Antibacterial Potential of *Nicotiana tabacum* L. var Virginia Pyrolysis Extract Against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa*

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Abstract. Tobacco plants are one of the main trade commodities in Indonesia. At present, the main production of tobacco is cigarettes. However, tobacco has active antibacterial compounds, such as phenols, alkaloids, and essential oils. Therefore, tobacco can be used in the health sector. This study was conducted to determine the effectiveness of the pyrolysis extract of *Nicotiana tabacum* L. var Virginia in inhibiting *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. This study uses a true experimental research design with tobacco extract samples obtained by pyrolysis at concentrations of 20%, 40%, 60%, 80%, and 100%. The antibacterial test carried out was the Kirby-Bauer diffusion method on Mueller Hinton Agar (MHA) media. One-Way ANOVA test results with p <0.05 indicate the effectiveness of tobacco pyrolysis extract in inhibiting *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The average yield of inhibition zones found in *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa* were 6.35 mm, 5.9 mm, 3.97 mm, and 5.025 mm. From these results, *Staphylococcus aureus* bacteria became the most sensitive bacteria with Virginia tobacco pyrolysis extract.

1. Introduction

Nosocomial infections can cause deterioration in the disease and even death in patients in hospitals and other health care facilities. Nosocomial infections occur after admission to the hospital environment for about 48 hours to 30 days after hospital treatment. *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* can cause these nosocomial infections [1], [2]. Nosocomial infections can be endogenous as from the patient himself or can be exogenous. Nosocomial infections can result in bacterial resistance. This condition is often due to the inappropriate
use of antibiotics in health care.[3] Therefore, alternative treatments are needed for nosocomial infections, namely by using plants as raw materials from herbal medicines.

Indonesia is a country with high biodiversity. Indonesia has around 30,000 plants, and 9,600 of them have medical properties, such as tobacco plants.[4] Tobacco is one of the most commonly found plants in Indonesia. The most common type of tobacco in Indonesia is the Virginia tobacco variant, with a percentage of 63% in all land in Indonesia.[5]

Part of the tobacco plant that is beneficial to health is tobacco leaf. Tobacco leaves have active antibacterial compounds. Besides being antibacterial, tobacco can also be useful as a biolarvacide.[6] In the previous study, it was proven that the ethanol extract of tobacco leaves, which was extracted by the maceration method. It had an inhibitory effect on the growth of *Streptococcus mutans* and *Porphyromonas gingivalis* bacteria.[7] Other research showed that tobacco extract could inhibit bacterial growth. Tobacco extract contained alkaloids, flavonoids, and terpenoids. These compounds can inhibit the growth of bacteria that cause nosocomial infections.[8]

In a study conducted by Pramono et al. (2018), tobacco extracts with concentrations of 20%, 40%, 60%, 80%, and 100% had only a small inhibitory effect on the bacteria *E. faecalis*, *E. coli*, *P. aeruginosa*, and *S.aureus*. The study uses the heat reflux method as an extraction method.[8] Therefore, this study uses extraction methods other than the heat reflux method, the pyrolysis method. Pyrolysis method is carried out by heating the simplicia in high temperatures without gas, especially oxygen. The result of pyrolysis is bio-oil. The advantage of this pyrolysis method is the high amount of active ingredient contained in the extract produced.[9] The antibacterial potential of the pyrolysis extraction of *Nicotiana tabacum* Var Virginia needs to be further investigated.

2. Materials and Methods

2.1 Experimental Design

A true experimental design was used in this study. Tobacco leaf extract (*Nicotiana tabacum* L. var *Virginia*) with various concentrations (20%, 40%, 60%, 80%, and 100% diluted in propylene glycol) is used against *E. faecalis* (ATCC 29212), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), and *S.aureus* (ATCC 25923).

2.2 Time and Place

The study was conducted at the Microbiology Laboratory, Faculty of Medicine, Pembangunan Nasional Veteran Jakarta University from May 2019 to January 2020. Virginia tobacco pyrolysis extract was obtained from the Department of Chemical Engineering, University of Indonesia.

2.3 The Process of Making Tobacco Extract by Pyrolysis Method

Virginia tobacco plant is a staple for making extracts. The material was obtained from the City of Ponorogo, East Java. Several steps are needed in making tobacco leaf extract by the pyrolysis method. First, the tobacco leaves are dried until the water content contained is less than 10%. The drying process is carried out so that the extraction results do not contain too much water.[10] After that, the tobacco leaves are chopped into smaller parts. This is done to improve the efficiency of the pyrolysis process. The next step is the pyrolysis reaction. The pyrolysis reaction is carried out in a closed pyrolysis reactor so that oxygen and other air cannot enter the reactor. Then, the simplicia is heated to a temperature of 500°C. After going through the heating process, tobacco leaves turn into gas and charcoal. Inside the cyclone separator, gas and charcoal are separated. The gas that has been separated from the charcoal will be cooled using cold water. The gas cooling process produces extracts in the form of bio-oil (hydrophobic). The pyrolysis extract produced has a concentration of 100%.[9] The tobacco extract was diluted to a concentration of 20%, 40%, 60%, and 80%. Dilution is carried out by mixing tobacco extract with propylene glycol (PG). PG has a molecular structure that can be hydrophilic and lipophilic to be used as a solvent for tobacco leaf extracts.[11]
2.4 Evaluation of Antibacterial Activity
Evaluation of antibacterial activity was carried out at the Microbiology Laboratory of the Faculty of Medicine, Pembangunan Nasional Veteran Jakarta University. The antibacterial test used in this study is the Kirby-Bauer diffusion method. Paper discs that were previously immersed in various tobacco extract concentrations were placed on the media of Mueller Hinton Agar (MHA) that had been inoculated with test bacteria. Next, the MHA was incubated at 37°C. This incubation was carried out for 24 hours in an incubator. After that, a digital caliper is used to measure the inhibition zone's diameter from the outer edge of the clear zone.

2.5 Data Analysis
Data processing in this study uses analytic methods with the One-Way ANOVA test. Before the One-Way ANOVA test, the data must be normally distributed and homogeneous data variations.[12] The data normality test is done using Shapiro-Wilk because the sample used is less than 50. The data is normally distributed when the value of $p > 0.05$. Next, the variance homogeneity test was performed using the Levene test. Data is said to be homogeneous if the value of $p > 0.05$. After the two conditions are met, the One-Way ANOVA test is carried out. There is a significant difference between the independent and dependent variables when the $p$-value $< 0.05$. If the $p$-value $< 0.05$, Bonferroni's posthoc test was done to see the differences between groups of concentrations of tobacco extract. The Kruskal-Wallis test is carried out if the two conditions for conducting One-Way ANOVA tests are not met.

3. Results and Discussion

3.1 Tobacco pyrolysis extract inhibits the growth of all four nosocomial bacteria
The results showed that the pyrolysis extract of Virginia tobacco leaves at concentrations of 20%, 40%, 60%, 80%, and 100% could inhibit the growth of $\text{E. faecalis}$, $\text{E. coli}$, $\text{P. aeruginosa}$, and $\text{S.aureus}$ concentrations. The study was conducted with four repetitions.

![Figure 1](image.png)

Figure 1. The inhibition zone against bacteria. The inhibition looks clear around the paper disc

The presence of a clear zone of inhibition around the disc paper indicates that tobacco leaf extract can be useful as an antibacterial. Tobacco leaf extract contains an alkaloid, flavonoid, and terpenoid. Alkaloids can interfere with the synthesis of the constituent components of peptidoglycan in bacterial cell walls so that the cell wall's integrity is disturbed.[8] Then, flavonoid, which is classified as one type of polyphenols, can inhibit bacterial growth by inhibiting metabolic energy, damaging cell membranes, and inhibiting nucleic acid synthesis.[13] Furthermore, the antibacterial mechanism of terpenoid compounds is to inhibit enzymes' function in the synthesis of bacterial cell walls.[7]
The average inhibition zone of tobacco extract by pyrolysis method against all 4 nosocomial bacteria

Consequently, the test bacteria with the largest to smallest inhibition zone diameters were *Staphylococcus aureus* (6.35 mm), *Enterococcus faecalis* (5.9 mm), *Pseudomonas aeruginosa* (5.025 mm), and *Escherichia coli* (3.975 mm). The largest inhibitory zone's average diameter was seen in *Staphylococcus aureus* bacteria at 6.35 mm at a concentration of 100%. The antibacterial power strength is categorized as moderate (6-10 mm) based on Moral es et al. (2003) classification. The results of the evaluation of inhibition zone diameter in other bacteria are included in the category of weak antibacterial ability (< 6 mm).[14]

*S. aureus* and *E. faecalis* are classified as Gram-positive bacteria, while *P. aeruginosa* and *E. coli* are classified as Gram-negative bacteria. The results showed that Gram-positive bacteria were more sensitive to tobacco leaf extracts compared to Gram-negative bacteria. This can be caused by the cell wall structure of Gram-positive bacteria that are different from Gram-negative bacteria. The structure of Gram-positive bacteria's cell walls is simpler compared to the structure of cell walls of Gram-negative bacteria. Also, Gram-negative bacteria have rigid cell walls.[15] The outer membrane of Gram-negative bacteria has hydrophilic porin, while tobacco leaf extract is hydrophobic. This makes diffusion of the active compound in tobacco extract into Gram-negative bacteria more difficult.[16] Other literary sources also say that polyphenol compounds are more effective as antibacterial agents in Gram-positive bacteria than Gram-negative bacteria. This happens because the structure of the cell walls of Gram-negative bacteria is more complex than Gram-positive bacteria. Therefore, the penetration of polyphenol compounds in tobacco leaf extracts into Gram-negative bacteria becomes slower.[17]

### 3.2 Statistical analysis results to see significant differences between concentration extract

Before the One-Way ANOVA test, the normality test and the homogenous variance test were performed. The results of the two statistical tests showed a value of p > 0.05. Then proceed with the One-Way ANOVA test. One-Way ANOVA test results showed a p-value < 0.05. These results mean that Virginia tobacco leaf extract has antibacterial properties against the four nosocomial bacteria. Next, Bonferroni's posthoc test was performed. In *E. coli*, there were no significant differences between all concentration groups. Then, in *P. aeruginosa* there are significant differences in almost all concentrations. *E. faecalis*, *E. coli*, and *S. aureus* have the largest average inhibition zone diameter at a concentration of 100%. This happens because, at a concentration of 100%, there are many active compounds in tobacco leaf extracts. Therefore, the diameter of the inhibition zone produced is also large. But in the bacterium *Pseudomonas aeruginosa*, the average yield of the largest inhibitory zone diameter
was obtained at a concentration of 80%. At a concentration of 100%, the diameter of the \textit{P. aeruginosa} inhibitory zone decreased. This shows that the concentration is not always directly proportional to the diameter of the inhibition zone. The speed of diffusion of the active compound into the media can be the cause.[18] The reduced speed of diffusion can be caused by the almost dense tobacco leaf extract’s consistency at a concentration of 100%.[8]

\textbf{Table 1.} Bonferroni’s posthoc test results

| Extract Concentration | Extract | \textit{S. aureus} Significance | \textit{E. faecalis} Significance | \textit{E. coli} Significance | \textit{P. aeruginosa} Significance |
|------------------------|---------|------------------------------|-------------------------------|-----------------------------|-------------------------------|
| 20%                    | 40%     | 1.000                        | 1.000                         | 1.000                       | 0.007*                        |
|                        | 60%     | 1.000                        | 1.000                         | 0.426                       | 0.001*                        |
|                        | 80%     | 0.571                        | 0.361                         | 1.000                       | 0.001*                        |
|                        | 100%    | \textbf{0.004*}              | \textbf{0.005*}               | 0.054                       | \textbf{0.001*}               |
| 40%                    | 20%     | 1.000                        | 1.000                         | 1.000                       | 0.007*                        |
|                        | 60%     | 1.000                        | 1.000                         | 1.000                       | 0.526                         |
|                        | 80%     | 1.000                        | 1.000                         | 1.000                       | \textbf{0.001*}               |
|                        | 100%    | \textbf{0.042*}              | 0.069                         | 0.699                       | \textbf{0.006*}               |
| 60%                    | 20%     | 1.000                        | 1.000                         | 0.426                       | \textbf{0.001*}               |
|                        | 40%     | 1.000                        | 1.000                         | 1.000                       | 0.526                         |
|                        | 80%     | 1.000                        | 1.000                         | 1.000                       | \textbf{0.003*}               |
|                        | 100%    | 0.133                        | 0.308                         | 1.000                       | 0.781                         |
| 80%                    | 20%     | 0.571                        | 0.361                         | 1.000                       | \textbf{0.001*}               |
|                        | 40%     | 1.000                        | 1.000                         | 1.000                       | \textbf{0.001*}               |
|                        | 60%     | 1.000                        | 1.000                         | 1.000                       | \textbf{0.003*}               |
|                        | 100%    | 0.887                        | 1.000                         | 1.000                       | 0.328                         |
| 100%                   | 20%     | \textbf{0.004*}              | \textbf{0.005*}               | 0.054                       | \textbf{0.001*}               |
|                        | 40%     | \textbf{0.042*}              | 0.069                         | 0.699                       | \textbf{0.006*}               |
|                        | 60%     | 0.133                        | 0.308                         | 1.000                       | 0.781                         |
|                        | 80%     | 0.887                        | 1.000                         | 1.000                       | 0.328                         |

*The mean difference is significant at 0.05 level.

\textbf{4. Conclusion}

Tobacco pyrolysis extract can inhibit \textit{E. faecalis}, \textit{E. coli}, \textit{P. aeruginosa}, and \textit{S. aureus} growth. However, the diameter of the inhibitory zone, which is produced still classified as weak to moderate. Further research on \textit{Nicotiana tabacum} Var Virginia pyrolysis extract is needed to improve antibacterial inhibition strength.

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