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ANTIMICROBIAL POTENTIAL OF *Acanthus ilicifolius MANGROVE* LEAF EXTRACT AGAINST PATHOGENIC BACTERIA *Escherichia coli*

Potensi Antimikroba Ekstrak Daun Mangrove *Acanthus ilicifolius* terhadap Bakteri Patogen *Escherichia coli*

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ABSTRACT

This research was motivated by the need for alternative natural treatments for bacterial infections, particularly Escherichia coli, a pathogen that causes gastrointestinal and urinary tract infections. The mangrove plant Acanthus ilicifolius is known to contain bioactive compounds such as flavonoids, alkaloids, and polyphenols, which have antibacterial potential. Therefore, this study aimed to determine the antimicrobial potential of Acanthus ilicifolius leaf extract against E. coli and to determine the most effective extract concentration in inhibiting its growth. The study was conducted experimentally in a laboratory using the agar diffusion method. Leaf samples were collected from the coast of Wara Selatan District, Takalala Village, Palopo City, then dried, ground, and extracted using a maceration method with 96% ethanol. The resulting extract was tested against E. coli at four concentrations: 15%, 30%, and 60%. Paper disks containing concentrated Acanthus ilicifolius extract were then placed on agar media that had been inoculated with E. coli test bacteria, then incubated for 24 hours at 37°C. After that, observations and measurements of the inhibition zone were carried out with a caliper. The results showed that Acanthus ilicifolius leaf extract had antibacterial activity against E. coli, which was indicated by the formation of an inhibition zone around the paper disk. The average inhibition zone ranged from 10.5 mm to 12.5 mm. A concentration of 30% produced the largest inhibition zone of 12.5 mm, indicating the highest effectiveness, although there was no tendency that higher concentrations produced larger inhibition zones. This indicates that the antibacterial effectiveness of the extract is not linear with increasing concentration.

Keywords: antimicrobial potential, mangrove leaf extract, *Acanthus ilicifolius, Escherichia coli,* pathogenic bacteria

ABSTRAK

Penelitian ini dilatarbelakangi oleh perlunya alternatif pengobatan alami terhadap infeksi bakteri, khususnya *Escherichia coli*, yang merupakan salah satu patogen penyebab penyakit

infeksi saluran pencernaan dan saluran kemih. Tanaman mangrove *Acanthus ilicifolius* diketahui memiliki kandungan senyawa bioaktif seperti flavonoid, alkaloid, dan polifenol yang berpotensi sebagai antibakteri. Oleh karena itu, penelitian ini bertujuan untuk mengetahui potensi antimikroba dari ekstrak daun *Acanthus ilicifolius* terhadap bakteri *E. coli* dan menentukan konsentrasi ekstrak yang paling efektif dalam menghambat pertumbuhannya. Penelitian dilakukan secara eksperimental di laboratorium dengan menggunakan metode difusi agar. Sampel daun dikumpulkan dari pesisir Kec.Wara Selatan Kelurahan Takalala Kota Palopo, kemudian dikeringkan, dihaluskan, dan diekstraksi menggunakan metode maserasi dengan etanol 96%. Ekstrak yang dihasilkan diuji terhadap bakteri *E. coli* dengan empat variasi konsentrasi, yaitu 15%, 30%, dan 60%. *Paper disk* yang mengandung kosentrasi ekstrak *Acanthus ilicifolius* , lalu diletakkan pada media agar yang telah diinokulasi bakteri uji *E. coli*, kemudian diinkubasi selama 24 jam pada suhu 37°C. Setelah itu dilakukan pengamatan dan pengukuran zona hambat dengan jangka sorong.Hasil penelitian menunjukkan bahwa ekstrak daun *Acanthus ilicifolius* memiliki aktivitas antibakteri terhadap *E. coli*, yang ditunjukkan dengan terbentuknya zona hambat di sekitar *paper disk*. Rata-rata zona hambat berkisar antara 10,5 mm hingga 12,5 mm.konsentrasi 30% menghasilkan zona hambat terbesar 12,5 mm, mengindikasikan efektivitas paling tinggi, meskipun tidak ada kecenderungan bahwa konsentrasi yang lebih tinggi menghasilkan zona hambat yang lebih besar. Hal ini menunjukkan bahwa efektivitas antibakteri ekstrak tidak bersifat linear terhadap peningkatan konsentrasi.

Kata Kunci: potensi antimikroba, ekstrak daun mangrove, *Acanthus ilicifolius, Escherichia coli,* bakteri patogen

INTRODUCTION

Acanthus ilicifolius is one type of plant that inhabits mangrove forest areas. This plant is commonly referred to as jeruju, daruju, daun api-api, and other local names. *Acanthus ilicifolius* is a true mangrove species that is widely utilized by local communities as a medicinal resource. Based on phytochemical test results of *Acanthus ilicifolius* leaf extract, it contains alkaloids, flavonoids, polyphenols, phenylethanoid glycosides, and coumarins (Latief *et al*., 2022). Mangrove *Acanthus ilicifolius* is a plant that differs morphologically from other mangrove species, as its stem, branches, and twigs are surrounded by fine and sharp spines, and it has flowers. It is one of the mangrove species associated with wild vegetation, usually growing near mangroves and very rarely found on land. It has a distinctive characteristic as a low-growing and hardy herb (Pringgenies *et al*., 2020).

Mangrove jeruju (*Acanthus ilicifolius*) can be utilized as jeruju tea beverage, which has a fresh taste and aroma similar to herbal drinks made from ginger. Thus, *Acanthus ilicifolius* not only serves an ecological function but also has economic and social value because it is used in traditional medicine to treat various diseases such as rheumatism, skin diseases, and respiratory disorders (Arifiana, 2023). On the other hand, *Acanthus ilicifolius* faces threats from human activities, including land conversion for agriculture, coastal development, and pollution. A decline in the population of *Acanthus ilicifolius* can negatively impact the stability of coastal ecosystems and the welfare of communities that depend on mangrove resources.

In the last five years, research on *Acanthus ilicifolius* has increased, focusing on the ecological benefits and herbal potential of this species. Recent studies indicate that extracts from this plant possess antioxidant, anti-inflammatory, and antimicrobial properties, which strengthen its potential use in modern medicine. In addition, a deeper understanding of the role of *Acanthus ilicifolius* in mangrove ecosystems has encouraged conservation and rehabilitation efforts of mangrove habitats globally (Prabowo *et al*., 2015). *Escherichia coli* is a rod-shaped bacterium that lives in the intestines of humans and warm-blooded animals. Most strains of *E.*

coli are harmless and are part of the normal intestinal microbiota. However, some strains can cause infections and diseases. Infection by (*E. coli*) is a significant global health problem, often causing urinary tract infections, diarrhea, and sepsis (Anggoro, 2013).

Acanthus ilicifolius, a mangrove plant known in traditional medicine, shows antimicrobial potential. Mangrove *Acanthus ilicifolius* extract has potential as an antimicrobial agent against the pathogenic bacterium *E. coli*. Other studies have shown that mangrove plants contain bioactive compounds such as flavonoids, alkaloids, and saponins, which may exert antimicrobial effects (Saptiani *et al*., 2013). This study aims to examine the antimicrobial potential of mangrove *Acanthus ilicifolius* leaf extract against the pathogenic bacterium *Escherichia coli*, with the expectation of providing solutions to the problem of antibiotic resistance and supporting the development of environmentally friendly natural-based therapies. The objective of this study is to determine the antimicrobial potential of *Acanthus ilicifolius* against *E. coli* and to identify the concentration at which the ethanol extract of *Acanthus ilicifolius* leaves most effectively inhibits the growth of *E. coli*. The results are expected to contribute to knowledge regarding bioactive compounds in mangrove plants and to serve as an alternative solution to the problem of antibiotic resistance.

METHODS

Research Location and Time

Sampling Location

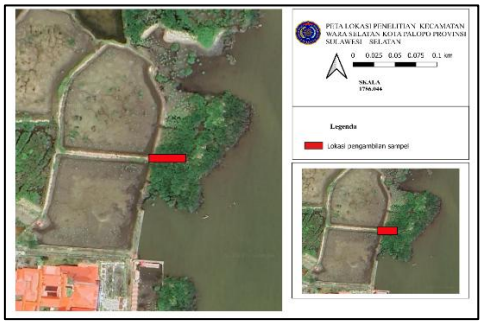

Figure 3.1 Research location, Palopo City, Wara Selatan District, Takalala Village

Leaf samples of *Acanthus ilicifolius* for this study were collected in the coastal area of South Wara District, Takalala Subdistrict. The City of Palopo has a relatively extensive coastal area, including mangrove forest regions that constitute the natural habitat of mangrove plants, including *Acanthus ilicifolius* (jeruju). This area was selected as the sampling location due to the presence of mangrove species that remain relatively natural and possess considerable potential as natural resources beneficial for research purposes, particularly for antimicrobial studies.

Sample Testing Location

After sample collection from the location in the City of Palopo, testing of the *Acanthus ilicifolius* leaf extract samples was conducted at the Microbiology Laboratory, Pharmacy Study Program, Muhammadiyah University of Palopo.

Time of Study

The study was conducted from May to July 2025 at the Microbiology Laboratory of the Pharmacy Study Program, Muhammadiyah University of Palopo.

Research Design

This study employed a laboratory experimental design using the agar diffusion method to test antimicrobial activity.

Tools and Materials

The equipment used included an analytical balance, blender, sterile forceps, petri dishes, micropipettes, incubator, glass jars, and a rotary evaporator.

The materials used were *Acanthus ilicifolius* leaves, nutrient agar (NA) media, nutrient broth (NB), and *Escherichia coli* bacterial culture, a positive control (chloramphenicol), and a negative control (paper disk).

Research Procedure

The research procedure regarding the antimicrobial potential of mangrove *Acanthus ilicifolius* leaf extract against the pathogenic bacterium *Escherichia coli* was carried out through several stages, including sample collection, preparation of *Acanthus ilicifolius* leaf extract, and antimicrobial testing.

Sample Collection

Sampling of *Acanthus ilicifolius* leaves was conducted on Sunday, 25 May 2025, in the morning before 10:00 a.m. WITA to maintain the stability of active compound content. The leaves collected were healthy, mature leaves with a fresh green color, free from damage, perforation, and disease symptoms. The samples were carefully collected and placed into clean plastic bags, after which the leaves were washed thoroughly under running water and then dried at room temperature until completely dry.

Preparation of *Acanthus ilicifolius* Leaf Extract

1. **Sample Preparation**
   a) **Collection and Preparation of *A. ilicifolius* Leaves**
   Fresh leaves were collected from their natural habitat in coastal or mangrove forest areas. The selected leaves were then washed thoroughly under running water to remove dirt and other contaminants.
   b) **Cleaning and Drying of *A. ilicifolius* Leaves**
   The cleaned leaves were dried through air-drying in a shaded area (natural drying). Drying aimed to reduce moisture content, thereby facilitating extraction and preventing spoilage.
2. **Sample Extraction Process**
   a) The dried and powdered *Acanthus ilicifolius* leaves were then extracted using the maceration method. A total of 100 grams of weighed leaf powder was placed into a glass jar and 1000 mL of 96% ethanol solvent was added. The mixture was then tightly sealed and stored at room temperature for three days. During the maceration process, the mixture was stirred twice daily to assist the solvent in maximally extracting bioactive compounds. After three days, the solution was filtered using filter paper to obtain the filtrate.
   b) After three days, the mixture was filtered using a clean cloth to separate the residue from the extract solution. The resulting filtrate was then collected in a clean, closed container and stored for the evaporation process.
   c) The evaporation process was carried out to separate the ethanol solvent from the macerated extract of *A. ilicifolius* leaves. At this stage, a simple rotary evaporator assembled manually was used, consisting of a heating vessel and a cooling system. As a cooling medium, approximately one gallon of water mixed with five packs of ice cubes was used to maintain a low temperature in the condensation section. The filtrate was placed into a round-bottom flask and then gently heated at a temperature of approximately 40–50°C. During heating, ethanol vapor rose and was cooled through the cooling system until it condensed and separated from the extract. This process was continued until all solvent had evaporated, leaving a viscous extract at the bottom of the flask. The concentrated extract was then collected and stored in a clean, closed

container at room temperature to maintain its quality prior to antibacterial testing (Sari *et al*., 2022).

Data Analysis

Data analysis was conducted descriptively by measuring the inhibition zones formed at each concentration of *Acanthus ilicifolius* leaf extract against *Escherichia coli* bacteria. The concentrations used were 15%, 30%, and 60%, each tested on three petri dishes (Dish 1, 2, and 3) to maintain data accuracy and consistency. The extract was applied onto sterile paper disks, which were then placed on agar media in petri dishes that had been inoculated with *E. coli* suspension. In addition, two types of controls were prepared, namely a positive control using antibiotics and a negative control without extract. Each control was also placed on separate dishes. After incubation for 24 hours at 37°C, the inhibition zones (clear zones) formed around the paper disks were observed. Measurements were performed using a caliper, and the results were recorded in millimeters (mm). Data from each dish were then analyzed descriptively to examine the relationship between extract concentration variation and its effectiveness in inhibiting *E. coli* growth. The larger the inhibition zone diameter, the higher the antibacterial activity of the extract. With the inclusion of positive and negative controls, the results could be compared more objectively (Ganesh & Vennila, 2010).

RESULTS

This study was conducted to evaluate the antimicrobial potential of ethanol extract of mangrove *Acanthus ilicifolius* leaves against the growth of the pathogenic bacterium *Escherichia coli*. To test antimicrobial activity, paper disks were used on agar media, with three variations of extract concentrations, namely 15%, 30%, and 60%. Observations were carried out by measuring the inhibition zones in millimeters (mm) formed around the paper disks after incubation for 24 hours at 37°C. The calculated data are presented as mean values of inhibition zone diameters and standard deviation (± SD) for each extract concentration, as shown in Table 1.

Table 1. Results and mean diameter of inhibition zones

Replica	Average inhibition zone (mm)				
	15%	30%	60%	K+	K-
C1	10.5	12.5	11.5	3.45	0.0
C2	10.5	10.5	12.5	3.45	0.0
C3	10.5	12.5	10.5	3.45	0.0
Average value	10.5	11.83	11.5	3.45	0.0
SD	0	0.94	0.81	0.0	0.0
Average ± SD	10.5±0	11.83±0.94	11.5±0.81	3.45±0.0	0.0±0.0

Table 1 shows the average inhibition zones of *Acanthus ilicifolius* leaf extract against *Escherichia coli* bacteria at concentrations of 15%, 30%, and 60%, as well as the positive control (K+) and negative control (K−). At a concentration of 15%, all replicates showed the same result, namely 10.5 mm. The 30% concentration exhibited the largest average inhibition zone, namely 11.83 mm, with two replicates reaching 12.5 mm. Meanwhile, at the 60% concentration, the average slightly decreased to 11.5 mm, because one replicate showed only 10.5 mm. The positive control (K+) produced an inhibition zone of 3.45 mm, whereas the negative control (K−) did not show any inhibition zone (0.0 mm). These results indicate that the 30% concentration was the most effective in inhibiting the growth of *E. coli*, although an increase in concentration did not always result in a greater inhibitory effect.

DISCUSSION

Antibacterial Activity of *Acanthus ilicifolius* Leaf Extract

Based on laboratory test results, *Acanthus ilicifolius* leaf extract demonstrated the ability to inhibit the growth of *Escherichia coli* bacteria, although its inhibitory activity was still classified as low to moderate. The inhibition zones formed ranged from 10.5 mm to 12.5 mm. As shown in Figure 4.1.1, the presence of a clear zone around the paper disk indicates the formation of inhibition zones resulting from the extraction assay of *Acanthus ilicifolius* leaves from each petri dish. According to the classification by Sandrawati *et al*. (2023), inhibition zone diameters ranging from 10 mm to 13 mm are categorized as moderate. The 30% concentration consistently produced the largest inhibition zone, namely 12.5 mm. This indicates that the active compounds at this concentration possessed the most effective ability to inhibit the growth of *E. coli*.

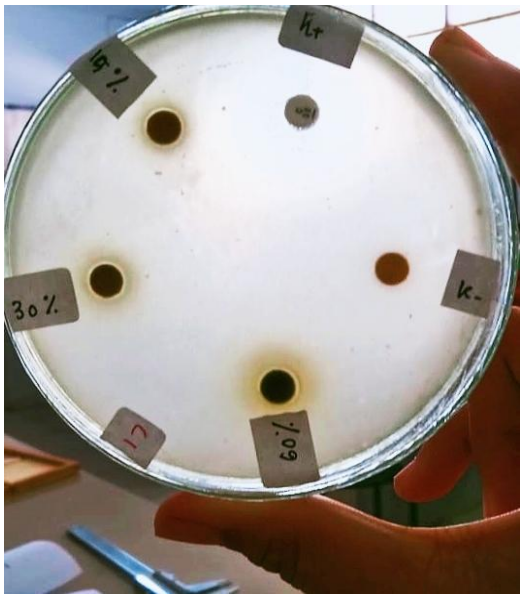

Figure 4.1 The image shows a clear zone around the paper disk.

The results in each petri dish showed inhibitory zone activity around the paper disk. The formation of inhibition zones by *Acanthus ilicifolius* leaf extract against *Escherichia coli* bacteria was observed. In petri dish 1, the 15% concentration produced a large inhibition zone diameter of 10.5 mm, which was categorized as low inhibition. Increasing the concentration to 30% caused the inhibition zone to increase to 12.5 mm, which falls into the moderate category; however, at a concentration of 60%, the inhibition zone decreased again to 11.5 mm, which is still classified as moderate. Based on the classification of inhibition strength, inhibition zones with diameters of 10–13 mm are categorized as moderate, those below 10 mm are classified as low, and those greater than 13 mm are considered strong or high. Thus, it can be concluded that the 30% concentration is the most optimal concentration and shows the highest antimicrobial activity against *E. coli* compared to other concentrations in this study.

These findings are consistent with various previous studies that have also reported the antibacterial potential of active compounds in *Acanthus ilicifolius*. According to Puspitasari (2022), ethanol extract of *A. ilicifolius* leaves was proven to be effective in inhibiting the growth of *Staphylococcus aureus* and *E. coli*, with inhibition zones ranging from 11 mm to 14 mm. This study also emphasized the role of compounds such as flavonoids and tannins as the main contributors to antibacterial activity. The study further emphasized the effectiveness of methanolic extract of *Acanthus ilicifolius* leaves against Gram-negative bacteria, where an

extract concentration of 50% showed an inhibition zone of 12.8 mm, indicating that the type of solvent and concentration greatly influence antibacterial effectiveness.

Andriani *et al*. (2020) reported that phytochemical compounds such as saponins, alkaloids, and flavonoids present in *A. ilicifolius* extract have synergistic effects in damaging bacterial cell walls, leading to cell lysis. This study found the highest inhibition zone of 13.2 mm against *E. coli*, similar to the observations at the 30% concentration in this study. Sravya *et al*. (2023) also showed that phenolic and terpenoid compounds in *A. ilicifolius* play an important role as antibacterial agents through mechanisms involving disruption of bacterial membrane permeability and inhibition of essential enzymes in microbial metabolism. This study strengthens the role of specific bioactive compounds in inhibiting *E. coli* bacteria.

Thus, the results of this study are consistent with and supported by various other studies over the past five years, which indicate that *Acanthus ilicifolius* leaves indeed contain various active compounds with potential as antibacterial agents. Although the inhibition zones observed were classified as moderate, these findings open opportunities for further development through increasing extract concentration, purification of bioactive compounds, and exploration of synergistic effects with other antibacterial agents to achieve higher effectiveness.

CONCLUSION

Based on the results of the research conducted, it can be concluded that mangrove *Acanthus ilicifolius* leaf extract has potential antimicrobial activity against pathogenic *Escherichia coli* bacteria, as indicated by the formation of inhibition zones in the paper disk diffusion test on agar media. The inhibition zones ranged from 10.5 mm to 12.5 mm, depending on the concentration of extract used. The 30% concentration produced the highest average inhibition zone (12.5 mm) compared to the other concentrations.

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