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Inhibitory Test of Water Hyacinth Leaf Extract *(Eichhornia Crassipes)* on the Growth of *Escherichia Coli* and *Propionibacterium Acnes Bacteria*

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Abstract. Antimicrobial resistance has accelerated the search for alternative antibacterial agents derived from natural products. Water hyacinth (Eichhornia crassipes) is an invasive aquatic weed that is abundant in Indonesian waters, yet rich in secondary metabolites such as flavonoids, tannins, alkaloids, and saponins with antibacterial potential. This study aimed to evaluate the inhibitory activity of the ethanol extract of water hyacinth leaves against Escherichia coli (Gram-negative) and Propionibacterium acnes (Gram-positive). A descriptive-exploratory laboratory design was used. Leaf samples collected from Lake Tondano were extracted by maceration in 96% ethanol and concentrated using a rotary evaporator. Antibacterial activity was assessed by the well-diffusion method at 100% extract concentration, with 30% amoxicillin as the comparator, incubated at 37°C for 24 hours. The extract produced mean inhibition zones of 7.0 mm against E. coli and 5.7 mm against P. acnes, both classified as moderate according to the Davis & Stout scheme, whereas amoxicillin produced zones of 8 mm and 7 mm. The ability of the extract to inhibit both Gram-positive and Gram-negative bacteria indicates a broad spectrum of activity. These findings confirm that water hyacinth leaves are a promising source of natural antibacterials while adding value to aquatic weed management.

Keywords: Antibacterial; Eichhornia Crassipes; Escherichia Coli; Inhibition; Propionibacterium Acnes.

1. INTRODUCTION

Infectious diseases are still one of the biggest health burdens in the world, especially in developing countries with uneven sanitation conditions and access to health services. Infections are generally caused by pathogenic microorganisms, and bacteria occupy an important position due to their ability to transmit, adhesion to the surface of host cells, as well as invasion into body tissues. Bacterial pathogenesis is a series of processes that start from the initiation of infection to the appearance of clinical symptoms when bacterial activity has caused structural and functional damage to the host tissue (Jawetz et al., 2016). The most common treatment of infections is the administration of antibiotics, but irrational use practices have raised serious global problems.

The excessive use of antibiotics, inappropriate dosage, and long duration encourages the development of resistance of microorganisms to various types of antibiotics (multidrug resistance). The consequence is a decrease in the effectiveness of treatment, an increase in the rate of illness and death, and a swelling of health costs. Resistance is also triggered by the massive use of broad-spectrum antibiotics and their use in plants and animals which ultimately have an impact on humans. Increasing resistance demands the availability of new or alternative antibacterial agents to fight infections, especially those caused by bacteria that have become

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resistant to conventional antibiotics (Rameshkumar et al., 2016). It is in this context that the exploration of natural materials as an antibacterial source becomes very relevant.

Escherichia coli is a rod-shaped Gram-negative bacterium that is a normal flora in the digestive tract of humans and animals, but a number of its pathogentypes are pathogenic. The diarrheagenic *groups of E. coli* such as ETEC, EPEC, EHEC, EIEC, EAEC, and DAEC are responsible for acute to chronic diarrhea, while extraintestinal groups such as UPEC and NMEC cause urinary tract infections and meningitis (Croxen & Finlay, 2010). This bacteria is also known as a sanitary indicator so its presence in water and food indicates fecal contamination. On the other hand, *Propionibacterium acnes* is a facultative anaerobic Gram-positive bacterium that is the normal flora of the skin and plays an important role in the pathogenesis *of acne vulgaris*. These bacteria produce lipases that break down sebum triglycerides into free fatty acids, triggering inflammation and the formation of acne lesions that impact the quality of life of sufferers (Zahrah et al., 2018).

In response to the limitations of synthetic antibiotics, the World Health Organization advocates the use of natural ingredients as herbal remedies to address various health problems. Treatments based on natural ingredients are seen as safer, easier to obtain, and have a lower risk of side effects. In line with that, research on plants containing antibacterial compounds is growing rapidly, including the use of plants that have been considered weeds (Munier & Muflihah, 2021). This shift in perspective opens up opportunities for the valorization of aquatic weeds into pharmacological resources with economic value.

Water hyacinth (*Eichhornia crassipes*) is a floating aquatic plant that grows very quickly and is known to damage aquatic ecosystems because it covers the water surface, lowers dissolved oxygen levels, accelerates siltation, and causes ecological, economic, and social losses. Its population continues to increase in various lakes and reservoirs in Indonesia, including Lake Tondano in North Sulawesi (Degaga, 2019). But behind its image as a weed, water hyacinth holds great potential as a medicinal ingredient. This plant contains secondary metabolites such as flavonoids, alkaloids, tannins, phenol components, saponins, and terpenoids that have the potential to be antioxidants, antibacterial, antifungal, and anticancer (Lata & Dubey, 2014; Shanab et al., 2010).

A number of studies have proven the antibacterial activity of hyacinth extract. Jaikumar et al. (2012) and Jayanthi et al. (2013) reported antimicrobial activity of *E. crassipes* extract against some test bacteria. Susmitha et al. (2019) found that ethanol extract of hyacinth leaves inhibited acne-causing bacteria with an increased inhibition zone with concentration. Sidharta et al. (2021) and Juliatri et al., (2023) shows its effectiveness against *Porphyromonas*

gingivalis, while Firman et al., (2024) and Dwiningrum et al., (2025) Develop anti-acne gel and cream preparations made from this extract. These studies confirm the consistency of the antibacterial potential of water hyacinth on various bacterial targets.

However, studies that directly compare the inhibition of water hyacinth leaf extract against Gram-negative and Gram-positive bacteria at the same time, especially *E. coli* and *P. acnes*, using samples from Lake Tondano and the sewage diffusion method at 100% concentrations are still very limited. Testing against two different types of cell walls is important to assess the broad spectrum of an extract's activity. This gap is the basis of this research. Based on this description, this study aims to test and measure the inhibition of ethanol extract of hyacinth leaves (*Eichhornia crassipes*) against the growth of *Escherichia coli* and *Propionibacterium acnes*. The results of the study are expected to provide scientific information on the antibacterial potential of water hyacinth leaves, provide basic data for the development of raw materials for natural medicines, as well as support the productive and sustainable use of aquatic weeds.

Antimicrobial resistance is now seen as one of the most pressing global public health threats. World health agencies place it as a priority because of its cross-sectoral impact, ranging from simple infection therapy failures, extended treatment periods, to increased mortality and cost burdens. In Indonesia, this problem is exacerbated by the high practice of self-medication, the purchase of antibiotics without a prescription, and the use of non-prescription antibiotics. The condition exerts selective pressure that expands populations of resistant bacteria, including the increasingly reported multiresistant strain of E. coli and acne-causing bacteria that are beginning to show a decreased response to conventional topical antibiotics. Therefore, the provision of new antibacterial candidates from abundant and inexpensive sources is becoming a strategic necessity.

Lake Tondano as one of the largest lakes in North Sulawesi is experiencing ecological stress due to the massive growth of water hyacinths. This weed cover degrades water quality, disrupts fishing activities and water transportation, and accelerates sedimentation. The removal of water hyacinth biomass has been treated more as waste. In fact, if the biomass can be processed into products with pharmacological value, environmental problems can be turned into economic opportunities. The concept of aquatic weed valorization is the basis for the selection of water hyacinth leaves from Lake Tondano as the object of research, as well as placing this study at the intersection between applied microbiology and sustainable environmental management.

The urgency of the search for natural antibacterial is also supported by the large burden of the two types of infections that are the focus of the research. Diarrhea associated with E. coli is still a major cause of pain, especially in children in developing countries, and is closely related to food security and water sanitation (Croxen & Finlay, 2010). On the other hand, *acne vulgaris* triggered by *P. acnes* is not a fatal disease, but it decreases the confidence and quality of life of sufferers because it affects physical appearance (Zahrah et al., 2018). Both conditions are very common in the community, so the availability of inexpensive and easily accessible natural antibacterial agents has real practical value.

2. LITERATURE REVIEW

Eceng Gondok (Eichhornia crassipes)

Water hyacinths (*Eichhornia crassipes*) belong to the *family Pontederiaceae*, class *Monocotyledonae*, and are closed-seeded aquatic plants (*angiosperms*) that live floating or taking root in shallow waters. The flowers are arranged in inflorescences containing ten to thirty blue-violet flowers with golden yellow patches that attract pollinating insects, while the root system is dark in color. The plant reproduces generatively through seeds and vegetatively through stolons; The vegetative method is the main mechanism that causes the population to grow rapidly in a short time so it is often treated as a weed (Degaga, 2019).

Water hyacinths grow optimally in nutrient-rich waters, especially nitrogen, phosphate, and potassium. Its massive growth decreases the volume and quality of the water. Phytochemically, hyacinth leaves contain flavonoids, alkaloids, tannins, phenol components, saponins, and terpenoids, as well as a variety of amino acids and minerals (Nyananyo et al., 2007; Rorong & Suryanto, 2010). The content of bioactive compounds scattered throughout the organs makes it a potential candidate as a traditional medicinal ingredient, including as a natural antibacterial agent (Munier & Muflihah, 2021).

Taxonomically, hyacinths belong to *the Kingdom Plantae*, Division *Spermatophyta*, Class *Monocotyledonae*, Order *Pontederiales*, Family *Pontederiaceae*, Genus *Eichhornia*, with species *Eichhornia crassipes*. Vegetative reproduction through stolons produces seedlings identical to their parents in a short period of time, while generative reproduction through seeds that can survive many years allows for long-distance propagation. The combination of these two mechanisms makes water hyacinth a weed that is difficult to control, but at the same time ensures the abundant and sustainable availability of biomass as raw material for extracts.

Escherichia Coli* dan *Propionibacterium Acnes

Escherichia coli adalah bakteri batang Gram-negatif, fakultatif anaerob, tidak berspora, anggota famili *Enterobacteriaceae*. Sebagian besar strainnya komensal, namun strain patogen menyebabkan diare, infeksi saluran kemih, hingga meningitis. Dinding sel bakteri Gram-negatif memiliki lapisan *peptidoglikan* tipis yang dilapisi membran luar mengandung lipopolisakarida (Croxen & Finlay, 2010). E. coli also serves as an indicator of fecal contamination in water and food (Maradesa et al., 2020).

Propionibacterium acnes (now *Cutibacterium acnes*) is a Gram-positive bacterium that includes the normal flora of the skin and grows slowly. This bacterium plays a central role in the pathogenesis *of acne vulgaris* through the production of lipases that break down free fatty acids so that they trigger inflammation in *the polyzebasea follicles* (Zahrah et al., 2018). The Gram-positive cell wall has a thick *layer of peptidoglycan* reinforced *teic acid*, in contrast to the thinner Gram-negative so it is relatively more susceptible to exposure to antibacterial compounds (Radji et al., 2011).

Antibacterial, Mechanism of Action, and Resistance

Antibacterial is a substance that inhibits (bacteriostatic) or kills (*bactericidal*) bacteria. Based on their working mechanism, antibacteria can inhibit cell wall synthesis, interfere with cell membrane permeability, inhibit protein synthesis, damage nucleic acids, and interfere with cell metabolism such as folic acid formation (Jawetz et al., 2016). The spectrum of antibacterial activity is differentiated into narrow and broad; broad-spectrum agents capable of inhibiting both Gram-positive and Gram-negative bacteria (Ryan et al., 2014).

Antibiotic resistance is the ability of microorganisms to survive an agent that was previously effective at inhibiting or killing them. Resistance can be pharmacological, clinical, or microbiological, and continues to increase due to the selective pressure of antibiotic use. This phenomenon is the main reason for the search for alternative antibacterial sources from natural materials (Rameshkumar et al., 2016).

Bioactive Compounds as Antibacterial and Extraction Methods

The antibacterial activity of plant extracts is determined by the group of compounds they contain. Flavonoids work by damaging the permeability of the cell wall, interacting with DNA, and interfering with energy transduction on the cytoplasmic membrane. Tannins inhibit extracellular enzymes and bind to the substrate needed for bacterial growth, while alkaloids disrupt the constituent components of peptidoglycan so that the cell wall becomes brittle. Saponins lower the surface tension of the cell wall and increase membrane permeability until cell component leakage occurs (Huda et al., 2019; Novita, 2016; Patil et al., 2015).

Extraction is the process of withdrawing the target compound using a solvent. Maceration is a simple method of immersing simplicia in an organic solvent; In principle, the breakdown of cell walls and membranes due to pressure differences so that secondary metabolites are dissolved. This method was chosen because the procedure is easy and maintains the stability of the thermobilable compounds. 96% ethanol is widely used because it is universal with a wide polarity range, safe, and easy to vaporize (Fakhruzy et al., 2020; Tutik et al., 2022). Antibacterial activity is generally measured through the inhibition zone by the sumpray diffusion method, then categorized according to its diameter (Davis & Stout, 1971; Hudzicki, 2009).

Method of Diffusion of Well and Classification of Inhibitory Power

In vitro antibacterial activity testing generally uses diffusion methods, both disc (disk diffusion) and well diffusion. The drainage method was chosen in many studies because the extracts in liquid or viscous form are easy to insert into the holes made in the media, and the contact of the extracts with the media is wider so that the diffusion of the active compounds takes place more effectively (Umar et al., 2023). Activity is assessed from the diameter of the clear zone (barrier zone) that forms around the ridge after the incubation period.

The magnitude of the inhibition zone is converted into an antibacterial strength category. Commonly used classifications divide the resistance into weak (diameter <5 mm), medium (5–10 mm), strong (11–20 mm), and very strong (>20 mm) (Davis & Stout, 1971). This classification facilitates comparisons between treatments and between studies, although the absolute value of the inhibition zone is still influenced by technical factors such as media thickness, inoculum density, and diffusion ability of compounds.

Framework of Thought and Hypothesis

The line of thought of this research departed from the problem of bacterial infections that attack various organs, ranging from the skin to the digestive tract. P. acnes-induced acne and diarrhea associated with *E. coli* represent two types of pathogenic bacteria with different cell wall structures, namely Gram-positive and Gram-negative. Increasing resistance to conventional antibiotics is driving the need for alternative natural remedies that utilize ingredients from nature with a lower risk of side effects. Water hyacinths in Lake Tondano are thought to have antibacterial potential because they are rich in secondary metabolite compounds that are able to inhibit the growth of pathogenic bacteria. On that basis, inhibition testing was performed on *P. acnes* (Gram-positive) and *E. coli* (Gram-negative) using the sewage diffusion method to measure the ability of the extract's active compounds to inhibit the growth of both bacteria.

The selection of two bacteria with different cell wall types aimed to assess the breadth of the extract's activity spectrum, rather than just its effectiveness on a single target. Thus, this framework of thinking links the theory of natural material extraction, the mechanisms of bacterial cellular toxicity, and the clinical need for alternative antibacterials. Based on this framework, the research hypothesis is formulated as follows: ethanol extract of the leaves of water hyacinth (*Eichhornia crassipes*) can inhibit the growth of *Escherichia coli* and *Propionibacterium acnes bacteria*.

3. RESEARCH METHODS

This research is an exploratory descriptive research carried out experimentally at the Microbiology Laboratory, Department of Biology, Faculty of Mathematics, Natural Sciences, and Earth, Manado State University, for three months. The tools used include rotary evaporators, autoclaves, Laminar Air Flow, incubators, petri dishes, analytical scales, micropipettes, hotplates, and rulers. The main ingredients are water hyacinth leaves (*Eichhornia crassipes*) from Lake Tondano, *Escherichia coli* and *Propionibacterium acnes* isolates, 96% ethanol, Nutrient Agar media (Nutrient Broth and agar), sterile aqueducts, and amoxicillin antibiotics as a comparison.

The entire appliance is sterilized using an autoclave at 121°C for 15 minutes. Samples of fresh green hyacinth leaves were taken from Lake Tondano, washed with running water, and drained. A total of 2 kg of leaves are dried in the oven at 60°C for 3 hours, then mashed into simplicia powder. Extraction is carried out by the maceration method: the powder is soaked in 1 liter of 96% ethanol for 1×24 hours in a dark room, then filtered. The filtrate is concentrated using a rotary evaporator at 40°C until a viscous extract is obtained, which is then stored in the refrigerator.

The growth medium is made by weighing 8 g of Nutrient Broth and 2 g of agar dissolved in 100 mL of aqueducts, heated until homogeneous, and then sterilized at 121°C for 15 minutes. The test medium is prepared by pouring 15 mL of media that has been inoculated with bacterial suspension into a petri dish filled with a ring. After solidifying, the ring is removed aseptically so that a suction is formed. The comparator is made from one amoxicillin tablet that is crushed and dissolved in sterile aqueducts until a 30% amoxicillin solution is obtained.

The antibacterial test uses the suction diffusion method. 100% concentration of hyacinth leaf extract and 30% amoxicillin solution are dripped into the well on the medium that has been planted with bacteria. The petri dish is then incubated at 37°C for 24 hours. After incubation, the diameter of the barrier zone around the ridge is measured using a ruler. The

measurement of the overlapping zones is carried out by measuring the radius from the center of the well to the edge of the clear zone and then multiplying by two, while the oval-shaped zone is calculated from the average of the longest and shortest axis (Hudzicki, 2009). Each treatment was carried out in three repetitions.

The data were analyzed descriptively quantitatively by calculating the average diameter of the inhibition zone from three replicates. Resistive force is categorized according to classification Davis & Stout (1971), namely weak (<5 mm), medium (5–10 mm), strong (11–20 mm), and very strong (>20 mm). The test bacterial suspension is prepared from *E. coli* and *P. acnes isolate* stocks, then inoculated into the media before being poured into a petri dish so that the bacteria are evenly distributed on the surface of the medium. All stages of planting, ring removal, and extract dripping are carried out aseptically in the Laminar Air Flow near the flame to prevent contamination. Sterilization of tools and media aims to ensure that the barrier zones that form are actually caused by the activity of the extract, not by contaminant microorganisms.

The concentration of 100% extract was selected as the maximum dose to determine the highest potential of the extract in inhibiting bacterial growth, while 30% amoxicillin was used as a comparative positive control. Each treatment was performed in three replicas on the same cup, bacteria, and time to improve the reliability of the data, and the reported value was the average diameter of the inhibition zone of all three replicas. Incubation at 37°C for 24 hours was chosen because it was close to the optimal temperature of the growth of both test bacteria.

4. RESULTS AND DISCUSSION

Extraction

The maceration process of 2 kg of hyacinth leaf simplicia in 1 liter of 96% ethanol for 1×24 hours in a dark room, followed by condensation with a rotary evaporator at 40°C, yields a thick extract of 10 grams. The extract has physical characteristics in the form of a thick texture, dark blackish green color, and a strong characteristic aroma of the plant. This characteristic signifies the attraction of secondary metabolite compounds from the leaf matrix into the ethanol solvent.

The dark blackish-green color and strong distinctive aroma of the extract indicate the attraction of chlorophyll along with phenolic compounds and other secondary metabolites into the solvent. These organoleptic characteristics are in line with the phytochemical profile of hyacinth leaves which are reported to contain flavonoids, tannins, alkaloids, saponins, and phenol components (Nyananyo et al., 2007; Rorong & Suryanto, 2010). The concentrated

texture indicates that most of the solvent has evaporated in the condensation process so that the concentration of active compounds in the extract is relatively high. Thus, the viscous extract obtained is suitable for direct use as a test material at 100% concentrations without further dilution.

Inhibition Against *Escherichia Coli*

Testing for *E. coli* was done on three petri dishes with bacteria and the same treatment at the same time. The inhibition zone formed in each successive iteration was 5.7 mm; 8.2 mm; and 7.2 mm, with an average of 7.0 mm. In comparison, amoxicillin 30% produced a consistent inhibition zone of 8 mm on all three replicates. More data are presented in Table 1.

Table 1. Diameter of the inhibition zone of hyacinth leaf extract against Escherichia coli.

Treatment	Ul. I (mm)	Ul. II (mm)	Ul. III (mm)	Average (mm)	Category
Ekstrak E. crassipes 100%	5,7	8,2	7,2	**7,0**	Medium
Amoksisilin 30%	8	8	8	**8,0**	Medium

Based on Table 1, water hyacinth leaf extract at a concentration of 100% yields an average inhibition zone of 7.0 mm against *E. coli*, classified as a medium category according to the classification Davis & Stout (1971). This value is only 1 mm different from 30% (8.0 mm) amoxicillin which is also in the medium category. The formation of clear zones around the wells indicates the inhibition of the extract's antimicrobial compounds against the growth of bacterial cells. Variations between replicas (5.7–8.2 mm) indicate a reasonable diversity that is commonly encountered in diffusion testing due to differences in diffusion diffusion in the media.

In more detail, the three replicas in *E. coli* showed a range of inhibition zones of 5.7–8.2 mm with a difference between replicas of 2.5 mm. This diversity is normal in the well diffusion method because it is influenced by the thickness of the media, the homogeneity of the inoculum, and the different diffusion rate of the extract in each cup. Despite the variations, all repeats still produce clear zones, so an average of 7.0 mm can be considered to represent the activity of the extract against these bacteria consistently.

When compared to positive controls, the average extract inhibition zone (7.0 mm) was equivalent to about 87.5% of the 30% (8.0 mm) amoxicillin inhibition zone. This achievement is relatively high considering that the extract is a complex mixture with unrefined levels of active compounds, while amoxicillin is a single antibiotic with a specific work target. The clear zone in *E. coli* appears relatively spherical and evenly distributed around the radius, indicating that the diffusion of the active compound is taking place in all directions in a balanced manner.

Inhibitory Forces Against Propionibacterium Acnes

The test for *P. acnes* was also carried out in three replicas with the same concentration and type of bacteria. The consecutively formed inhibition zone is 5 mm; 5 mm; and 7 mm, with an average of 5.7 mm. 30% amoxicillin as a comparator produces an inhibition zone of 7 mm; 8 mm; and 8 mm, with an average of 7.7 mm. A summary of the results is presented in Table 2.

Table 2. Diameter of the inhibition zone of hyacinth leaf extract against Propionibacterium acnes.

Treatment	Ul. I (mm)	Ul. II (mm)	Ul. III (mm)	Average (mm)	Category
Ekstrak E. crassipes 100%	5	5	7	**5,7**	Medium
Amoksisilin 30%	7	8	8	**7,7**	Medium

Table 2 shows that 100% concentration of hyacinth leaf extract yields an average inhibition zone of 5.7 mm against *P. acnes*, which according to the classification Davis & Stout (1971) It is classified as a medium category because it is in the range of 5–10 mm. 30% amoxicillin yields an average of 7.7 mm which is also moderate. It should be noted that in the original report the average *P. acnes* was rounded to 5 mm; recalculations of all three repetitions (5; 5; 7 mm) yielded 5.7 mm, so the medium classification remained consistent.

In *P. acnes*, two of the three repeats produce an inhibition zone of 5 mm and one repeat 7 mm, resulting in a range of 5–7 mm with a difference between repeats of only 2 mm. This uniformity indicates a relatively stable bacterial response to the extract. An average of 5.7 mm puts the extract's activity at the lower limit of the medium category, slightly above the weak category threshold (<5 mm).

The average extract inhibition zone against *P. acnes* (5.7 mm) is equivalent to about 74% of the amoxicillin inhibition zone (7.7 mm). The larger difference compared to *E. coli* indicates that at the same concentration, the extract is relatively less effective at penetrating the defenses of *P. acnes*. The clear zone on part of the *P. acnes* cup does not appear to be completely round, so measurements are made by evenly aligning the longest and shortest shafts according to the procedure (Hudzicki, 2009).

A direct comparison of the two bacteria showed that at identical extracts concentrations (100%), the inhibition zone against *E. coli* was about 1.3 mm larger than for *P. acnes*. This pattern was consistent across all replicas and was an important finding that showed the influence of cell wall type on the magnitude of the inhibition response, as described in the discussion section.

Overall, water hyacinth leaf extract was able to form an inhibitory zone against both test bacteria. The inhibition against *E. coli* (7.0 mm) is greater than that of *P. acnes* (5.7 mm). This pattern suggests that at the same concentration, the extract was relatively more effective at inhibiting Gram-negative bacteria than the Gram-positive bacteria in this study. In both bacteria, the extract inhibition zone was still below the amoxicillin inhibition zone, but the difference was not large (1 mm for *E. coli* and 2 mm for *P. acnes*), even though the extract concentration (100%) was far different from the comparative concentration (30%).

The clear zones formed around the wells in both tests became a visual indicator of antibacterial activity. In *E. coli*, the clear zone appears relatively evenly around the rim, while in *P. acnes* the clear zone tends to be narrower and in some cups it is not completely round, so measurements are made with a pivotal average approach. The consistency of the formation of zones throughout the repetition confirms that the inhibition activity is not a random event, but rather a result of the presence of active compounds in the extract.

Refers to classification Davis & Stout (1971) As summarized in the method section, the two average values of the extract inhibition zone are in the range of 5–10 mm so they are both categorized as moderate. The equalization of these categories makes it easy to read the results: although the absolute values against *E. coli* and *P. acnes* are different, they are at the same level of antibacterial potency. This shows that at 100% concentrations, the extract has a categorically equivalent effectiveness against both Gram-negative and Gram-positive bacteria.

In addition to the exposed area, bacterial growth still appears tight in the part of the media that is far from the exposure. This indicates that the bacterial inoculum is viable and grows normally, so that the clear zone formed is really the result of inhibition by the extract and amoxicillin, not due to the failure of bacterial growth in general. The absence of foreign microorganism growth in the media also indicates that the aseptic and sterilization procedures have gone well. The finding that unrefined coarse extracts are already capable of producing medium-category inhibitory zones is a promising early indication. In general, the fractionation and purification process will increase the level of active compounds per unit volume so that it has the potential to enlarge the inhibition zone. Thus, the values of 7.0 mm and 5.7 mm obtained in this study can be seen as the lower limit of the antibacterial potential of water hyacinth leaves which is still open to be optimized.

Thus, the results of the study answer both formulations of the problem: hyacinth leaf extract has an inhibition against *E. coli* of 7.0 mm (medium category) and against *P. acnes* of 5.7 mm (medium category). The ability to inhibit two bacteria with different cell wall types is

a key finding that will be discussed further in the discussion section, especially with regard to the wide spectrum of extract activity.

Discussion

Water hyacinth leaf samples obtained from Lake Tondano were extracted by maceration method using 96% ethanol. The selection of maceration is based on the simplicity of the procedure and its ability to maintain the stability of the active compounds that are thermolabile so that they do not get damaged. 96% ethanol was chosen because of its universal properties with a wide polarity range that effectively attracts target compounds such as flavonoids, tannins, alkaloids, and saponins, in addition to its safety factors and ease of evaporation during extract concentration (Fakhruzy et al., 2020; Tutik et al., 2022). Perolehan ekstrak kental sebesar 10 gram dengan warna hijau tua kehitaman mengindikasikan terekstraknya pigmen dan senyawa fenolik dalam jumlah memadai.

The antibacterial activity of the extract is characterized by the formation of clear zones around the rim. This zone is formed because antimicrobial compounds diffuse into the medium and inhibit the growth of surrounding bacterial cells. The inhibition mechanism is closely related to the group of compounds contained in the extract. Flavonoids damage the permeability of the cell wall and interact with DNA and interfere with energy transduction on the cytoplasmic membrane, so that bacterial metabolism and motility are disrupted, causing toxic effects. Tannins work by inhibiting extracellular enzymes and taking over the substrate necessary for bacterial growth. Alkaloids interfere with the constituent components of peptidoglycan so that the cell wall becomes brittle and the cell dies, while saponins lower the surface tension of the cell wall and increase the permeability of the membrane until the cell contents leak (Huda et al., 2019; Novita, 2016; Patil et al., 2015).

Flavonoids are seen as the compounds that play the most role in the antibacterial activity of hyacinth leaf extract. These compounds form complexes with extracellular proteins and cell walls, damaging membrane permeability, and are able to interact with DNA and disrupt energy transduction on the cytoplasmic membrane. As a result, nutrient transport is disrupted, bacterial metabolism and motility are inhibited, leading to toxic effects that kill cells (Jayanthi et al., 2013; Novita, 2016).

Other compounds work in a complementary manner. Tannins, which are polar polyphenols, inhibit extracellular enzymes and bind to substrates important for bacterial growth, even exhibiting antiviral and antifungal properties (Patil et al., 2015). Alkaloids interfere with the constituent components of peptidoglycan so that the cell wall loses its rigidity and the cell dies in a hypotonic environment. Saponins lower the surface tension of the cell

wall and increase membrane permeability, while steroids disrupt the stability of the lipid membrane to the point of triggering cell leakage (Huda et al., 2019). The combination of these various mechanisms is thought to work synergistically so that the extract is able to inhibit two bacteria with different cell wall types.

The greater resistance to *E. coli* (7.0 mm) than to *P. acnes* (5.7 mm) can be explained by the difference in the cell wall structure of the two bacteria. Gram-positive bacteria such as *P. acnes* have a thick, stiff peptidoglycan layer reinforced with teic acid, while Gram-negative bacteria such as *E. coli* have a thinner peptidoglycan. According to Radji et al. (2011), This thinner structure under certain conditions makes the cell more susceptible to damage when exposed to certain antibacterial compounds. In addition, differences in the composition of the target compounds on the membrane and the diffusion ability of each metabolite can affect the size of the barrier zone formed in each bacterium.

Interestingly, although the concentration of the extract (100%) is much higher than that of amoxicillin (30%), the inhibitory zone of the extract remains slightly below the comparator. This is natural because amoxicillin is a pure antibiotic with a specific target of action on cell wall synthesis, while extracts are a complex mixture of various compounds with relatively low levels of each and must diffuse together through the media. The small difference between the extract and the antibiotic actually shows that the antibacterial potential of hyacinth leaf extract is promising enough to be further developed, for example through fractionation or purification of active compounds to increase its effectiveness.

These findings are in line with previous studies. Susmitha et al. (2019) Firman et al. (2024) Firman et al. (2024)*P. acnes*. Sidharta et al. (2021) and Juliatri et al. (2023) prove the effectiveness of the extract against *Porphyromonas gingivalis*, while Dwiningrum et al. (2025) shows the activity of the extract cream against *Staphylococcus aureus*. The consistency of the results across studies confirms that the secondary metabolite content of water hyacinth is indeed responsible for its antibacterial activity. Quantitatively, the amount of inhibition zones in these studies varied according to the concentration and form of the preparation. Susmitha et al. (2019) recorded inhibition zones of 9.3 mm, 10.8 mm, and 15.2 mm respectively at concentrations of 5%, 10%, and 15% against acne-causing bacteria. Firman et al. (2024) obtained a zone of 15.2 mm against *P. acnes* and 16.7 mm against *Staphylococcus aureus* at a gel concentration of 15%, while Dwiningrum et al. (2025) reported cream inhibition zones of 3.81 mm and 3.69 mm (weak category) in the initial two formulations and 5.95 mm in the 30% concentrated formulation. This comparison confirms that the concentration and shape of the preparation greatly determine the amount of activity produced.

It should be noted that some of the literature has found that Gram-positive bacteria are more sensitive to antibacterial compounds because they do not have an outer membrane as an additional barrier. In this study, *Gram-negative E. coli* showed a larger inhibition zone, so the results were not always uniform with the general pattern. This difference reminds us that the sensitivity of bacteria is not determined by the type of Gram alone, but also by the composition of the extract's active compounds, their affinity for targets on the membrane, and their diffusion ability in the media. Therefore, the interpretation of the difference in the inhibition zone should be carried out carefully and supported by further testing such as the determination of the Minimum Inhibition Concentration and the Minimum Kill Concentration.

From a therapeutic development perspective, the extract's ability to inhibit gastrointestinal pathogens as well as skin pathogens expands its possible applications, for example as a candidate for topical anti-acne preparations and natural sanitizing agents. However, the transition from in vitro activities to real applications requires stages of safety testing, preparation stability tests, and standardization of active compound levels for consistent product quality and effectiveness.

The difference in the size of the barrier zone between studies can be traced from a number of factors. Lingga dan Rustama (2005) states that increased concentration of extracts tends to enlarge the inhibition zone. Other factors include the origin of the sample and the environmental conditions where it is grown that affect the secondary metabolite levels, the type of solvent and extraction method, the test method (well or disc), and the sensitivity of the test bacterial strain. In this study, a single concentration of 100% was used so that it could not describe the dose-response relationship; This is one of the limitations that need to be refined in follow-up research with multi-level concentration variations.

The extract's ability to inhibit both *E. coli* (Gram-negative) and *P. acnes* (Gram-positive) is an important indication that hyacinth leaf extract has a broad spectrum of activity. Broad-spectrum antimicrobials are able to affect a wide range of bacteria with different cell wall types (Ryan et al., 2014). This broad spectrum is advantageous in terms of therapeutic coverage, but in clinical use it needs to be accompanied by caution because broad-spectrum agents have the potential to affect the normal flora of the body. For the context of herbal medicine development, this trait shows the flexibility of the extract's utilization against more than one pathogen target. Practically, the results of this study provide two contributions at once. First, from a pharmacological perspective, water hyacinth leaves have proven to have the potential to be a natural source of antibacterial that can be an alternative in the midst of increasing antibiotic resistance. Second, from an environmental perspective, the use of aquatic

weeds which has so far only been seen as detrimental can provide economic added value while supporting the management of aquatic ecosystems such as Lake Tondano. This approach to weed valorization is in line with the principle of sustainable resource utilization.

The position of these findings becomes clearer when compared to other plant extracts tested against *E. coli*. Dewi Yani et al., (2024) reported the ethanol extract activity of enau root against *E. coli* and *Staphylococcus aureus*, while Umar et al., (2023) showed the inhibition of *Eucheuma spinosum* red seaweed extract against *E. coli* by the sumpray diffusion method. The medium category inhibition shown by hyacinth leaf extract places it as a competitive natural antibacterial candidate, especially since its raw materials are abundant and can be obtained without cultivation costs. The relevance of the findings to the local context is also worth underlining. Maradesa et al., (2020) detecting *E. coli contamination* in water sources in the North Sulawesi region, which illustrates the still high risk of exposure to this bacteria in the community. The availability of natural antibacterial agents based on water hyacinths that grow in the same lake opens up opportunities for solutions based on local resources, both for water sanitation purposes and the development of value-added health products.

This research has several limitations. The concentration of the extracts tested was only one level (100%), the analysis was quantitative descriptive without inferential statistical tests, and no quantitative phytochemical screening was carried out as well as the determination of the Minimum Inhibitory Concentration (KHM) and Minimum Kill Concentration (KBM). Therefore, further research is recommended to test for multi-level concentration variations, fractionation and identification of active compounds, determine KHM/KBM, and expand the spectrum of test bacteria so that the potential of hyacinth leaves as raw materials for natural medicines can be mapped more comprehensively.

5. CONCLUSIONS AND SUGGESTIONS

Ethanol extract of water hyacinth leaves (Eichhornia crassipes) has an inhibition against the growth of Escherichia coli and Propionibacterium acnes. At 100% concentration, the extract yielded an average inhibition zone of 7.0 mm against E. coli and 5.7 mm against P. acnes, both of which are classified as moderate by classification Davis & Stout (1971). The extract's ability to inhibit both Gram-negative and Gram-positive bacteria indicates a broad spectrum of activity. Thus, water hyacinth leaves have the potential to be developed as a natural antibacterial source, as well as an opportunity for productive use of aquatic weeds. Further research is recommended using concentration variation, fractionation of active compounds, determination of KHM/KBM, and testing for other pathogenic bacteria.

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