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Efficacy Test of Tetanus Leaf (*Leea aequata* L.) Ethanol Extract against Bacteria (*Pseudomonas aeruginosa)*

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Abstract

Antibiotic-resistant nosocomial infections are caused by the opportunistic bacterium Pseudomonas aeruginosa. The purpose of this study is to use the disc diffusion method to assess the antibacterial efficacy of Tetanus (Leea aequata L.) leaf ethanol extract against Pseudomonas aeruginosa. The antibacterial activity was evaluated at 25%, 50%, and 75% concentrations after the leaves were extracted by maceration with 96% ethanol. The findings indicated that the antibacterial activity was depending on concentration, with respective minimum inhibition level measuring 12.97 mm, 16.7 mm, and 21.06 mm. The negative control (DMSO) displayed no inhibition zone, but the positive control (gentamicin) displayed KHM measuring 25.03 mm. The data distribution did not differ significantly, according to statistical analysis ($p > 0.05$). Bioactive substances that can harm bacterial cell structures, including flavonoids, alkaloids, tannins, and saponins, were thought to be responsible for this antibacterial action. According to these results, tetanus leaf extract may be used as a natural antibacterial agent and as a substitute for antibiotics in the treatment of Pseudomonas aeruginosa infections

Keywords: *Pseudomonas aeruginosa*, tetanus leaf, ethanol, antimicrobial, disc diffusion technique

Abstrak

Infeksi nosokomial yang resisten terhadap antibiotik disebabkan oleh bakteri oportunistik Pseudomonas aeruginosa. Tujuan dari penelitian ini adalah untuk menggunakan metode difusi cakram untuk menilai efikasi antibakteri ekstrak etanol daun Tetanus (Leea aequata L.) terhadap Pseudomonas aeruginosa. Aktivitas antibakteri dievaluasi pada konsentrasi 25%, 50%, dan 75% setelah daun diekstraksi dengan metode maserasi menggunakan etanol 96%. Hasil penelitian menunjukkan bahwa aktivitas antibakteri tergantung pada konsentrasi, dengan Kadar Hambat Minimum (KHM) masing-masing berukuran 12,97 mm, 16,7 mm, dan 21,06 mm. Kontrol negatif DMSO tidak menunjukkan adanya zona hambat, tetapi kontrol positif gentamisin menunjukkan adanya KHM sebesar 25,03 mm. Sebaran data tidak berbeda secara signifikan, menurut analisis statistik ($p > 0,05$). Zat bioaktif yang dapat merusak struktur sel bakteri, termasuk flavonoid, alkaloid, tanin, dan saponin, diduga bertanggung jawab atas aksi antibakteri ini. Berdasarkan hasil ini, ekstrak daun tetanus dapat digunakan sebagai agen antibakteri alami dan sebagai pengganti antibiotik dalam pengobatan infeksi Pseudomonas aeruginosa.

Kata kunci: *Pseudomonas aeruginosa, daun tetanus, etanol, antibakteri, metode difusi cakram*

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INTRODUCTION

Since the colonial era, there have been substantial developments in the advancement of global health science, particularly with the rise of tropical medicine that emerged during the colonization of European nations. The WHO started to define and direct the idea of global health in the 1940s[1]

Gram-positive and Gram-negative bacteria are single-celled microorganisms that differ in the structure of their cell walls. Regarding pathogenicity, certain bacteria possess unique capacity to adhere to host cells and cause a range of pathological responses within the human body[2].

Pseudomonas aeruginosa, a member of the opportunistic pathogenic bacterial group, is one of the bacteria that has a

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significant clinical impact. Nosocomial infections are frequently linked to this bacterium, particularly in patients with compromised immune systems or other concomitant conditions as diabetes mellitus[1]. Due to their high level of resistance to several conventional medicines, *Pseudomonas aeruginosa* infections are major concern[1,3].

This high level of antibiotic resistance makes treatment more difficult and raises the possibility of health issues that raise patient morbidity and death rates. *Pseudomonas aeruginosa* infections, which frequently affect patients receiving long-term medical care in hospitals, include bloodstream infections, pneumonia, and urinary tract infections[1,3].

Tetanus (*Leea aequata L.*) is one plant that may be able to heal bacterial illnesses. In Indonesia and other regions of Southeast Asia, this herb has long been used to cure infectious infections and inflammation[4].

Flavonoids, tannins, saponins, and alkaloids are among the bioactive substances found in tetanus leaf extract that contribute to its antibacterial properties. These substances can help stop the formation of harmful germs because of their antibacterial, anti-inflammatory, and antioxidant properties[5]. Tetanus leaf extract has been shown in numerous studies to effectively prevent the growth of *Escherichia coli* and *Staphylococcus aureus*, however there is currently little study on *Pseudomonas aeruginosa*[6].

Therefore, more investigation is required to determine whether tetanus leaf extract can effectively combat these bacteria that are resistant to antibiotics. Finding substitutes based on natural materials is becoming more and more crucial in the fight against antibiotic resistance. Numerous medicinal plants found in Indonesia, a nation rich in biodiversity, have long been utilized to treat a range of infectious disorders. Tetanus (*Leea aequata* L.*)* is one of the plants that may be able to treat bacterial illnesses[7].

In Southeast Asia, especially Indonesia, this herb has traditionally been used to cure infectious infections and inflammation[4]. Utilizing tetanus leaf extract has advantages for the economy and environment in addition to its efficacy as an antibacterial agent. Extracts made from natural components have the potential to be more cost-effective and ecologically friendly than manufactured antibiotics. Tetanus leaf extract is therefore one of the prospective substitutes in the creation of antibacterial treatment that is safer and more long-lasting[8].

The growing number of nosocomial infections brought on by Pseudomonas aeruginosa highlights how urgent it is to do research on remedies based on natural ingredients. The purpose of this study is to evaluate how well Tetanus leaf ethanol extract inhibits *Pseudomonas aeruginosa* bacterial development[9]. It is anticipated that this research will help create herbal remedies that are more successful in treating bacterial illnesses resistant to antibiotics, which will help address the growing

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demand for safer and more sustainable treatments.

METHODOLOGY

This research method was conducted using an experimental study approach, to observe the impact of changes in concentration on the diameter of the inhibition zone of *Pseudomonas aeruginosa* bacteria studied. Tetanus leaves (*Leea aequata* L.) from Patumbak, West Deli Tua Village, Deli Serdang Regency were used as samples.

Tools and Materials

The glassware used consisted of measuring cup (Pyrex), beaker glass (Pyrex), erlenmeyer (Pyrex), test tube (Pyrex), petri dish, paper disc, tweezers, spatula, oven, Bunsen flame, ose needle, autoclave, laminar air flow cabinet, incubator, spatula, vortex, and hot plate. The materials consist of sodium agar media (NA: Himedia), dimethylsulfoxide (DMSO), ethanol extract of tetanus leave, *Pseudomonas aeruginosa* bacteria that have been cultured from the Integrated Laboratory of Prima Indonesia University.

Preparing the Sample

Old tetanus leaves were collected from Patumbak, West Deli Tua Village, Deli Serdang Regency. After being collected and washed with running water, the tetanus leaves were dried in a drying cabinet and pulverized into simplisia powder using a blender.

Tetanus Leaf Ethanol Extract Preparation

Using a 96% ethanol solvent, the maceration process was used to create an ethanol extract of tetanus leaves. One kilogram of powdered tetanus leaf was macerated for three days at a solvent ratio of 1:5, and then repeated for one day at a ratio of 1:3. The filtered filtrate was concentrated at 78°C using an evaporator. After that, a thick extract was produced by evaporating the filtrate[10] .

Sterilization of Equipment and Supplies

Sterilizing glassware involved heating it in an oven at 170°C to 180°C for around two hours, while cleaning ose needles and tweezers directly over a Bunsen flame. The medium was sterilized for 15 minutes at 121°C in an autoclave [11].

Getting the Test Solution Ready Focus

To obtain concentration variations of 25%, 50%, and 75%, 10 g of thick extract was weighed and mixed with 1 mL of DMSO to prepare a 100% concentration. From this 100% stock solution, various concentrations were prepared by diluting: for 75%, 0.75 mL of the stock was mixed with 0.25 mL of DMSO; for 50%, 0.5 mL of the stock was mixed with 0.5 mL of DMSO; and for 25%, 0.25 mL of the stock was mixed with 0.75 mL of DMSO. This procedure resulted in the desired concentrations for testing[12].

Making a Suspension of Test Bacteria

In a test tube, 10 ml of 0.9% physiological NaCl solution was added to one ose of pure culture to create a bacterial suspension, which was then

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swirled until it was uniform. The turbidity of the solution was adjusted using the McFarland standard as a guide to make sure the number of bacteria was within the proper range and to avoid providing false information about resistance or susceptibility to antimicrobial medications[13].

Test of Antibacterial Activity

To assess the antibacterial activity, the test bacterial solution was spread out over toughened NA media using the disc diffusion method. 0.1 ml of bacterial suspension is flattened, inoculated, and allowed to dry using an ose needle. Tetanus leaf extract at 25%, 50%, and 75% concentrations should be placed on the media. Three repeats of the positive control (gentamicin ointment), three repetitions of the negative control (DMSO), and three repetitions for each extract concentration make up one petri. The test was run three times (triplo) ten times following a 24 hour incubation period at 37°C[14].

$$\text{Activity Index} = \frac{\text{diameter of the extract zone}}{\text{diameter of the control zone}}$$

Data Analysis

The test findings, which were all presented as mean values from three repeats with standard deviation (SD), were assessed using SPSS version 22.

RESULT AND DISCUSSION

The results of the antibacterial efficacy test of an ethanol nanoemulsion of Tetanus (*Leea aequata* L.) leaf extract against the growth of *Pseudomonas aeruginosa* bacteria are shown in the table 1 below. The results of the antibacterial efficacy test of an ethanol nanoemulsion of Tetanus (*Leea aequata* L.) leaf extract against the growth of *Pseudomonas aeruginosa* bacteria are shown in the table below.

Tabel 1. Measurement Information for Disc Diffusion Inhibition Diameter

Concentration of Extract (%)	Inhibition Zone (mm)
K^-	0
25	12.97
50	16.7
75	21.06
K^+	25.03

K^- : Negative control (Dymethylsulfoxide)
K^+ : Positive control (gentamycin ointment)

Sig P is > 0.05, there is no difference. Notable distinction between the normal distribution and the data distribution.

Tetanus leaf extract (*Leea aequata* L.) was used as a test solution with concentrations of 25%, 60%, and 75%. In addition, the controls tested against bacteria are positive and negative controls. control positive uses Gentamycin and control negative uses 2% DMSO. The zone of inhibition formed in the disc paper area will be calculated using a caliper tool by paying attention to the formation of millimeters (mm).

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Based on the results of the zone of inhibition incubated for 24 hours against antibacterial disc diffusion method Tetanus leaf extract (*Leea aequata* L.) showed concentration-dependent antibacterial activity. The absence of inhibition zone (0 mm) in the negative control using DMSO indicates that DMSO lacks antibacterial activity. In contrast, gentamicin antibiotic produced an average inhibition zone of 25.03 mm in the positive control, indicating the efficacy of the antibiotic in preventing bacterial growth.

These antibacterial testing results demonstrate the potential of Tetanus leaf extract as a natural antibacterial agent. The prominent efficacy, especially at 50% and 75% concentrations, suggests that the extract's active ingredients can support antibacterial activity although their inhibitory potency is not comparable to gentamicin. Flavonoids, alkaloids, tannins, and saponins in the extract are some of the compounds that may contribute to the mechanism of inhibiting bacterial growth. Tannins work as antibacterials by precipitating proteins that result in protein denaturation in bacterial cells[15].

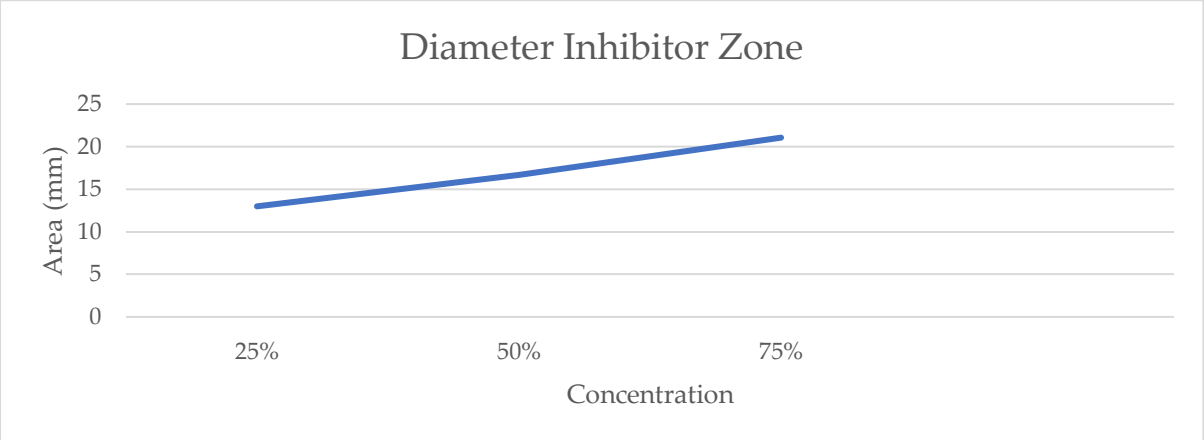

Figure 1. Diameter Chart of Zone of Inhibition of *Pseudomonas aeruginosa* Bacteria Growth.

Based on the analysis of variance, the inhibition zone obtained from the extract concentration shows the results of all P-values greater than the significance level of 0.05 (α = 5%), which means that there is no significant difference between the data distribution and the normal distribution. This indicates that Pseudomonas aeruginosa is significantly inhibited by bioactive chemicals present in the ethanol extract of tetanus leaves. These bacteria often cause infections in diabetic foot wounds and ulcers, which are difficult to heal due to peripheral neuropathy and poor vascularization.

In addition, *Pseudomonas aeruginosa* is known to be a significant contributor to nosocomial infections and pneumonia in hospitals, which increases morbidity and mortality rates among

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patients who already have underlying medical problems. Clinically, the efficacy of this extract can be used to treat diabetic ulcers, which are chronic wounds where *Pseudomonas aeruginosa* infections often result in life-threatening consequences. In addition, given the high rate of antibiotic resistance of *Pseudomonas aeruginos*a strains in hospital settings, its application in the prevention of nosocomial infections is also beneficial[16].

CONCLUSION

With an activity value of 21.6, ethanol extract of tetanus leaf (*Leea aequata* L.) showed antibacterial activity against *Pseudomonas aeruginosa*. With an inhibition zone width of 12.99 mm, the 75% concentration produced the largest inhibition zone, while the 25% concentration showed the least amount of inhibition. These findings suggest that tetanus leaves have antibacterial therapeutic potential; therefore, it is recommended to separate the bioactive components for further research relating to potential health effects. Since tetanus leaves have been shown to have antibacterial properties, their use by the public should not be a cause for concern.

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