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Antibacterial Activity Testing of Methanol Extract of Yellow Rope Barb (*Anamirta cocculus*)

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Abstract: Indonesia has abundant natural wealth. One of the endemic plants of Papua which is often used in traditional medicine is yellow rope (*A. cocculus*). This study aims to determine the antibacterial activity of methanol extract of yellow rope bark (*A. cocculus*) against *P. acnes, S. aureus,* and *E. coli* bacteria using an experimental microbiological approach in the laboratory, and the experiment model may also serve as a contextual laboratory-based learning resource in microbiology education. The antibacterial activity test was carried out using the disc diffusion method which was made in 3 concentrations, namely 5%, 10% and 15%. The results of measuring the diameter of the inhibition zone showed that each concentration was able to inhibit the growth of P. acnes bacteria with an average diameter of 2,816 mm (Weak), 6,733 mm (Medium), 9,333 mm (Medium), and control (+) clindamycin 2 µg 14,483 mm (Strong). S. aureus bacteria with an average diameter of 3.133 mm (Weak), 6.35 mm (Medium), 8.235 mm (Medium) and control (+) clindamycin 2 µg 20.97 mm (Very strong). E. coli bacteria showed an average diameter of 3.875 mm (Weak), 6.175 mm (Medium), 8.566 mm (Medium) and control (+) ciprofloxacin 5 µg 20.783 mm (very strong). This research shows that the methanol extract of yellow stem bark (*A. cocculus*) has antibacterial activity.

Keywords: *A. cocculus*; Antibacterial; *E. coli*; *P. acnes; S. aureus*

Introduction

Indonesia is a large country that has abundant natural wealth. One form of natural wealth is various types of plants with varying sizes, from small to large. These plants have economic potential and can be used as raw medicinal materials. The research process begins with an empirical study regarding using plants as medicine. Research conducted by the Indonesian people regarding the use of medicinal plants from generation to generation is the first step in exploring the potential of these plants (Edy & Parwanto, 2019). Apart from that, research on chemical content, healing potential, toxicity properties, and formulation into medicines are some of the main focuses in research on plants that are believed to have medicinal properties (Edy, 2022).

Infectious disease is a type of disease caused by pathogenic bacteria that enter and multiply in the body. This disease is one of the leading causes of death throughout the world, including in Indonesia. One way to treat patients with infectious diseases is through antibiotics. Examples of several bacteria that can cause infections include *P. acnes*, *S. aureus*, *and E. coli* (Hafifah, 2022).

Antibiotics play an important role in eliminating infection-causing bacteria. However, the emergence of antibiotic resistance is a new problem in infection therapy, so it is necessary to develop new drugs made from natural ingredients to overcome this problem. Natural sources such as plants have the potential to be used as raw materials for the development of new drugs because the phytochemical content in these plants has various pharmacological activities, including

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antibacterial. In addition, administering antibacterials derived from natural ingredients is expected to minimize the side effects usually caused by chemical antibiotics (Putri, 2021).

Antibacterials can kill or inhibit the growth of pathogenic bacteria that cause infections. This antibacterial substance can be isolated from synthesizing secondary metabolites in microbes, animals, or plants. Antibacterial substances isolated from plants are generally used as alternatives in medicine. The antibacterial properties of these plants are also used in traditional medicine (Hafifah, 2022).

One of the endemic plants of Papua that has potential as a medicine used in traditional medicine is yellow rope (*Anamirta cocculus*), which has long been used traditionally to treat malaria and contains interesting active compounds (Ratnasari, 2018). Yellow rope (*A. cocculus*) contains various active compounds, such as alkaloids, saponins, and flavonoids. Previous research has identified the presence of alkaloids in the roots and stems of this plant, according to research (Hardiansyah et al., 2024). The yellow rope bark (*A. cocculus*) methanol extract was positive for containing flavonoids, alkaloids, terpenoids, and tannins. Although yellow string has been used traditionally in medicine, research on the antioxidant potential of its bark is limited (Erawati et al., 2024).

Alkaloids can inhibit bacterial growth through various mechanisms. Research by Karunakaran et al. (2022), shows that alkaloids can interfere with the synthesis of nucleic acids and proteins, damage bacterial cell membranes and walls, and disrupt the metabolism of the bacteria themselves. One way this compound works is by inhibiting the formation of peptidoglycan in cell walls, which prevents the cell wall layers from forming perfectly. This results in inhibition of the function of the FtsZ protein in the cell division process. Furthermore, alkaloids can disrupt bacterial metabolism, with ATP (adenosine triphosphate) as the primary target. ATP plays a crucial role in several enzymatic reactions, so inhibition of ATP can hurt bacterial metabolism, leading to their biological death (Shari, 2024).

The mechanism of action of flavonoid compounds as antibacterials can be divided into three main ways. First, flavonoids inhibit nucleic acid synthesis, disrupting cell membrane function. Second, these compounds inhibit bacterial cell metabolism (Mutmainnah et al., 2020). Flavonoid compounds have phenyl rings (A and B), which can interact with hydrogen bonds, forming complexes with nucleic acid bases. This results in interference in the DNA and RNA synthesis process in bacteria. Flavonoid compounds can inhibit cytochrome C reductase activity, which has an impact on blocking metabolic processes. They also disrupt energy metabolism by blocking oxygen use, weakening the bacteria's ability to survive (Nomer et al., 2019).

Terpenoid compounds work as antibacterial agents by damaging membranes, which is what lipophilic chemicals do. When terpenoids interact with porins (transmembrane proteins) in the bacterial cell wall's outer membrane, they create potent polymer bonds that harm the porins and lessen the bacterial cell wall's permeability. Bacterial cells thus go hungry and either stunt or die (Amalia et al., 2017).

Tannins have almost the same antibacterial effect as terpenoids, exerting osmotic or physical pressure on bacterial cell walls, leading to cell lysis. In addition, tannin compounds can inactivate enzymes and adhesins in bacterial cells and interfere with protein transport across cell membranes. It is known that tannins can interfere with polypeptide formation, so cell walls do not form ultimately (Sulaiha et al., 2022).

In addition to contributing to the development of science, this research can also help science education by providing contextual materials for biology laboratory activities, especially those related to natural antibacterial agents.

Method

Research Procedures

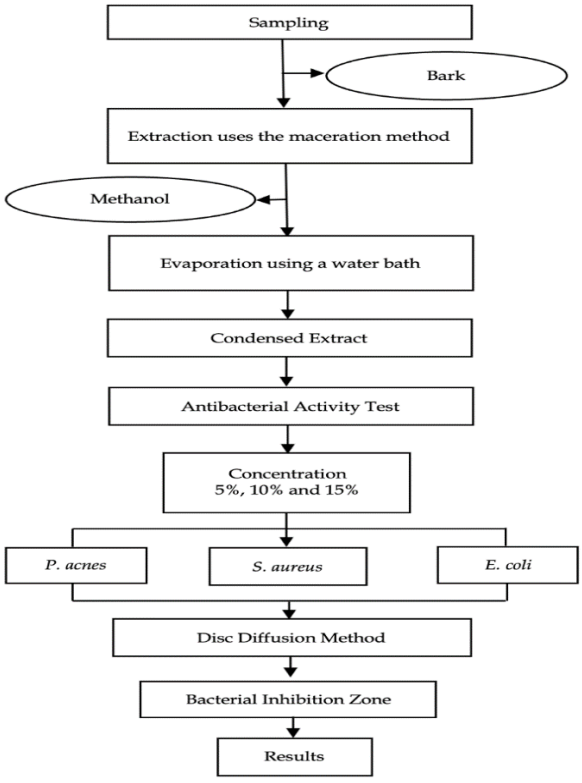

Figure 1. Research procedures

Tools and Materials

The tools used in this research were aluminium foil, autoclave (All American), sieve, blender, bunsen, stirring rod, glass bottle, petri dish (*Pyrex*), funnel, Erlenmeyer (*Pyrex*), beaker (*Pyrex*), measuring cup (*Pyrex*), hand scoop, hot plate, incubator, oscillating needle, vernier caliper, cotton, label paper, filter paper, disc paper, laminar airflow (BIOBASE -V1300), micro poppet, dropper pipe, tweezers, plastic wrap, test tube rack, marker, mattress strap, analytical scale, test tube, jar, and water bath. The materials used in this research were 2 µg clindamycin disk antibiotics (oxoid CT0064B), 5 µg ciprofloxacin antibiotics (oxoid CT0425B), aqua pro injection (ikapharmindo), sterile quads (Otsuka), *P. acnes* bacteria, *S. aureus* bacteria, *E. coli* bacteria, yellow tali bark extract (*A. cocculus*), methanol, nutrient agar (NA) media (oxoid CM0003), (NaCl) sodium chloride 0.9%.

Sample Preparation

Yellow rope bark (*A. cocculus*) samples were taken in the East Misool area, Raja Ampat Regency. The sample used in this research was yellow rope bark (A. cocculus), which was taken in the morning. After the sample was obtained, the stages of wet sorting, washing, cutting, drying, and dry sorting were carried out; the sample was ground using a blender and weighing. Next, extraction is carried out using the maceration method to obtain a thick extract. After evaporation, it produces a dry extract, the soaking is calculated, and then fractionation of the yellow string bark extract is carried out using methanol solvent (Hardiansyah et al., 2024).

Sample Processing

A sample of yellow rope (*A. cocculus*) 5 kg was cleaned of impurities, washed, drained, and dried in an oven at 50°C for 8 hours. Yellow Rope Bark (*A. cocculus*) was ground using a blender until powder was obtained from Yellow Rope Bark (*A. cocculus*) to be used as a sample (Laswati, 2017).

Making Extracts

500 grams of yellow rope bark simplicia (*A. cocculus*) were put into a jar container and then soaked in 2000 mL of methanol solvent in a ratio of 1:4 until the simplicial was completely submerged. The maceration container was closed and left for 3x24 hours, stirring occasionally, and protected from direct sunlight (Fabanyo et al., 2023).

Making Nutrient Agar Media

A total of 7 grams of Nutrient Agar was dissolved in 250 mL of sterile distilled water using an Erlenmeyer flask. Next, homogenize on a hot plate until it boils. The boiling Nutrient Agar (NA) is covered with aluminium foil. Nutrient Agar media was sterilized in an autoclave at 121°C for 15 minutes. Then, pour 5 mL into a sterile test tube. The media is poured in warm conditions (40-45°C) and then tilted at a slope of 30-45°C. The mouth of the test tube was blocked with cotton wrapped in sterile gauze, then waited until the medium solidified. Media preparation was carried out aseptically in laminar airflow (Usman, 2020).

Preparation of Test Bacteria

P. acnes, *S. aureus*, and *E. coli* bacteria. Those obtained from pure culture were taken one dose each and then inoculated by streaking on the Nutrient medium so that it was slanted. After that, the inoculation was incubated at 37°C for 24 hours. After the test bacteria have been rejuvenated for 24 hours, a sterilized tube needle is used to collect the bacteria. Next, the bacteria were incubated in a test tube containing 10 mL of 0.9% NaCl solution. This process is continued with homogenization until the turbidity of the solution reaches a level equivalent to the McFarland standard (3 x 108 CFU/ml) (Usman, 2020).

Antibacterial Activity Test

Antibacterial activity testing was carried out using the agar diffusion method using discs. The initial step is to prepare a bacterial suspension. Next, prepare the agar nutrient media that will be used. Pour about 15-20 mL of Nutrient agar medium into a petri dish and allow it to solidify. After the medium hardens, one dose of bacteria is inoculated, which is measured according to McFarland standards. Apply evenly using a sterile cotton swab with a zig-zag movement on the surface of the solidified media. Wait a few minutes for the bacterial suspension to soak into the agar medium.

The sterilized disc is then transferred aseptically using sterile tweezers into the previously prepared test solution. The test solution consists of a control (-), aqua pro injection, and an extract suspension at concentrations of 5%, 10%, and 15%. This process is left for 15 minutes until it reaches a saturation point. After that, the soaked discs were aseptically transferred using sterile tweezers to Nutrient Agar (NA) medium, which had been inoculated with *P. acnes*, *S. aureus*, and *E. coli*. The transfer process was carried out sequentially, starting from the control disk (+) containing clindamycin disk 2 µg and ciprofloxacin 5 µg, followed by the control disk (-) containing aqua pro injection. Then, discs containing methanol extract solutions from Yellow Rope Bark (A. cocculus) in various concentrations were placed in the same petri dish, with a distance between the discs of around 1-2 cm from the edge of the petri dish. After all treatments, the petri dish was incubated for 1 x 24 hours at 37°C. Replication was carried out three times.

Treatment to determine antibacterial activity was carried out aseptically in laminar airflow (Putri, 2021).

Observation and Measurement

After a day, the diameter of the clear zone that had developed around the disc was then measured with a calliper. The formula below was used to determine the inhibition zone's diameter (Fiana et al., 2020).

$$\text{Inhibition zone} = \frac{(Dv-Dc)+(Dh-Dc)}{2} \quad (1)$$

Information:

D_v : Inhibition Zone

D_h : Horizontal Diameter

D_c : Disc Diameter

Data Analysis

The data collection method applied in this research is through experimental tests that rely on quantitative data. This quantitative data includes antibacterial power resulting from antibacterial activity testing.

Results and Discussion

Extraction Results

The process of extracting the bark of yellow rope (A. cocculus) using the maceration method using methanol solvent produces a thick extract with a soaking value that can be seen in (Table 1).

Table 1. Results of Extraction of Yellow Rope Bark (A. cocculus)

Simplicity	Sample weight (Kg)	Powder weight (grams)	Extract weight (grams)	Soaking (%)
Yellow Rope Bark	5 kg	500 grams	59 grams	11.8%

The results obtained in Table 1 showed that Simplicia powder weighed 500 grams, and the thick extract weighed 59 grams. From the thick extract, the yield of methanol extract from yellow rope bark (*A. cocculus*) was 11.8%. According to the Indonesian pharmacopoeia, which states that the yield requirement is not less than 10%, the higher the yield value indicates that the extraction method used is more effective (Senduk et al., 2020).

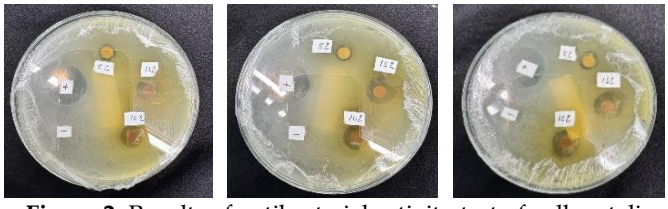

Figure 2. Results of antibacterial activity test of yellow tali bark extract against bacteria *p. acnes*

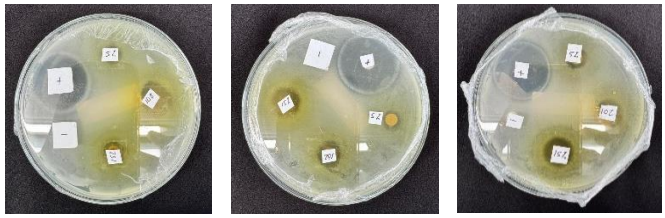

Figure 3. Results of antibacterial activity test of yellow tali bark extract against bacteria *S. aureus*

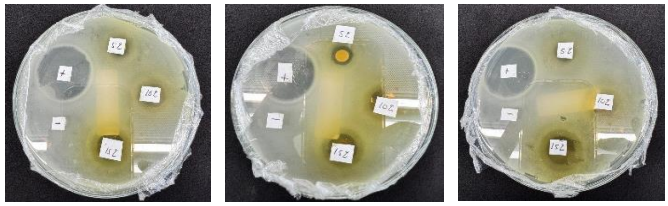

Figure 4. Results of antibacterial activity test of yellow tali bark extract against bacteria *E. coli*

Antibacterial Activity Test Results

Testing the antibacterial activity of yellow rope bark (*A. cocculus*) against *P. acnes*, *S. aureus*, and *E. coli* bacteria. This was carried out using the disc diffusion method on extracts of yellow string bark with varying concentrations of 5%, 10% and 15% to determine the inhibitory zone produced with the comparison used as a positive control, namely clindamycin disk 2 µg, ciprofloxacin 5 µg and the negative control was aqua pro injection, the appearance of the inhibitory zone was marked by the formation of a clear zone around the disk.

In Figure 5, the antibacterial test results show that the higher the concentration of yellow rope bark extract (*A. cocculus*), the larger the diameter of the inhibition zone against all types of bacteria tested. A concentration of 15% showed the highest effectiveness compared to concentrations of 5% and 10%, with the largest inhibition zone found in *P. acnes* bacteria, followed by *S. aureus* and *E. coli*. This shows that *P. acnes* is the most sensitive bacteria to the active compounds in the extract, while *E. coli* is the most resistant bacteria among the three. This difference in antibacterial activity is likely due to differences in cell wall structure. Gram-negative bacteria such as *E. coli* have a thicker and more complex lipopolysaccharide layer, making them more resistant to antibacterial compounds. The variation in inhibition zones could be explored by students to analyze the correlation between extract concentration and antibacterial efficacy, enhancing their data interpretation skills.

Research examining the antibacterial activity of tali king (*A. cocculus*) bark extract showed that the methanol extract could inhibit bacterial growth. *Propionibacterium acnes* bacteria showed an inhibition zone in the methanol extract by forming an inhibition zone around the disc. At a concentration of 5% methanol extract, replication 1 obtained a value of 2.55 mm, replication 2 obtained a

value of 3.7 mm, and replication 3 obtained a value of 2.2 mm, with an average value of 2.816 mm in the (Weak) category. At a concentration of 10% methanol extract with replication 1, a value of 6.95 mm was obtained, replication 2 obtained a value of 6.8 mm, and replication 3 obtained a value of 6.45 mm, with an average value of 6.733 mm in the (Medium) category. Furthermore, at a concentration of 15% methanol extract with replication 1, a value of 7.6 mm was obtained, replication 2 obtained a value of 9.85 mm, and replication 3 obtained a value of 10.55 mm, with an average value of 9.333 mm in the (Medium) category. In the positive control using the antibiotic *clindamycin* 2 µg with replication 1, a value of 14.15 mm was obtained, replication 2 obtained a value of 14.65 mm, and replication 3 obtained a value of 14.65 mm, with an average value of 14.483 mm in the (Strong) category. Meanwhile, in the negative control Aqua pro injection, there was no zone of inhibition around the disc.

Table 2. Results of Observations of the Inhibition zone of Methanol Extract of Yellow Rope Bark (*A. cocculus*)

Concentration (%)		Average Zone of Inhibition		
		P. acnes	*S. aureus*	*E. coli*
	R 1	2.55 mm	2.3 mm	3.47 mm
5 %	R 2	3.7 mm	2.75 mm	3.35 mm
	R 3	2.2 mm	4.35 mm	4.8 mm
Average		2.82 mm	3.13 mm	3.87 mm
Category		Weak	Weak	Weak
	R 1	6.95 mm	5.9 mm	5.95 mm
10 %	R 2	6.8 mm	5.75 mm	6.22 mm
	R 3	6.45 mm	7,4 mm	6.35 mm
Average		6.73 mm	6.35 mm	6.17. mm
Category		Medium	Medium	Medium
	R 1	7.6 mm	9.2 mm	10.02 mm
15 %	R 2	9.85 mm	8.12 mm	8.32 mm
	R 3	10.55 mm	7.38 mm	7.35mm
Average		9.333 mm	8.23 mm	8.57 mm
Category		Medium	Medium	Medium
Control (-) Aqua pro injection	R 1 2 3	0 mm	0 mm	0 mm
Control (+)	R 1	14.15 mm	20.9 mm	
clindamycin 2 µg	R 2	14.65 mm	20.85 mm	
	R 3	14.65 mm	21.17 mm	
Average		14.48 mm	20.97 mm	
Category		Strong	Very strong	
Kontrol (+)	R 1			19.9 mm
ciprofloxacin 5 µg	R 2			22.05 mm
	R 3			20.4mm
Average				20.78 mm
Category				Very strong

Information: R= Replication 1, 2 and 3; Control (+)= clindamycin disk 2 µg and ciprofloxacin 5 µg; Control (-)= aqua pro injection

Staphylococcus aureus bacteria showed an inhibition zone in the methanol extract by forming an inhibition zone around the disc. Methanol extract with a concentration of 5% methanol extract with replication 1 obtained a value of 2.3 mm. In contrast, replication 2 obtained a value of 2.75 mm, and replication 3 obtained a value of 4.35 mm, with an average value of 3.133 mm in the (Weak) category. At a concentration of 10% methanol extract with replication 1, a value of 5.9 mm was obtained, replication 2 obtained a value of 5.75 mm, and replication 3 obtained a value of 7.4 mm with an average value of 6.35 mm in the (Medium) category. Furthermore, at a concentration of 15% methanol extract, replication 1 obtained a value of 9.2 mm, replication 2 obtained a value of 8.125 mm, and replication 3 obtained a value of 7.38 mm, with an average value of 8.235 mm in the (Medium) category. In the positive control using the antibiotic *clindamycin* 2 µg with replication 1, a value of 20.9 mm was obtained, replication 2 obtained a value of 20.85 mm, and replication 3 obtained a value of 21.175 mm, with an average value of 20.97 mm category (Powerful). Meanwhile, in the negative control, Aqua pro injection, there was no zone of inhibition around the disc.

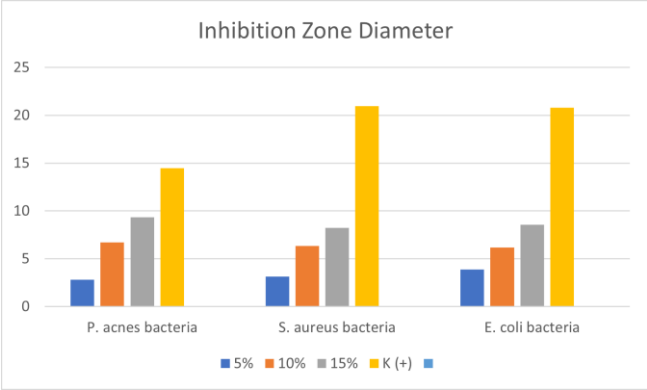

Figure 5. Bar chart of the results of observations of the inhibition zone of methanol extract of yellow rope bark (*A. cocculus*) against *P. acnes, S. aureus, and E. coli*

Furthermore, *Escherichia coli* bacteria showed the existence of an inhibition zone in the methanol extract by forming an inhibition zone around the disc. Methanol extract with a concentration of 5% methanol extract with replication 1 obtained a value of 3.475 mm. In contrast, replication 2 obtained a value of 3.35 mm, and replication 3 obtained a value of 4.8 mm, with an average value of 3.875 mm in the (Weak) category. At a concentration of 10% methanol extract with replication 1, a value of 5.95 mm was obtained, replication 2 obtained a value of 6.225 mm, and replication 3 obtained a value of 6.35 mm, with an average value of 6.175 mm in the (Medium) category. Furthermore, at a concentration of 15% methanol extract, replication 1 obtained a value of 10.025 mm, replication 2 obtained a value of 8.325 mm, and replication 3 obtained a value of

7.35 mm, with an average value of 8.566 mm in the (Medium) category. In the positive control using the antibiotic ciprofloxacin 5 µg with replication 1, a value of 19.9 mm was obtained, replication 2 obtained a value of 22.05 mm, and replication 3 obtained a value of 20.4 mm, with an average value of 20.783 mm category (Very Strong). Meanwhile, in the negative control, Aqua pro injection, there was no zone of inhibition around the disc.

The antibacterial activity test showed different variations against each type of bacteria, namely *P. acnes*, *S. aureus,* and *E. coli*. This difference is influenced by the amount of secondary metabolite content and the concentration contained in the sample. In addition, the diffusion speed of antibacterial agents in the agar medium also plays an important role. Because more active chemicals are contained at higher concentrations, the diffusion rate improves (Suhaera et al., 2022).

Plant secondary metabolites can vary depending on several factors, including the growing environment, species differences, physiological conditions (old and young), and chemical properties. Environmental factors such as air temperature, relative humidity, solar radiation, wind, soil temperature, water availability, and light intensity during photosynthesis are important in regulating plant physiology, anatomy, and life cycles. Plants tend to adapt to natural changes by adapting. These environmental factors have a significant effect on the production of secondary metabolites of the yellow rope bark plant (*A. cocculus*) (Nurulita, 2017).

The inhibition zone formed is related to the group of secondary metabolite compounds, flavonoids, alkaloids, terpenoids, and tannins. This indicates. Research by Karunakaran et al. (2022), shows that alkaloids can interfere with the synthesis of nucleic acids and proteins, damage bacterial cell membranes and walls, and disrupt the metabolism of the bacteria themselves. One way this compound works is by inhibiting the formation of peptidoglycan in cell walls, which prevents the cell wall layers from forming perfectly. The alkaloids in the bark of the yellow string stem have antibacterial properties against gram-positive and gram-negative bacteria by inhibiting bacterial DNA (Maisarah et al., 2023).

Flavonoids exert antibacterial effects by forming plant complexes with extracellular proteins and solvents. As a result, flavonoids can damage bacterial cell membranes, leading to the release of intracellular compounds. Flavonoid compounds inhibit bacterial growth through various mechanisms. One of these mechanisms damages the bacterial wall and permeability of microsomes and lysosomes (Rani et al., 2023). Flavonoids trigger structural changes in bacteria, which ultimately cause toxic effects due to these structural changes. The flavonoid group of compounds can denature proteins, thereby damaging bacterial cell walls and allowing hydroxyl groups to penetrate damaged bacterial cell walls. Lipophilic flavonoid compounds have antibacterial capabilities by damaging bacterial cell membranes and walls, which can ultimately result in cell death (Kaban et al., 2023).

The antibacterial properties of terpenoid compounds are based on the harm that lipophilic chemicals cause to membranes. When terpenoids interact with porins (transmembrane proteins) in the bacterial cell wall's outer membrane, they create potent polymer bonds that harm the porins and lessen the bacterial cell wall's permeability. Bacterial cells thus go hungry and either stunt or die. The antibacterial activity test aims to determine the ability of yellow rope bark extract (*A. cocculus*) to inhibit bacterial growth. The formation of a clear zone around the disc indicates inhibitory ability. This clear zone indicates the antibacterial activity of the extract tested. The bacteria used in this research were *P. acnes*, *S. aureus*, and *E. coli*. The choice of this bacteria as the test bacteria was because this bacteria is a type of gram-positive *P. acnes* bacteria, which is associated with chronic inflammatory processes in the skin glands and plays a role in the development of acne (Sifatullah & Zulkarnain, 2021). *E. coli* bacteria are among the most common nosocomial pathogens that cause urinary tract infections. *S. aureus* bacteria are infectious agents that are significantly responsible for mortality and morbidity (Mulangsri et al., 2022).

Conclusion

Yellow stem bark (*A. cocculus*) methanol extract exhibits antibacterial activity at 5%, 10%, and 15% concentrations. The average diameter of the inhibitory zone for each concentration of *P. acnes* bacteria was 2.82 mm (weak category), 6.73 mm (medium category), and 9.33 mm (medium category). The inhibition zone of *S. aureus* bacteria is 3.13 mm (weak category), 6.35 mm (medium category), and 8.23 mm (medium category) on average. In addition, the average diameter of the inhibitory zone of *E. coli* bacteria falls into one of three categories: 3.87 mm (weak), 6.17 mm (medium), and 8.57 mm (medium). Yellow rope bark extract has been shown to have varying antibacterial activity, depending on the concentration and type of bacteria tested. A concentration of 15% was shown to be the most effective in inhibiting bacterial growth, with *P. acnes* as the most sensitive bacteria to the treatment. This difference in effectiveness indicates that the active compounds in the extract work differently according to the characteristics of each target bacteria. Therefore, this extract has the

potential to be developed as a natural antibacterial agent, especially to overcome bacteria that cause skin problems such as *P. acnes*. This research not only contributes to the scientific understanding of microbiology, but also has great potential to be applied as an experimental learning model, both in health education environments and in the community. Through direct practice, this activity can improve laboratory skills while deepening understanding of the role and function of microorganisms in everyday life.

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Author Contributions
Conceptualization, WON, and AMM; methodology, AMM, and LH; writing—preparation of the original draft, WON, AMM, and LH; writing—review and editing, WON, AMM, and LH. All authors have read and approved the published version of the manuscript.

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Conflict of interest
The author declares no conflict of interest.

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