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# Antibacterial Activity Test of Ethanol Extract of Fiddlehead (*Diplazium esculentum*) Agains the Growth of *Staphylococcus aureus* and *Escherichia coli* Bacteria in Vitro

Uji Aktivitas Antibakteri Ekstrak Etanol Pakis Sayur *(Diplazium esculentum)* terhadap Pertumbuhan Bakteri *Staphylococcus aureus* dan *Escherichia coli* secara *in Vitro*

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Abstrak

Penyakit infeksi merupakan salah satu isu kesehatan masyarakat yang signifikan di negara maju dan berkembang. Penyakit infeksi diantaranya disebabkan oleh bakteri *Staphylococcus aureus* dan *Escherichia coli* yang menyebabkan tingginya angka kesakitan dan kematian. Pengobatan penyakit infeksi yang disebabkan oleh bakteri patogen umumnya menggunakan antibiotik, namun penggunaan antibiotik yang terus menerus dapat memberikan efek samping seperti terjadinya resistensi antibiotik. Pakis sayur merupakan salah satu tanaman obat yang sering dimanfaatkan untuk mengobati penyakit infeksi. Kandungan senyawa metabolit sekunder yang terkandung dalam *Diplazium esculentum* antara lain saponin, steroid, alkaloid, tanin, glikosida, triterpenoid, fenol, flavon, dan flavonoid. Penelitian ini bertujuan untuk mengetahui pengaruh aktivitas bakteriostatik ekstrak etanol D. *esculentum* dan konsentrasinya yang efektif dalam menghambat pertumbuhan patogen S. *aureus* dan E. *coli*. Penelitian dilaksanakan menggunakan Rancangan Acak Kelompok Lengkap (RAKL) dengan empat konsentrasi ekstrak etanol pakis sayur, yaitu 20 %, 33 %, 43 %, dan 50 %. Sebagai kontrol positif digunakan antibiotik kloramfenikol dan kontrol negatif menggunakan akuades steril. Data yang berupa diameter zona hambat yang terbentuk dari masing-masing perlakuan dianalisis menggunakan uji non parametrik *Kruskal Wallis* dan dilanjutkan dengan uji *Post Hoc Mann Whitney*. Hasil penelitian menunjukkan bahwa konsentrasi ekstrak etanol D. *esculentum* menunjukkan aktivitas bakteriostatik terhadap bakteri S. *aureus* pada konsentrasi 43% dan 50% dengan zona hambat sebesar 6.52 mm dan 5.55 mm, serta bakteri E. *coli* pada konsentrasi 43% dan 50% dengan zona hambat sebesar 5.11 mm dan 4.37 mm, namun tidak efektif dalam menghambat pertumbuhan bakteri S. *aureus* dan E. *coli*.

**Kata kunci:** *Staphylococcus aureus, Escherichia coli, Diplazium esculentum,* bakteriostatik

Abstract

*Infectious diseases are a significant public health issue in both developed and developing countries. Infectious diseases are caused by the bacteria Staphylococcus aureus and Escherichia coli, which cause high rates of morbidity and mortality. Treatment of infectious diseases caused by pathogenic bacteria generally uses antibiotics, but the continuous use of antibiotics can have side effects such as the occurrence of antibiotic resistance. Diplazium esculentum is one of the medicinal plants that is often used to treat infectious diseases. The content of secondary metabolite compounds contained in D. esculentum includes saponins, steroids, alkaloids, tannins, glycosides, triterpenoids, phenols, flavones, and flavonoids. This study aims to determine the effect of bacteriostatic activity of ethanol extract of D. esculentum and its effective concentration in inhibiting the growth of pathogens S. aureus and E. coli. The study was conducted using a Randomised Complete Block Design (RAKL) with four concentrations of ethanol extract of vegetable fern, namely 20%, 33%, 43%, and 50%. As a positive control, the antibiotic chloramphenicol was used and the negative control used sterile distilled water. Data in the form of inhibition zone diameter formed from each treatment were analysed using Kruskal Wallis non-parametric test and continued with Mann Whitney Post Hoc test. The results of the study showed that the concentration of ethanol extract of D. esculentum showed bacteriostatic activity against S. aureus bacteria at concentrations of 43% and 50% with inhibition zones of 6.52 mm and 5.55 mm, as well as E. coli bacteria at concentrations of 43% and 50% with inhibition zones of 5.11 mm and 4.37 mm, but was not effective in inhibiting the growth of S. aureus and E. coli bacteria.*

***Keywords***: *Staphylococcus aureus*, *Escherichia coli*, *Diplazium esculentum*, bacteriostatic

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## INTRODUCTION

Infectious diseases are a significant health concern in both developed and developing countries. Infection occurs when microorganisms, which are microscopic organisms that can consist of one or more cells, such as bacteria, enter and grow in the body [1]. This process causes damage to the body and produces specific symptoms. Microorganisms that can cause disease in humans are called pathogens, one of which is pathogenic bacteria [2]. Some pathogenic bacteria that can cause infections are *Staphylococcus aureus* and *Escherichia coli*.

*Staphylococcus aureus* is a bacterium that appears blue when tested using the Gram method and can cause various diseases characterized by signs such as inflammation, necrosis, and abscess formation. These types of infections can range from mild skin infections, such as boils, to severe pneumonia [3]. *Escherichia coli* is a pink-colored bacterium when tested using the Gram staining method, does not produce oxidase, is rod-shaped, and belongs to the Enterobacteriaceae family. This bacterium is part of the intestinal flora of humans and other mammals. However, *Escherichia coli* is also known as an opportunistic microbe. Such conditions mean that the bacterium can cause disease when the immune system is compromised or when it spreads to organs or tissues outside the intestine. In individuals with weakened immune systems, *Escherichia coli* can multiply uncontrollably, causing infection [4]. Various types of infectious diseases caused by *Escherichia coli* include infections of the digestive system (such as diarrhea), urinary tract infections, and meningitis [5].

Antibiotics are needed to treat infections caused by pathogenic bacteria. Antibiotics are compounds composed of small molecules that are bacteriostatic or bactericidal against sensitive microbes, particularly bacteria [6]. Criteria for the appropriate use of antibiotics include cost-effective use, increased clinical effectiveness, minimized drug toxicity, and minimized development of resistance [7]. Synthetic antibiotics are no longer effective because many bacteria have developed resistance, while researchers struggle to discover new drugs. Antibiotic resistance can occur due to excessive and careless use of antibiotics. Researchers consider bacteria resistant when even high doses of antibiotics are unable to stop them. The inappropriate use of antibiotics leads to antibiotic resistance, changes in microorganisms, and mutations or the presence of genes that render them resistant to antibiotics [8].

The vegetable fern [*Diplazium esculentum* (Retz.) Swartz] is a medicinal plant. Researchers or practitioners can use vegetable ferns to treat various infectious diseases caused by bacteria, such as dysentery, digestive disorders, diarrhea,

swollen glands, and several types of skin infections [9]. The secondary metabolite compounds contained in *Diplazium esculentum* include saponins, steroids, alkaloids, tannins, glycosides, triterpenoids, phenols, flavones, and flavonoids [10]. Based on research by Fandini, the ethanol extract of D. esculentum showed moderate antibacterial activity against Gram-negative (*Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus epidermidis*) bacteria. Phytochemical screening revealed the presence of alkaloids, steroids, polyphenols, tannins, flavonoids, and saponins, which are known to damage cell walls and disrupt cell membrane permeability [22].

The purpose of this study was to determine the bacteriostatic activity and effective concentration of *Diplazium esculentum* ethanol extract in inhibiting the growth of *Staphylococcus aureus* and *Escherichia coli* pathogens.

## METHODS

This study used a completely randomized block design (CRBD) with two treatments. The first treatment was vegetable fern ethanol extract, with a positive control treatment using 0.6% chloramphenicol (K+), a negative control using distilled water (K-), vegetable fern ethanol extract at concentrations of 20% ($K_{20}$), 33% ($K_{33}$), 43% ($K_{43}$), and 50% ($K_{50}$). The second treatment involved testing microbes, specifically *Staphylococcus aureus* ($B_S$) and *Escherichia coli* ($B_E$). Each treatment unit was repeated three times. The variable measured was the inhibition zone formed on the medium.

### Production of Vegetable Fern Extract

A total of 300 g of vegetable fern leaf powder was dissolved in 1500 mL of ethanol solvent. Maceration was carried out for 1 x 24 hours three times, followed by re-maceration twice at room temperature. The mixture was then filtered using a Buchner funnel, and the filtrate was evaporated using a rotary vacuum evaporator at 40 °C until a thick extract was obtained [11].

### In vitro Antibacterial Activity Test

The researchers conducted antibacterial testing of the vegetable fern ethanol extract using the Kirby–Bauer disk diffusion method. They prepared 5 mm disks from sterile filter paper and soaked the sterilized disks in various concentrations of extract for 24 hours. Afterward, they transferred 0.1 mL of a 24-hour suspension culture—adjusted to the 0.5 McFarland turbidity standard in 0.9% NaCl—onto a dish containing Mueller–Hinton Agar (MHA). The researchers used a sterile cotton swab to apply the suspension and spread it evenly across the entire surface of the medium, ensuring uniform distribution of the inoculum. The inoculum is left to dry for several minutes. Then, discs are placed on the medium at various concentrations and incubated at 37°C for 24 hours. Then, the clear zone formed around the disc was measured using calipers in

millimetres (mm) [12]. The diameter of the inhibition zone was measured using the formula:

$$D = \frac{(Dv - Dc) + (Dh - Dc) + (Dd - Dc)}{3}$$

Description:

D : Inhibition Zone Diameter

Dv : Vertical Inhibition Zone Diameter

Dh :Horizontal Inhibition Zone Diameter

Dd : Diagonal Inhibition Zone Diameter

Dc : Disc Paper Diameter

The results of the inhibition zone calculations were then used in the classification criteria for the effectiveness of antibacterial substances as follows [13]:

a. Diameter < 5 mm: Weak inhibition.
b. Diameter 5-10 mm: Moderate inhibition.
c. Diameter 11-20 mm: Strong inhibition.
d. Diameter > 20 mm: Very strong inhibition.

## RESULTS AND DISCUSSION

The diameter of the growth inhibition zone of Staphylococcus aureus and Escherichia coli treated with vegetable fern ethanol extract as an antibacterial agent is presented in Table 1.

**Table 1.** Average Diameter of Inhibition Zone against the Growth of *Staphylococcus aureus* and *Escherichia coli*

| Treatment | *Staphylococcus aureus* | | *Escherichia coli* | |
|---|---|---|---|---|
| | **Average Diameter of Inhibition Zone (mm) ± SD** | **Antibacterial Category** | **Average Diameter of Inhibition Zone (mm) ± SD** | **Antibacterial Category** |
| K- | 0 ± 0[c] | No inhibitory activity | 0 ± 0[c] | No inhibitory activity |
| 20 % | 5.71 ± 0.46[b] | Moderate | 0 ± 0[c] | No inhibitory activity |
| 33 % | 5.71 ± 0.43[b] | Moderate | 0 ± 0[c] | No inhibitory activity |
| 43 % | 6.52 ± 1.02[b] | Moderate | 5.11 ± 1.25[b] | Moderate |
| 50 % | 5.55 ± 0.24[b] | Moderate | 4.37 ± 0.40[b] | Low |
| K+ | 28.19 ± 0.64[a] | Very strong | 32.25 ± 2.03[a] | Very strong |

**Note:** Values followed by the same letter in the same column indicate no significant difference with the Mann-Whitney Post Hoc test at α= 5%.

Based on the data in Table 1, the antibacterial activity of vegetable fern (*Diplazium esculentum*) ethanol extract against the growth of *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) was determined using the disk diffusion method. In *Staphylococcus aureus*, the extract demonstrated the ability to inhibit bacterial growth, with an inhibition zone ranging from 5.55 mm to 6.52 mm, which was significantly smaller than that of the positive control (28.19 mm). The largest inhibition zone was obtained at a concentration of 43%, indicating that increasing the concentration can enhance antibacterial activity to a certain point. Still,

at a concentration of 50%, antibacterial activity decreased slightly, possibly due to solubility factors or interactions of active compounds in the extract that affect its effectiveness. The antibacterial effectiveness of the extract is the result of a "team effort" by secondary metabolites, rather than a single "star player." The higher the concentration of the extract, the more team members are ready to attack, thereby increasing the effectiveness of bacterial killing [14].

Meanwhile, against *Escherichia coli*, extracts at low concentrations (20% and 33%) did not show antibacterial activity (inhibition zone of 0 mm), indicating that these concentrations were not sufficient to inhibit bacterial growth. However, at higher concentrations of 43% and 50%, the extract began to show activity with inhibition zones of 5.11 mm and 4.37 mm, although still much lower than the positive control (32.25 mm) [9]. Based on the research by Husna *et al*., alkaloids, terpenoids, and saponins in *D. esculentum* are active substances that act as antibacterial agents by damaging cell walls and disrupting membrane permeability [12]. This indicates that *Escherichia coli* is more resistant to the extract than *Staphylococcus aureus*, which may be due to differences in cell wall structure between Gram-negative and Gram-positive bacteria, where Gram-negative bacteria have a more complex outer membrane that acts as a barrier to antibacterial compounds [9]. This is in line with the research by Fandini, which states that the antibacterial activity of 80% ethanol extract of vegetable fern leaves (*Diplazium esculentum*) against the Gram-negative pathogen *Pseudomonas aeruginosa* (Gram-negative) was lower than that against the pathogen *Staphylococcus epidermidis* (Gram-positive), as indicated by inhibition zone diameters of 5.47 and 5.69 mm, respectively [22]. The differences in the results of the antibacterial activity test of *Diplazium esculentum* ethanol extract against *Staphylococcus aureus* and *Escherichia coli* are due to several main factors related to the biological and structural characteristics of the two bacteria. Gram-positive bacteria, such as *Staphylococcus aureus*, have relatively thick and straightforward cell walls, composed mainly of peptidoglycan layers that reach approximately 40 layers and form a rigid structure. These peptidoglycan layers allow antibacterial compounds to penetrate and bind directly to target proteins in the cell wall, causing damage and lysis of bacterial cells [15].

In contrast, Gram-negative bacteria, such as *Escherichia coli*, have more complex and thinner cell walls, containing only about 10% peptidoglycan, but are coated by an outer membrane that contains lipopolysaccharides (LPS), lipoproteins, and porin proteins. This outer membrane acts as a physical and chemical barrier, limiting the penetration of antibacterial compounds.

Active compounds must pass through porins to reach peptidoglycan and the plasma membrane, so antibacterial effectiveness against *Escherichia coli* tends to be lower and requires higher concentrations to demonstrate activity. In addition, Escherichia coli possesses additional defence mechanisms, such as efflux pumps and degrading enzymes, that can neutralize or remove toxic compounds, thereby increasing resistance to natural antibacterial agents [15].

Several factors that influence antibacterial activity include the quality of the extract, its concentration, the composition of antibacterial compounds, the dispersibility of the extract, and the type of bacteria targeted for inhibition. Extract quality is significantly influenced by chemical factors, including the type and concentration of active compounds present, as well as the extraction method and type of solvent used [16]. Additionally, biological variations, such as plant origin, also have an effect. Environmental factors such as temperature, humidity, sun exposure, wind, plant temperature, oxygen availability, and light intensity that affect photosynthesis also influence the physiological function, anatomy, and life cycle of plants. Young leaves typically contain antibacterial compounds, whereas older leaves have different compositions [17]. In this study, the researchers collected stem and leaf samples without considering the age of the plants. Several other factors, such as the solubility of the active substance, diffusion rate, incubation temperature, and heat absorption rate of the Petri dish incubator, may vary depending on the thickness of the Petri dish [14]. The factor causing differences in the inhibition rate is the metabolite content in the extract that has not been thoroughly mixed with the solution, resulting in an extract content that is not optimal [18].

In basic chemistry theory, one factor that affects reaction speed is the concentration of the solution. The higher the concentration of a solution, the faster the reaction rate. Thus, the higher the concentration of the extract, the greater its ability to inhibit microorganisms. However, the author's research yields different results. The concentration treatment did not show any increase in inhibitory power against the two test bacteria. At the highest concentration level, there was actually a decrease in resistance [19].

This finding is consistent with the experiment conducted by Shinta [14], which shows that the diameter of the inhibition zone decreases, even when it is at its maximum. An increase in concentration may not be directly proportional to the diameter of the inhibition zone. The low diffusion ability of the compound may result from excessive extraction, which limits its movement into the agar medium. Antibacterial substances will diffuse from

the paper disc into the medium containing the test bacteria, producing an inhibition zone as a sign of their inhibitory power.

Chloramphenicol antibiotics are capable of forming very high inhibition zones because chloramphenicol is a commercial antibiotic that can kill a wide range of bacteria, meaning that it can inhibit or kill both Gram-positive and Gram-negative bacteria. Chloramphenicol interferes with protein synthesis in bacteria by binding to the 50S subunit of the bacterial ribosome, specifically at the peptidyl transferase centre, which is involved in the formation of peptide bonds between amino acids during the translation process. By binding at this site, chloramphenicol inhibits the activity of the peptidyl transferase enzyme, thereby preventing the formation of new polypeptide chains [20]. Bacterial ribosomes have a different structure and composition than human ribosomes, so chloramphenicol specifically targets bacterial ribosomes (50S and 30S) without directly interfering with human ribosomes (60S and 40S). Chloramphenicol can inhibit protein synthesis in human cell mitochondria by suppressing the activity of the 55S mitochondrial ribosomal subunit, which is similar to bacterial ribosomes, thereby disrupting mitochondrial respiration and the proliferation of specific eukaryotic cells. The activity of this antibiotic can cause severe side effects, including bone marrow suppression and aplastic anemia, in humans [21]. The negative control group did not form a zone because the distilled water solution lacked active compounds that function as antimicrobials.

The data in Table 1 show that vegetable fern ethanol extract has potential as a natural antibacterial agent, exhibiting moderate activity against *Staphylococcus aureus* and limited activity against *Escherichia coli* at concentrations above 40%. This finding aligns with previous studies reporting the antibacterial activity of *Diplazium esculentum* ethanol extract against several pathogenic bacteria, which has a phytochemical content including alkaloids, steroids, polyphenols, tannins, flavonoids, and saponins, contributing to its antibacterial effect [22]. According to Marfu'ah *et al*. [23], these compounds work by damaging the integrity of the bacterial cell membrane and wall, causing cell contents to leak and bacterial death, inhibiting the synthesis of proteins and nucleic acids that are important for bacterial growth and reproduction, and disrupting adhesion and biofilm formation, which are essential for bacterial virulence. The active compounds in bioantibacterials penetrate the simpler cell walls of Gram-positive bacteria (without an outer membrane) more easily than those of Gram-negative bacteria, which have a double outer membrane. The outer membrane of *Escherichia coli* acts as a physical and

chemical barrier to the bioactive compounds in the extract, resulting in lower extract effectiveness against *Escherichia coli* [24].

## CONCLUSION

The study's results showed that ethanol extracts of vegetable ferns (*Diplazium esculentum*) had an effect on the growth of *Staphylococcus aureus* bacteria at all concentrations tested. At the same time, *Escherichia coli* was only affected at concentrations of 43% and 50%. The extracts tested were not effective in inhibiting the growth of *Staphylococcus aureus* and *Escherichia coli* bacteria. It is necessary to identify the active compounds responsible for the antibacterial activity. Future studies need to examine the effectiveness of *Diplazium esculentum* extract at higher concentrations. Additional research is needed to determine the efficacy of *Diplazium esculentum* extract using various methods.

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## CONFLICT OF INTEREST

I hereby declare that there is no conflict of interest in the writing of this scientific paper.

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