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In Vitro Study of The Activity of Yellow Rope (*Anamirta cocculus*) Extract As An Antibacterial

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Abstrak: *A. cocculus* is an endemic plant to Papua, which is often used as medicine. This study aims to determine the antibacterial activity of the ethanol extract of *A. cocculus* stems against *P. acne, S. aureus*, and *E. coli* bacteria. This research also has the potential to be used as a contextual learning resource in biology classes, especially on the topic of antibacterial and secondary metabolite testing, using experimental methods. The antibacterial activity test was carried out using the disk diffusion method, which was performed in 3 concentrations, namely 5%, 10%, and 15%. The positive control used 2 µg of clindamycin antibiotic and 30 µg of chloramphenicol antibiotic; the negative control used Aqua Pro injection. Measurement of the diameter of the inhibition zone shows that *A. cocculus* extract can inhibit bacterial growth, concentration 5% (*P. acnes* = 9.18mm/medium; *S. aureus* = 9.17/medium, and *E. coli* = 3.91/weak), concentration 10% (*P. acnes* = 11.86mm/strong; *S. aureus* = 11.88/strong, and *E. coli* = 5.17mm/medium), and concentration 15% (*P. acnes* = 11.56/strong; *S. aureus* = 12.9/strong, and *E. coli* = 5.86/medium).

Kata kunci: Antibacterial; *Anamirta cocculus*; *Propionibacterium acnes*; *Staphylococcus aureus*; *Escherichia coli*

Introduction

Infectious diseases remain a significant challenge in the health sector, especially in developing countries (Savitri et al., 2019). Infections are caused by various microorganisms, especially pathogenic bacteria, often called disease germs. Examples of common bacteria include Propionibacterium acne, Staphylococcus aureus, and Escherichia coli. To address germs that lead to infections, various antimycobacterial have been developed, both at the cellular and molecular levels (Pratiwi 2017).

Antibacterial is a compound used to inhibit bacteria. Antibacterials are usually present in an organism as secondary metabolites. Antibacterial chemicals often work by causing cell damage, altering the permeability of membranes, interfering with protein synthesis, and preventing enzyme activity (Septiani *et al*., 2017).

The most significant potential natural resources in Indonesia are in Papua province (Sujarta *and* Dirgantara 2019). One plant that can be used as herbal medicine is yellow rope (*Anamirta cocculus*), a group of creeping plants with yellow stems. This bitter herbal plant has been used as a malaria medicine for generations in Papua (Taam et al., 2020).

Flavonoids and alkaloids are secondary metabolite compounds known to be found in yellow rope (*A. Cocculus*) (Erawati, Muslihin, *and* Hardia 2024). Previous research reported the content of flavonoids, alkaloids, terpenoids, and tannins (Hardiansyah *et al*., 2024). The general mechanism of action of antibacterials is to inhibit bacterial cell wall synthesis, disrupt cell membrane permeability, disrupt cell metabolism, damage nucleic acids, and impede cell protein synthesis (Sadiah *et al*., 2022).

Alkaloids typically work against bacteria by interfering with the peptidoglycan's constituent parts in bacterial cells. Cell death will result from incomplete formation of the bacterial cell wall layer (Siti *and* Lanny 2021), The antibacterial activity of flavonoids stems from

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their capacity to interact with bacterial cell membranes, affect their bioactivity, and decrease their fluidity—all of which are directly linked to cytoplasmic membrane disruption. To exert its antibacterial properties, tannins lyse bacterial cells. This occurs because the polypeptide walls of bacterial cells are targeted by tannins, which results in less-than-ideal cell wall development and bacterial cell death (Purba *et al*., 2020). However, it is thought that membrane-damaging lipophilic molecules mediate the antibacterial actions of terpenoid compounds (Amalia *et al*., 2017). This study also has the potential to be used as a contextual learning resource in biology classes, particularly on the topic of antibacterial testing and secondary metabolites.

Method

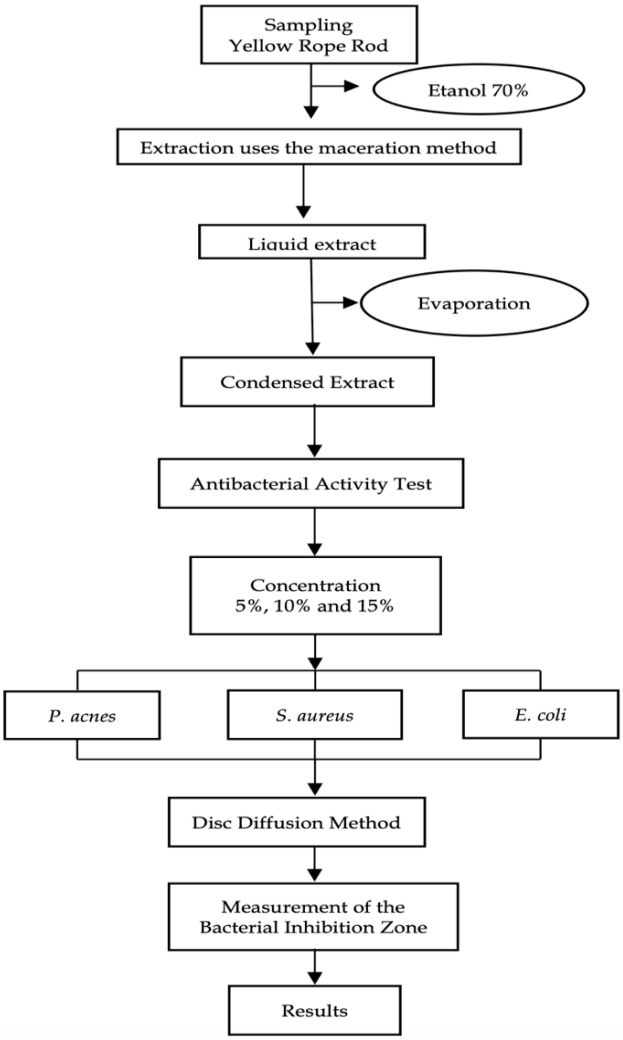

Figure 1. Research procedures

Tools and materials

The tools used in this research include: autoclave (All American No. 50X), petri dish, Erlenmeyer (pyrex), beaker (pyrex), hot plate, incubator, oscillating needle, vernier caliper, paper disc, laminar airflow (BIOBASE-V1300), micropipette, and water bath. The materials used in this study were the antibiotic clindamycin disk 2 µg (oxoid CT0064B), the antibiotic chloramphenicol disk 30 µg (oxoid CT0013B), aqua pro injection (ikapharmindo), sterile distilled water (otsuka), *P. acne* bacteria, *S. aureus* bacteria, *E. coli* bacteria, yellow rope (*A. cocculus*), 70% ethanol, nutrient agar (NA) media (oxoid CM0003), sodium chloride (NaCl) 0.9%.

Sample Preparation

Samples of yellow string stems (*A. cocculus*) taken from Misol Raja Ampat were first processed using the wet sorting method and then washed using running water. After that, the samples were chopped into small pieces and dried in an oven at 50℃ for 8 hours. After drying, the samples were sorted again in a dry state before finally being ground using a blender (Kusuma *et al*. 2020).

Yellow Rope Extraction

500 grams of yellow rope stem simplicia (*A. cocculus*) was put into the vessel for the maceration process. 70% ethanol was added until the simplicia was submerged entirely, and then the simplicia was left for 3 × 24 hours in a closed vessel and protected from light while occasionally stirring and filtering. After the first extraction, the dregs were remacerated again for 2 days using a new 70% ethanol solvent. The filtrate is then processed to produce a thick extract. Evaporation was carried out using a water bath at a temperature of 50℃ (Widayanti *et al*. 2023).

Making Nutrient Agar Media

An Erlenmeyer flask containing 7 grams of nutrient agar was used to dissolve it in 250 ml of sterile distilled water. The mixture was then homogenized on a hot plate until it boiled. After boiling, the nutrient agar (NA) was covered with aluminum foil. The nutrient agar media was sterilized in an autoclave at 121℃ for 15 minutes. (Arni, Irwandi, *and* Hardia 2024). After sterilization, 5 ml of the media is poured into a sterile test tube in warm conditions (40-45℃). The test tube is then tilted at an angle of 30-45°. The mouth of the tube was plugged with cotton wrapped in aluminum foil and left until the medium solidified. The entire process of making the media is carried out aseptically in laminar airflow (Usman 2020).

Preparation of Test Bacteria

One dose of *P. acnes, S. aureus,* and *E. coli* bacteria obtained from pure culture was then taken for each. The culture was inoculated by streaking on a nutrient agar medium. After that, the media was incubated at 37℃ for

1 × 24 hours. After the test bacteria have been rejuvenated for 24 hours, a sterile tube needle is used to collect the bacteria. Then, each type of bacteria was inoculated into a test tube containing 10 ml of 0.9% NaCl solution. Next, the mixture was homogenized until the turbidity was by the Mc Farland turbidity standard (3×10^8 CFU/ml) (Usman 2020).

Making Test Solutions

The negative control in this study used paper disks treated with aqua pro injection, while the positive control used 2 µg clindamycin antibiotic dist and 2 µg chloramphenicol antibiotic disks. Meanwhile, the concentration solution was made with 5%, 10%, and 15% yellow rope stem extract (*A. cocculus*), then dissolved in 10 ml of aqua pro injection and shaken until homogeneous (Arni et al., 2024).

Antibacterial Activity Test

Antibacterial activity was tested using the agar diffusion method with disk discs. First, a bacterial suspension is prepared. Next, the nutrient agar medium is prepared by pouring 15-20 ml into a petri dish and leaving it until it solidifies. After the medium hardens, each bacteria is inoculated using one dose, which has been measured according to McFarland standards. The smearing is carried out evenly using a sterile cotton swab in a zig-zag pattern over the surface of the solid media, and the suspension is left for a few minutes to absorb into the agar media.

The sterile disc is then aseptically transferred into the previously prepared test solution using sterile tweezers. The negative control consisted of paper discs treated with Aqua Pro injection, while the positive controls were clindamycin discs and chloramphenicol discs. Next, for the control treatment, the discs were soaked in the extract suspension with concentrations of 5%, 10%, and 15% for 15 minutes (until saturated). After that, the soaked discs were separated aseptically using sterile tweezers and placed into a nutrient agar medium containing P. acnes, S. aureus, and E. coli bacteria. This process begins with a positive control disc, a negative control, and finally, a disc with ethanol extract from yellow string stems (*A. cocculus*) with concentrations of 5%, 10%, and 15%. The discs were placed in a petri dish with a distance of 1-2 cm between the discs. After placing all the discs, the Petri dishes were incubated for 1 × 24 hours at 37℃, with replication 3 times. All antibacterial activity testing procedures were carried out aseptically in laminar airflow (Arni et al., 2024).

Observation and Measurement

After a day, the diameter of the clear zone that had developed around the disc was then measured with a calliper and the following formula was applied:

$$\text{Inhibition zone} : \frac{D1+D2}{2} - x \quad (1)$$

Information:
D1 : Vertical Diameter
D2 : Horizontal Diameter
X : Disc paper

Results and Discussion

Table 1. Yield results of yellow rope stem extract (*A. cocculus*)

Simplicia	Sample Weight (Kg)	Powder Weight (gram)	Extract Weight (gram)	Yield (%)
Yellow Rope Rod	1.5 kg	500 gram	56 gram	11.2

The results obtained in Table 1 showed that the weight of the simplicial powder was 500 grams, and the weight of the thick extract was 56 grams. From this extract, the yield of yellow rope (*A. cocculus*) stem extract was 11.2%. The higher the yield value, the more significant the amount of extract generated and the more successful the extraction method, according to the Indonesian Herbal Pharmacopoeia, which stipulates that the yield requirement is not less than 10% (Senduk et al.,2020).

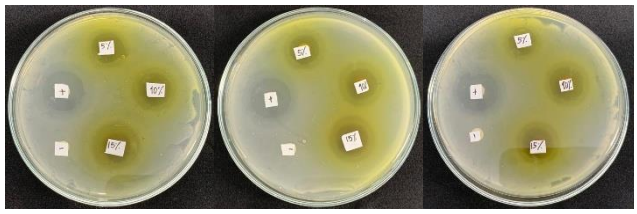

Figure 2. Test results of the antibacterial activity of yellow rope extract against *P. acnes*

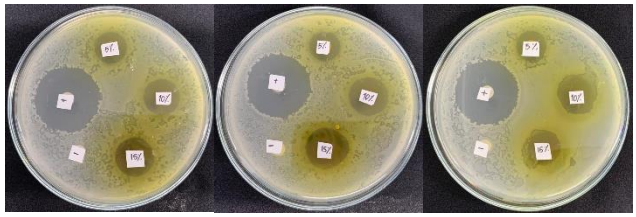

Figure 3. Test results of the antibacterial activity of yellow rope extract against *S. aureus*

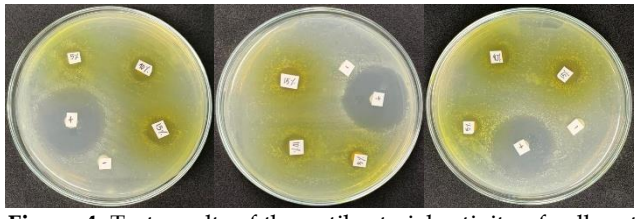

Figure 4. Test results of the antibacterial activity of yellow tali stem extract against *E. coli*

Table 2. Yellow rope stem (*A. cocculus*) 70% ethanol extract test results for antibacterial activity against *P. acnes, S. aureus*, and *E. coli* bacteria

Concentration (%)		Average Zone of Inhibition		
		P. acnes	*S. aureus*	*E. coli*
5 %	R 1	10.1 mm	9.1 mm	4.5 mm
	R 2	9.75 mm	9.32 mm	3.72 mm
	R 3	7.7 mm	9.1 mm	3.75 mm
Average		9.18 mm	9.17. mm	3.91 mm
Category		Medium	Medium	Weak
10 %	R 1	12.35 mm	11.05 mm	5.35 mm
	R 2	11.17 mm	12.7 mm	5,3 mm
	R 3	12.05 mm	11.9 mm	4,85 mm
Average		11.86 mm	11.88 mm	5.17 mm
Category		Strong	Strong	Medium
15 %	R 1	21 mm	12.35 mm	5.62 mm
	R 2	13.1 mm	14.05 mm	6.35 mm
	R 3	12.7 mm	12.3 mm	5.6 mm
Average		15.6 mm	12.9 mm	5.86 mm
Category		Strong	Strong	Medium
(-)	R 1	0 mm	0 mm	0 mm
(+) Clinda	R 1	23.5 mm	25,9 mm	
	R 2	17 mm	25.3 mm	
	R 3	17,9 mm	25.7 mm	
Average		19.47 mm	25.63 mm	
Category		Strong	Very strong	
(+) Kloram	R 1			20.9 mm
	R 2			19.75 mm
	R 3			24.5 mm
Average				21.72 mm
Category				Very strong

Information:
R : Replication
(-) : Negative Control (Aqua pro injeksi)
(+) Clinda : Positive Control (*Clindamycin disk* 2 µg)
(+) Kloram : Positive Control (*chloramfenikol disk* 30 µg)

In Table 2, the largest inhibition zone is shown by clindamycin and chloramphenicol as positive controls, and no inhibition zone was formed in the negative control Aqua Pro injection. As broad-spectrum antibiotics that can eradicate both gram-positive and gram-negative bacteria, clindamycin and chloramphenicol were selected as positive controls. If the diameter of the inhibitory zone produced by bacteria is less than 20 mm, they are considered resistant (Utomo *et al*. 2018). Clindamycin produced an average diameter value of 19.466 mm against *P. acnes* bacteria and 25.63 mm against S. aureus bacteria in this investigation. Chloramphenicol's average diameter value against E. coli bacteria is 21.72 mm.

The parameter measured in the antibacterial activity test forms an inhibitory zone around the paper disc soaked in yellow rope stem extract (*A. cocculus*). The inhibition zone is a clear area where bacteria do not grow. The research results can be seen after the paper discs are soaked in solutions of various concentrations. The disc paper is placed on nutrient agar (NA) media, which has been inoculated with a bacterial suspension. Results can be observed after the media is incubated at 37℃ for 24 hours to see bacterial growth, which is indicated by forming a clear zone around the paper disc.

According to Table 2, the inhibition zone for *P. acnes* bacterium at a 5% concentration is 9.18 mm on average. The inhibition zone that forms at a 10% concentration is 11.86 mm in diameter, and at a 15% concentration, it is 15.6 mm. For *S. aureus* bacteria, the average inhibitory zone value was 9.17 mm at 5% concentration, 11.88 mm at 10% concentration, and 12.9 mm at 15% concentration. In contrast, the average inhibitory zone value for *E. Coli* bacteria was 3.99 mm at 5% concentration, 5.17 mm at 10% concentration, and 5.86 mm at 15% concentration.

The bacterial inhibition power is classified as weak if the inhibition zone formed is less than 5 mm in diameter, medium if the inhibition zone formed is between 5 and 10 mm in diameter, strong if the inhibition zone formed is between 11 and 20 mm, and very strong if the inhibition zone formed is greater than 20 mm. These standards indicate that yellow rope stem extract (*A. cocculus*) has medium-strong antibacterial activity against *P. acnes* bacterium. The medium-substantial parameters also apply to *S. aureus* bacterium. Furthermore, the weak-moderate threshold applies *to E. coli* bacterium. Many variables, including the type of bacteria being suppressed, extract concentration, chemical composition, and extract diffusion power, affect antibacterial efficacy (Goetie et al., 2022).

Alkaloids, flavonoids, terpenoids, and tannins are among the secondary metabolites found in yellow rope stem extract (A. cocculus), according to earlier studies *(Erawati et al., 2024; Hardiansyah et al*., 2024). The presence of secondary metabolite compounds in yellow rope stem extract (*A. Cocculus*) can be related to the antibacterial properties of each compound, with different mechanisms. Alkaloids have antibacterial properties with a mechanism of action that inhibits cell wall synthesis, which will cause cell lysis, resulting in cell death. By creating complex molecules with soluble and extracellular proteins that contribute to bacterial cell membrane disruption and subsequent release of intracellular chemicals, flavonoids exhibit an antibacterial action (Maisarah *et al*., 2023; Putri *et al*., 2022). Porins, transmembrane proteins found in the bacterial cell wall's outer membrane, are reacted with by terpenoid chemicals to produce their antibiotic effects. Consequently, the porins are harmed by the formation of strong polymer linkages. Because damaged porins prevent bacterial cell walls from being permeable, bacteria are unable to obtain nutrients, which prevents them from growing or dying (Siti *and* Lanny 2021).

Tannins can also have antibacterial effects by blocking the movement of protein enzymes in the inner layers of cells and deactivating microbial cell adhesins and enzymes. Also, because of osmotic and physical pressure, tannins cause bacterial cells to lyse, which results in their death (Goetie *et al*., 2022).

These findings provide empirical data that can be transformed into student worksheets (LKPD) to explore experimental designs, the mechanism of action of plant-based antibacterials, and laboratory techniques in microbiology.

Conclusion

A. cocculus extract has the ability to inhibit bacterial growth, concentration 5% (*P. acnes* = 9.18mm/medium; *S. aureus* = 9.17/medium, and *E. coli* = 3.91/weak), concentration 10% (*P. acnes* = 11.86mm/strong; *S. aureus* = 11.88/strong, and *E. coli* = 5.17mm/medium), and concentration 15% (*P. acnes* = 11.56/strong; *S. aureus* = 12.9/strong, and *E. coli* = 5.86/medium). Moreover, the experiment model used in this study is adaptable for science education laboratories to enhance students' understanding of microbiological assays and ethnopharmacological research.

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Author Contributions
This research article was written by three researchers with their respective contributions: "Conceptualization, DT and AMM; methodology, DT and AMM; formal analysis, DT; data curation, AMM and LH; writing, preparation of original draft, DT, AMM and LH; writing, review, literature review, and editing, LH; All authors have read and approved the published version of the manuscript.

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Conflicts of Interest
No conflicts of interest are disclosed by the writers.

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