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# Antimicrobial Activity of *Pecut Kuda* Leaf Extract *(Stachytarpheta jamaicensis (L. Vahl)* against *Mycobacterium smegmatis*

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**Abstract:** *Mycobacterium smegmatis* infrequently causes infection, but it is easy to be pathogenic in immunosuppressed patients. Many reported that *M. smegmatis* resistance to several antibiotics became an impetus for searching for new antimicrobials. Therefore, this study aims to prove the effect of *Pecut Kuda* leaf extract (*Stachytarpheta jamaicensis (L.) Vahl*) on the growth of *M. smegmatis mc2 155*. This research is an experimental study with a post-test control group design. The susceptibility test was carried out using the two-fold microdilution method and resazurin staining. The concentration of *Pecut Kuda* leaf ethanol extract was prepared in the concentration range of 10000.0 – 625.0 µg/ml. Phytochemical analysis of the content of saponins, tannins, flavonoids, and alkaloids was also carried out on *Pecut Kuda* leaf ethanol extract. *Pecut Kuda* leaf ethanol extract can inhibit the growth of *M. smegmatis* with a minimum inhibitory concentration (MIC) of 5000 µg/ml (very weak activity) because, at the highest concentration of 10000 µg/ml, *M. smegmatis* still cannot be killed. Furthermore, Pecut Kuda leaf ethanol extract contains saponins, tannins, flavonoids, and alkaloids which are known to have antibacterial activity. However, further evaluation is needed to maximize the antibacterial activity of Pecut Kuda leaf extract, for example, by fractionating the extract.

**Keywords:** antibacterial agent; *Mycobacterium smegmatis*; phytochemical analysis; *Stachytarpheta jamaicensis (L.) Vahl*

## INTRODUCTION

*Mycobacterium smegmatis* is an acid-fast bacillus classified as a rapidly growing (RGM) Non-Tuberculosis Mycobacteria.[1] These bacteria infrequently cause infection but can easily infect immunosuppressed patients. Infections due to *M. smegmatis* are non-tuberculous infections and have broad clinical manifestations such as hypersensitivity, pneumonitis, asthma and bronchitis, skin infection, wounds, and glands.[2] The prevalence of non-tuberculous infections in Indonesia has increased since 2000. The incidence of these infections in Soetomo Hospital from 2014 to 2015 reached 5.78%. These infections increased in 2017, reaching 25% of patients.[3] The most common clinical manifestation caused by *M. smegmatis* is pulmonary infection.[4]

Treatment of non-tuberculous infection consists of prophylaxis and medication.[5] In terms of drug therapy, antibiotics such as erythromycin, clarithromycin, rifampin, rifabutin, and ethambutol can be used to treat this infection.[6] However, there have been many reports of resistance of *M. smegmatis* to several antibiotics such as ampicillin, amoxicillin, linezolid, erythromycin, streptomycin, and tetracycline.[7] It has become an impetus for searching for new antimicrobials, especially those from natural products. One source of natural products that can be explored is medicinal plants. *Pecut Kuda* leaves (*Stachytarpheta jamaicensis (L.) Vahl*) is a plant that has the potential as an antibacterial. The antibacterial activity of *Pecut Kuda* extract has been tested against several bacteria. *Pecut Kuda* leaf ethanol extract with a concentration of 12.5% is known to inhibit the growth of *Escherichia coli*. In addition, Pecut Kuda leaf extract with a concentration of

80% can also inhibit the growth of Gram-positive bacteria *Streptococcus pyogenes*, with an average inhibition zone diameter of 29.2 mm. The extract is known to contain compounds that have the potential as antimicrobials, including flavonoids, tannins, saponins, and alkaloids.[8,9]

Research on the effect of Pecut Kuda leaf (*S. jamaicensis (L.) Vahl*) extract on *M. smegmatis* has not been carried out until now. Therefore, this research was conducted to identify the potential of Pecut Kuda leaf extract to inhibit the growth of *M. smegmatis*. In addition, phytochemical analysis was carried out to qualitatively identify the content of chemical compounds in the *Pecut Kuda* leaves.

## MATERIAL AND METHOD

The test bacteria used in this study was *M. smegmatis* mc2 155 (ATCC 700084), obtained from the Microbiology Laboratory, Faculty of Medicine, Universitas Muhammadiyah Semarang. This research has been approved by the Health Research Ethics Commission of the Medical Faculty of Universitas Muhammadiyah Semarang under the number 160/EC/FK/2021.

### Secondary Metabolites Extraction of *Pecut Kuda* Leaves (*Stachytarpheta jamaicensis (L.) Vahl*)

First, *Pecut Kuda* leaves were dried under the sun with a covered cloth for approximately three days. Furthermore, 1 kg of *Pecut Kuda* leaves was prepared in the form of dried simplicia. The simplicia powder of *Pecut Kuda* leaves was soaked with 96% ethanol solvent for five days and stirred occasionally. After five days, the final result of the maceration process of dried *Pecut Kuda* Simplicia was filtered, and the supernatant was concentrated using a rotary evaporator (Ika® RV 10) at a temperature of 70 °C and a speed of 100 rpm.

### Phytochemical Analysis of *Pecut Kuda* (*Stachytarpheta jamaicensis (L.) Vahl*) Leaves Ethanol Extract

Phytochemical analysis was carried out to identify the content of chemical compounds in *Pecut Kuda* leaf ethanol extract. The test was conducted to qualitatively identify the presence of tannins, saponins, flavonoids, and alkaloids. In the tannin test, a total of 0.5 g of *Pecut Kuda* leaf extract was put into a test tube. 20 ml of distilled water was then added to the test tube and heated to boiling. The boiling solution was filtered and added with 1% FeCl3. The positive presence of tannins is indicated by a change in color to green or blue-black ink.[10-12]

Furthermore, in the saponins test, 100 mg of extract was mixed with 5 ml of water and shaken vigorously. If the solution forms a stable foam, it indicates the presence of saponins. To determine the presence of flavonoids, 0.5 ml of the extract was added with 5 ml of dilute ammonia and 2 ml of concentrated sulfuric acid. Flavonoid compounds can be seen from the appearance of a maroon color in the solution. Meanwhile, to identify alkaloid compounds, as much as 1 ml of *Pecut Kuda* leaf extract was added with 1% HCl in a test tube. The test tube was heated slowly, and the solution was filtered. 3 drops of Mayer and Wagner reagent can be added to the filtered supernatant. If a precipitate is formed in the solution, it is confirmed that the presence of alkaloids in the *Pecut Kuda* leaf extract is confirmed.[10-12]

### Antibacterial Activity Test of *Pecut Kuda* Leaves (*Stachytarpheta jamaicensis (L.) Vahl*) Ethanol Extract against *Mycobacterium smegmatis*

To determine the minimum inhibitor concentration (MIC) of *Pecut Kuda* leaf ethanol extract, the susceptibility test was carried out using the two-fold microdilution method. The concentration of *Pecut Kuda* leaf extract was prepared in the concentration range of 10000.0 – 625.0 µg/ml. The concentration of the *M. smegmatis* suspension in each well of the microplate was $1 \times 10^5$ CFU/mL. Furthermore, to determine the activity of *Pecut Kuda* leaf extract in inhibiting *M. smegmatis*, the microplate was incubated at 37 °C for 24 hours. After incubation, 0.0025% resazurin (Sigma Aldrich), as much as 20 µl, was added to each test well. The color change of resazurin from blue to pink became a parameter for the growth of *M. smegmatis*.[13]

As an antibiotic control, Rifampicin (Phapros Tbk) was used to determine the resistance pattern of *M. smegmatis*. The susceptibility test method of rifampicin against *M. smegmatis* was done using the same method to test the activity of *Pecut Kuda* leaf extract, but the concentration of rifampicin was prepared in the range of 2.0 – 0.0625 µg/ml. After obtaining the MIC value, the research continued to determine the minimum bactericidal concentration (MBC). The test suspension in each well of various concentrations of

*Pecut Kuda* leaf extract/rifampicin was taken as much as 10 µl. Furthermore, the suspension was grown in Mueller Hinton Agar (Merck Millipore) using the streak method. The Petri dishes were then incubated at 37 °C for 24 hours. The MBC value was determined from the smallest *Pecut Kuda* leaf extract/rifampicin concentration that caused the absence of *M. smegmatis* growth.

**RESULT**

The MIC value was determined from the smallest concentration of the microplate wells that did not change their color to pink. Based on the result of the susceptibility test, the MIC value of *Pecut Kuda* leaf (*Stachytarpheta jamaicensis (L.) Vahl*) extract in inhibiting the growth of *M. smegmatis* was 5000 µg/ml (Figure 1).

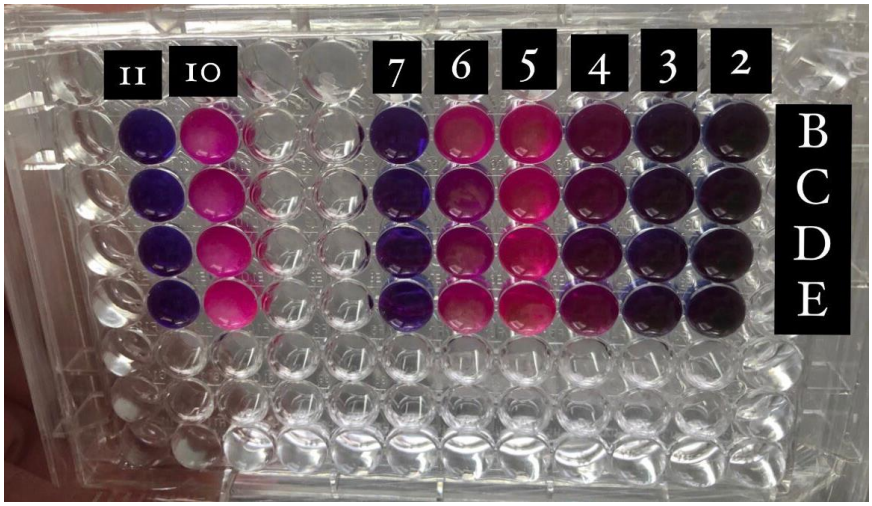


**Figure 1. The result of the minimum inhibitory concentration of *Pecut Kuda* Leaf (*Stachytarpheta jamaicensis (L.) Vahl*) extract against *M. smegmatis* carried out with two-fold microdilution and resazurin staining**

Note: B-E2 until B-E6 is a well containing *Pecut Kuda* leaf extract with a concentration range of 10000 - 625 $\mu g/ml$; B-E7 are well of *Pecut Kuda* leaf extract sterility control; B-E10 are *M. smegmatis* growth control; and B-E11 are media sterility control.

Furthermore, for the results of the susceptibility testing of rifampicin against *M. smegmatis*, the MIC value was 0.0625 µg/mL (Figure 2). Based on the MIC value, it can be concluded that *M. smegmatis* is still sensitive to rifampicin.[14]

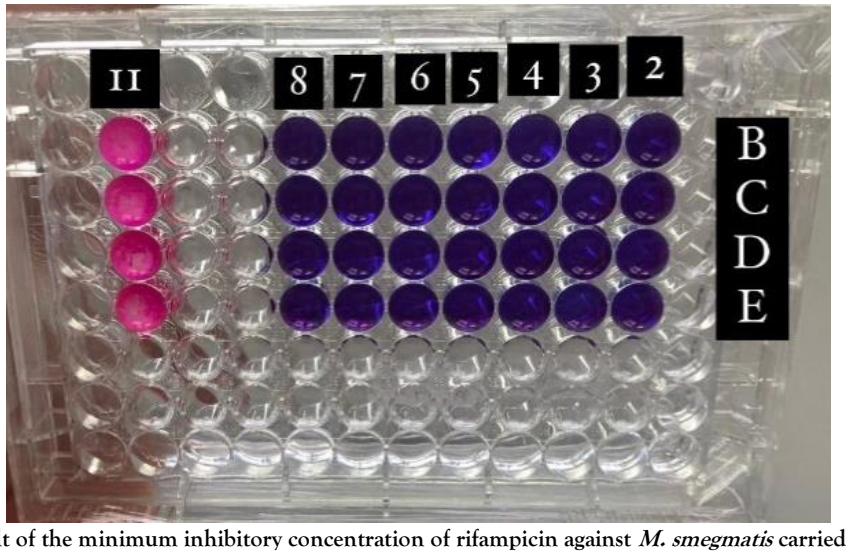


**Figure 2. The result of the minimum inhibitory concentration of rifampicin against *M. smegmatis* carried out with two-fold microdilution and resazurin staining**

Note: B-E2 until B-E7 is a well-containing rifampicin with a concentration range of 2 – 0.0625 µg/ml; B-E8 are well of rifampicin sterility control; B-E11 are *M. smegmatis* growth control.

Both *Pecut Kuda* leaf (*Stachytarpheta jamaicensis (L.) Vahl*) extract and rifampicin were still able to inhibit the growth of *M. smegmatis*. Therefore, the test was continued to determine the MBC value. Based on the results of *M. smegmatis* growth on agar media, it can be concluded that *Pecut Kuda* leaf extract was still unable to kill *M. smegmatis*. Meanwhile, at the smallest concentration used for the test, rifampicin had no *M. smegmatis* growth. Therefore, it can be concluded that the MBC value of rifampicin against *M. smegmatis* is 0.0625 µg/mL.

Because *Pecut Kuda* leaf extract had the potential to be developed as an antibacterial, a phytochemical analysis was carried out to determine the compound content. According to the results of the phytochemical analysis, it can be concluded that the *Pecut Kuda* leaf extract contained saponins, tannins, flavonoids, and alkaloids (Table I).

**Table I. Result of Phytochemical Analysis of Pecut Kuda (Stachytarpheta jamaicensis (L.) Vahl) Leaf Extract**

| Compound | Phytochemical Test Observation | Result |
|---|---|---|
| Saponins | Stable foam | + |
| Tannins | Dark blue | + |
| Flavonoids | Maroon | + |
| Alkaloids | Dissolving precipitation | + |

## DISCUSSION

Determination of the MIC value of *Pecut Kuda* (*Stachytarpheta jamaicensis (L.) Vahl*) leaf extract and rifampicin was carried out using the Resazurin Microplate Assay (REMA) method. This method is known to be utilized for the rapid screening of natural product compounds' antimycobacterial activity compared with the Crystal Violet Decolorization Assay (CVDA) method. In addition, the change in resazurin color from blue to pink due to *M. smegmatis* growth can simplify the interpretation of the test results.[13]

Based on the results of this study, *Pecut Kuda* leaf extract was able to inhibit *M. smegmatis* growth with a MIC value of 5000 µg/ml. However, *Pecut Kuda* leaf extract could still not kill the *M. smegmatis* at the highest test concentration of 10000 µg/mL. There has never been a study reporting the activity of *Pecut Kuda* leaf extract against *M. smegmatis*. However, *Pecut Kuda* leaf extract has been known to have antibacterial activity against other Gram-positive bacteria. *Pecut Kuda* leaf extract was able to inhibit the growth of *S. pyogenes*, and it has been developed into an antiseptic candy dosage form.[9,15] In addition, *Pecut Kuda* leaf extract can inhibit the growth of *Staphylococcus aureus*[16] and several other pathogenic bacteria such as *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*.[17] The ability of *Pecut Kuda* leaves extract to inhibit the growth of *M. smegmatis* can be categorized as very weak compared to its activity against other Gram-negative and positive bacteria. The MIC value of 5000 µg/mL was also very high compared to the MIC value of the control drug, rifampicin. Furthermore, according to another study, an extract can be developed as an antibacterial if the MIC value is less than 100 µg/mL.[18]

*Mycobacterium smegmatis* mc2 155 used in this study was a wild-type isolate, and it was proven that rifampicin could inhibit the growth and kill *M. smegmatis* at a concentration of 0.0625 µg/mL. Although the majority of *M. smegmatis* strains are not susceptible to tuberculosis drugs such as rifampicin, isoniazid, and ethambutol, the result is relevant to other research, which states that *M. smegmatis* strain ATCC 607 was reported to be susceptible to rifampicin with a MIC value of 0.97 - 1.6 µg/mL.[19] *Mycobacterium smegmatis* resistance pattern to rifampicin has been widely reported. The MIC value of rifampicin can reach 20 µg/mL in *M. smegmatis* resistant. *Mycobacterium smegmatis* itself can become resistant to rifampicin due to the mechanism of cellular permeability, enzymatic modification, and efflux system.[20]

Furthermore, according to the results of phytochemical tests, *Pecut Kuda* leaf extract contained saponins, tannins, flavonoids, and alkaloids. All of these compounds are known to have the potential as antibacterial. Flavonoids have antibacterial activity, as most phenols can inhibit protein synthesis and cause protein denaturation in cell walls. This activity is then followed by a disruption in the permeability of the bacterial cell wall.[21] In particular, flavonoids' effect on mycobacteria inhibits mycolic acid synthesis. Molecular docking tests of several flavonoids (quercetin and taxolin) showed their activity to inhibit mycobacterium DNA gyrase, preventing bacterial cells from dividing.[22] The effect of flavonoids on mycobacteria cell walls was also demonstrated by inhibiting the glutamate racemase enzyme, which inhibited peptidoglycan synthesis.[23] Meanwhile, saponins can have antibacterial activity by inhibiting cell membrane function, ultimately changing cell membrane permeability and damaging cell walls.[24] Until now, the mechanism of saponins against mycobacteria has never been reported. Furthermore, Alkaloids have a mechanism to inhibit cell wall

synthesis and interfere with peptidoglycan components, so bacterial cell walls are not formed properly.[25] Alkaloid activity in mycobacterium mainly depends on the ease of transporting substances through the cell membrane. Alkaloids are lipophilic compounds that can easily penetrate the walls of mycolic acid. The berberine derivative is an example of an alkaloid with antimycobacterial activity.[26,27] The last compound, tannin, has antibacterial activity related to its ability to activate microbial cell adhesion, inactivate enzymes, and interfere with protein transport in the inner layer of cells.[28]

When viewed from the mechanism of action of flavonoids, saponins, and alkaloids, *Pecut Kuda* leaf extract has the potential to be bactericidal. However, this study did not isolate compounds, so the type and amount of flavonoids, saponins, and alkaloids contained in *Pecut Kuda* leaf extract are unknown. Therefore, it cannot be ascertained why *Pecut Kuda* leaf extract identified in this study could not kill the *M. smegmatis*. Another possible reason for the lack of bactericidal effect of *Pecut Kuda* leaf extract is that *M. smegmatis* has N-acetylmuramic acid (MurNAc) and N-glycolylmuramic acid (MurNGlyc), which can increase drug efflux.[29] In addition, the cell wall of mycobacteria is different from that of Gram-positive and negative bacteria in general. Mycobacterial cell walls may consist of the mycoyl-arabinnogalactan-PG (mAGP) complex. Peptidoglycan is bound covalently to arabinogalactan, which is esterified with mycolic acid and becomes a lipid barrier in mycobacteria. It is very useful for controlling the cell wall's osmotic pressure and reduces the effectiveness of *Pecut Kuda* leaf extract as an antibacterial.[30] Despite all that, *Pecut Kuda* leaf (*Stachytarpheta jamaicensis (L.) Vahl*) still has the potential to be developed into antibacterial dosage forms. However, prior evaluation is needed to maximize the activity of the *Pecut Kuda* leaf extract, such as by fractionating the extracts to obtain purer active compounds.

## CONCLUSION

*Pecut Kuda* leaf (*Stachytarpheta jamaicensis (L.)* extract could inhibit the growth of *Mycobacterium smegmatis* with minimum inhibitory concentration (MIC) of 5000 µg/ml (very weak activity), and at the highest concentration of 10000 µg/ml, they were still not able to kill the *M. smegmatis*. Furthermore, *Pecut Kuda* leaf extract contained saponins, tannins, flavonoids, and alkaloids which were known to have antibacterial activity.

## CONFLICT OF INTEREST

None of the Conflicts of Interest

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