas aeruginosa*. Tujuan dari penelitian ini adalah untuk membandingkan efek dari ekstrak metanolik daun sirih hijau dan amikacin terhadap pertumbuhan koloni *Pseudomonas aeruginosa*. Penelitian ini menggunakan metode dilusi tabung dengan sampel *Pseudomonas aeruginosa* dari Laboratorium Mikrobiologi RSUD Dr. Saiful Anwar Malang. Hasil penelitian ini menunjukkan bahwa nilai MIC dan MBC yaitu 4800µg/mL untuk perlakuan dengan ekstrak metanolik daun sirih hijau. Sementara itu, perlakuan dengan amikacin mendapatkan nilai MIC 0,4µg/mL dan MBC 0,45µg/mL. Dari hasil regresi linier, ditemukan bahwa ekstrak metanolik daun sirih hijau memiliki nilai β-coefficient lebih mendekati -1 dibandingkan dengan β-coefficient amikacin. Hal tersebut menunjukkan bahwa ekstrak metanolik daun sirih hijau memiliki efek yang lebih baik dalam menghambat pertumbuhan koloni Pseudomonas aeruginosa.

Kata Kunci: Amikacin, antibakteri, daun sirih hijau, *Pseudomonas aeruginosa*

Correspondence: Irene Ratridewi. Department of Pediatric Faculty of Medicine Universitas Brawijaya Malang, Jl. Veteran Malang Malang Tel. +6282244126339 Email: irene24.fk@ub.ac.id

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INTRODUCTION

Healthcare-Associated Infections (HAIs) are a type of infection caused by infecting pathogens from hospitals or healthcare, appearing after 48 hours of care in a hospital setting or 30 days after receiving healthcare (1). A U.S. National Healthcare Safety survey in 2007 stated that *Pseudomonas aeruginosa* was the sixth-most common cause of HAIs such as ventilator-associated pneumonia (VAP) and bloodstream infections correlated to venous catheters (2). Research from the European Antimicrobial Resistance Surveillance System found that 18% of the *Pseudomonas aeruginosa* that had been isolated developed antimicrobial resistance (3).

Amikacin is one of the drugs of choice to treat *Pseudomonas aeruginosa* infections. It is categorized as an aminoglycoside, and it has a bactericidal characteristic (4). The bactericidal effect of amikacin is derived from the binding of the drug to the bacterial 30S ribosomal subunit, which further decreases protein synthesis (5). Antimicrobial side effects should also be considered in commencing therapy against bacterial infections. The most prominent adverse effect of aminoglycosides are nephrotoxicity and ototoxicity (6). The occurrence of antimicrobial resistance from *Pseudomonas aeruginosa* and the risk of drug side effects due to antimicrobial agent usage necessitates the study to look for alternatives, such as herbal agents. One of the renowned herbal agents with antimicrobial activity is *Piper betle* (7). Previous research stated that the methanolic extract of *Piper betle* showed antibacterial activity to some pathogens found in fish, such as *Aeromonas hydrophila, Escherichia coli, Pseudomonas aeruginosa,* and *Vibrio alginolyticus* (7). The methanolic extract of *Piper betle* results in the extraction of phytochemical compounds such as alkaloids, phenols, flavonoids, tannins, saponins, terpenoids, and steroids (7). Steroids are also known to be associated with the lipid membrane, and they cause liposome. Tannins can cause leakage of proteins and certain enzymes from cells (8). However, the difference in effects of antibacterial activity from the methanolic extract of *Piper betle* and amikacin as treatments for *Pseudomonas aeruginosa* infections is still not known. Thus, a comparison study needs to be conducted.

METHOD

This is an *in vitro* experimental study that was conducted in the Microbiology Laboratory in the period from January 2019 to April 2020. A sample of *Pseudomonas aeruginosa* was taken from the hospital. The objective of this study is to compare the efficacy of *Piper betle* and amikacin in stopping the growth of *Pseudomonas aeruginosa* colonies. The independent variable in this study is the different dosages of *Piper betle* extract and amikacin given in this experiment. The growth of *Pseudomonas aeruginosa* colonies is the dependent variable.

Identification of *Pseudomonas aeruginosa* was performed through the Gram test, the oxidase test, and evaluation of pigment production. Antimicrobial activity of *Piper betle* was tested with the tube dilution method and bacterial cultures on Mueller-Hinton agar plates. Subsequently, the growth of *Pseudomonas aeruginosa* colonies was confirmed, and the data were analyzed.

Data analysis was performed with Statistical Package for the Social Sciences (SPSS) version 25. The Shapiro-Wilk normality test was performed to find out the data distribution. Homogeneity was analyzed with Levene's statistical test. If the data distribution was normal and homogenous, One-Way ANOVA was performed to test the significance of *Piper betle* and amikacin against *Pseudomonas aeruginosa* colony growth. Subsequently, the linear regression test was performed to find out the correlation of *Piper betle* and amikacin dosage to colony growth.

RESULTS

The Gram test showed gram-negative rod-shaped bacteria. There were deep purple discolorations in the oxidase test, which meant a positive result. Pigment evaluation revealed a greenish color, marking pyocyanin pigment production in the Mueller-Hinton agar plates, and this is the hallmark of a *Pseudomonas aeruginosa* colony.

The results of the Shapiro-Wilk normality test showed that the data was normally distributed with $p>0.05$. The results of normality testing after exposure to *Piper betle* extract and amikacin can be seen in Table 1 and Table 2.

Table 1. Normality test results of *Piper betle* extract and growth of *Pseudomonas aeruginosa* colonies

Extract concentration (μg/mL)	p-value	α	Results
0	0.392	0.05	Normal
600	0.882	0.05	Normal
1200	0.806	0.05	Normal
1800	0.381	0.05	Normal
2400	0.311	0.05	Normal
3000	0.087	0.05	Normal
3600	0.257	0.05	Normal
4200	0.368	0.05	Normal

Table 2. Normality test results of amikacin and growth of *Pseudomonas aeruginosa* colonies

Amikacin concentration (μg/mL)	p-value	α	Results
0.00	0.470	0.05	Normal
0.05	0.518	0.05	Normal
0.10	0.090	0.05	Normal
0.15	0.245	0.05	Normal
0.20	0.726	0.05	Normal
0.25	0.633	0.05	Normal
0.30	0.537	0.05	Normal
0.35	0.444	0.05	Normal
0.40	0.848		

Tube dilution test was conducted with 4800μg/mL of *Piper betle* extract to define the minimum inhibitory concentration (MIC). Meanwhile, the MIC for amikacin exposure was established at 0.4μg/mL. Bacterial cultures were created to define the value of minimum bacterial concentration (MBC). The MBC from *Piper betle* extract was 4800μg/mL, whilst the MBC from amikacin exposure was 0.45μg/mL. The total number of bacterial colonies from culture results can be seen in Table 3 and Table 4.

Table 3. Number of *Pseudomonas aeruginosa* colonies after exposure to *Piper betle* extract

Concentration of *Piper betle* (μg/mL)	Mean of bacterial colony (10^{10} CFU/mL)	Mean ± SD
0	43.5	43.5±6.34
600	27.1	27.1±5.51
1200	25.2	25.2±7.95
1800	1.32	1.32±0.82
2400	1.24	1.24±0.75
3000	0.54	0.54±0.33
3600	0.35	0.35±0.11
4200	0.36	0.36±0.15
4800	0	0
5400	0	0

Table 4. Number of *Pseudomonas aeruginosa* colonies after exposure to Amikacin

Amikacin dosage (μg/mL)	Mean of bacterial colony (10^5 CFU/mL)	Mean ± SD
0	5,530,000	5,530,000±849,000
0.05	2,580,000	2,580,000±1,020,000
0.10	1,320,000	1,320,000±919,000
0.15	2,510	2,510±345
0.20	2,390	2,390±381
0.25	212	212±26.3
0.30	2.37	2.37±0.27
0.35	1.71	1.71±0.06
0.40	0.55	0.55±0.10
0.45	0	0

Homogeneity test was performed with Levene's statistical test, and the results showed homogenous data with p>0.05 (p=0.089). Parametric test by One-Way ANOVA was performed to determine the significance of *Piper betle* extract and amikacin toward the growth of *Pseudomonas aeruginosa* colonies. The result was p=0.00 with p<0.05. The result showed that there was a significant effect from exposure of *Piper betle* extract and amikacin on *Pseudomonas aeruginosa* colonies. Subsequently, the linear regression test was performed to find the correlation between *Piper betle* extract and amikacin and the growth of *Pseudomonas aeruginosa* colonies. The resulting β-coefficient for the *Piper betle* extract was -0.853, while the β-coefficient for amikacin was -0.754. Both of the results showed negative correlation.

DISCUSSION

The sample for this study was obtained from the Microbiology Laboratory of the hospital. Bacterial identification was made through the Gram test, the oxidase test, and pigment production evaluation. Gram staining revealed gram-negative rod bacteria. In gram-negative bacteria, the peptidoglycan layer is thinner compared to gram-positive bacteria. There was also the lipopolysaccharide component, which can produce endotoxins when the bacteria undergo the lysis process (8). The oxidase test results were positive, marked by a bluish color on the oxidase stripe. The cytochrome oxidase enzyme that resulted from the process is known to catalyze oxidation of cytochrome-C and reduce oxygen to form water (9). Final identification of the bacteria was made through pigment evaluation. Culture results on Mueller-Hinton agar plates showed changes in color, which was greenish. This marked the production of pyocyanine. Pyocyanin is known for its role in decreasing immunoglobulin secretion and hindering proliferation of lymphocytes, and thus being able to decrease immune response that protects the body from *Pseudomonas aeruginosa* infections (10).

A previous study tested the antimicrobial effect of *Piper betle* extract on *Pseudomonas aeruginosa* with the microtiter broth dilution method and showed a MIC of 150mg/mL. The study also tested the inhibition zone of 7.2 mg *Piper betle* extract using filter paper of 6 mm size. The results showed an inhibition zone of 10 mm (11). The study confirmed the antibacterial activity of *Piper betle* extract against *Pseudomonas aeruginosa*.

In this study, *Pseudomonas aeruginosa* was tested with exposure to amikacin using the tube dilution method. The results showed a MIC value of 0.4 μg/mL and an MBC value of 0.45μg/mL. The antibacterial effect of amikacin is derived from its ability to disrupt protein synthesis by binding to the bacterial 30s ribosomal subunit. This bind can impair ribosomal functions and inducing errors in the translation process (12). Another study compared the efficacy of amikacin with aztreonam, cefepime, ceftazidime, ceftolozane, ceftriaxone, ciprofloxacin, imipenem, meropenem, piperacillin, and tobramycin against isolates of multi-drug resistant *Pseudomonas aeruginosa*, conducted *in vitro*. The study involved sample collection from 50 hospitals in the U.S., consisting of 814 samples from blood-borne and air-borne infections in total. It showed that amikacin is the most potent antibiotic against *Pseudomonas aeruginosa* infection with a MIC ≤16μg/mL (13). However, there is a risk of antibiotic resistance.

The linear regression test that was performed in this study showed negative β-coefficients, which confirm the negative correlation between the dependent and independent variables. From the results, it could be confirmed that increased doses of amikacin did not significantly affect the growth of *Pseudomonas aeruginosa* colonies. The results showed that the antibacterial effect of *Piper betle* extract is superior compared to amikacin against *Pseudomonas aeruginosa* infections, with a β-coefficient value of nearly -1.

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