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Antibacterial Effectiveness Test of Wrap Leaf Extract (*Smilax rotundifolia*) Against *Escherichia coli* and *Propionibacterium acnes* Bacteria

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Abstract: Diarrhea and acne, caused by the bacteria *Escherichia coli* and *Propionibacterium acnes*, are common illnesses in children and adults. Antibiotics can overcome bacterial infections, but cases of antibiotic resistance are increasing. This study aims to determine the effect of wrap leaf extract (*Smilax rotundifolia*) on the growth of *P. acnes* and *E. coli*. This research can support microbiology-based science learning that utilizes biodiversity for students. Phytochemical tests showed that the extract contained flavonoids, alkaloids and saponins. The results of antibacterial testing showed that wrap leaf extract was able to inhibit the growth of *P. acnes* bacteria from concentrations of 10% and 15% with an average inhibition of 5.31 mm and 6.42 mm, and the positive control (Amoxicillin) had an inhibition of 7.26 mm. However, *E. coli* did not show any inhibition zone. The Mann-Whitney test results showed that the wrapper leaf extract has antibacterial effectiveness by comparing the negative control with a concentration of 10% and 15% and the positive control with a concentration of 10%. There is a difference between the concentration comparison where the Sig.<0.05 value. Then, comparing the positive control with a concentration of 15% produces a Sig.>0.05 value, meaning there is no difference between the two.

Keywords: Acne; Antibacterial; *Propionibacterium acnes; S. rotundifolia*

Introduction

Diseases that are often found in children and adolescents are diarrhoea and acne. Approximately 1.7 billion cases of diarrhoea occur in children worldwide each year. 19.589 cases of diarrhoea were found in West Papua Province. Diarrhoea was one of the 10 most common diseases among outpatients at the Mariat District Inpatient Medical Center in 2018, affecting 289 patients (Bambungan, 2020). The causes of diarrhoea include contamination of food with microorganisms and poor hygiene. *Escherichia coli* is one type of microorganism that causes diarrhoea. *E.coli* bacteria are usually found in the bodies of humans and warm-blooded animals, especially in the digestive tract. If the number of *E.coli* increases in the digestive tract or if it is present outside the intestine, these bacteria will become pathogenic (Hutasoit, 2020).

Diarrhea is a liquid bowel movement that occurs more than three times a day and usually lasts two days or more. In children, the consistency of feces is more important than the frequency of defecation. This is because defecation in infants is more frequent than in adults, up to five times a day (Fitri Melanie Ramadhina, Immawati, 2023).

Acne is a skin problem experienced by more than 85% of teenagers worldwide. Although not life-threatening, the presence of acne has a significant psychological impact on sufferers. It can affect how they assess themselves, react to situations, and perceive their surroundings, ultimately impacting their quality of life (Winato *et al.*, 2019). Acne is a skin disease caused by a buildup of oil that causes the pores of the facial skin to become clogged, triggering bacterial activity and inflammation of the skin (Sifatullah & Zulkarnain, 2021). The bacteria *P. acnes*. It is one of the factors that cause

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acne. Under normal conditions, these bacteria are not harmful to the skin. However, changes in skin conditions can cause these bacteria to invade and cause skin problems (Retnaningsih *et al*., 2019).

Infections caused by *E. coli* in humans can be treated with antibiotics such as amoxicillin and cotrimoxazole (Nuraeni Putri et al., 2023). Antibiotics such as clindamycin, erythromycin, and doxycycline are commonly used to treat acne (Madelina & Sulistiyaningsih, 2018). In addition, ingredients such as benzoyl peroxide, azelic acid, and retinoids are often used to treat acne. Although effective, chemical medications may pose a risk of side effects. These include antibiotic resistance, irritation, allergic reactions, and potential organ damage (Winato *et al*., 2019). Therefore, the development of new antibacterial drugs, especially those based on herbal plants, becomes very important to reduce the side effects caused by the use of antibiotics (Geofani, Septianingrum, and Dianita., 2022).

Indonesia is a country that has a tropical climate, so many types of plants grow in several regions that can be used as medicines. One very popular plant is the wrap leaf plant (*S. rotundifolia*), found in eastern Indonesia, especially in Papua. This plant thrives in coastal areas and is known for its extraordinary properties. In Papuan people, wrap leaf (*S. rotundifolia*) can be used as a virility medicine (Firawati & Iqbal Pratama, 2018). These leaves resemble betel leaves, spread in hot climates, and hang from trees and shrubs. There are several types of wrapper leaves used by the Papuan people, one type that is commonly used is creeping leaves and there are three leaves so it is known as three-finger leaves. The wrapper leaf is used by Papuans, especially men over 17, and is part of the culture (Chrystomo et al., 2016). From the results of phytochemical screening, there are various types of secondary metabolites, namely flavonoids, alkaloids, and saponins found in ethanol extracts of wrapper leaves (*S. rotundifolia*) (A. Wulandari et al., 2022).

Based on previous research, this study tested the antibacterial effectiveness of an ethanol extract of wrap leaves (*S. rotundifolia*) against the bacteria that cause acne, *Propionibacterium acnes*

This research can be used to develop microbiology-based science teaching materials or as a basis for teaching various science concepts to students, including using endemic plants in Papua.

Method

Tools and Materials

The materials used were amoxicillin, distilled water, aqua pro injection, *E. coli* culture, *P. acnes* culture, Pb2 acetate, Mayer reagent, 1% H_2SO_4 (sulfuric acid), 1% $BaCl_2$ (barium chloride), 70% ethanol, Nutrient agar, and wrap leaves (*S. rotundifolia*). The tools used in this research are an autoclave, mesh 60 sieves, stirring rod, blender, petri dish, hand scoop, ose needle, label paper, hot plate, incubator, filter paper, bunsen lamp, mask, tube rack, split, sterile swab, test tube, analytical balance and water bath.

Sample Preparation

The wrapper leaf plant comes from the Aimas area, Sorong Regency, Southwest Papua Province. The part used is the leaves. The leaves taken are intact and undamaged. The leaves taken are then cleaned from foreign objects that are still attached, and the cleaned leaves are cut into small pieces and washed using running water. The leaves that have been cleaned are then dried in an oven at 40°C. After the drying process is complete, the leaves are sorted to remove dirt that is still attached. After sorting, the leaf samples were pulverized using a blender and sifted using a 60-mesh sieve (Puspita & Prasetya, 2023).

Sample Extraction

The sample extraction process was carried out using the maceration (cold) method with 70% ethanol solvent. In this process, the simplisia powder was soaked in 70% ethanol thrice for 24 hours with occasional stirring. During the maceration process, the environment was kept dark and away from sunlight (Puspitasari et al., 2023). After the maceration stage, the solution is filtered with filter paper; the resulting extract liquid evaporates using a water bath until it reaches a thick extract consistency. The extract that has reached the desired consistency is weighed and transferred into a porcelain cup before being stored in a desiccator (Zukhri & Nurhaini, 2019).

Phytochemical Screening

Identification of flavonoid compounds

The flavonoid compounds are identified by taking a spatula of thick extract and dissolving it in distilled water, then adding 1-2 drops of Pb2 acetate. Positive contains flavonoids if the solution turns yellow-brown (Kurnianto, Rahman, and Farmasi, 2021).

Identification of saponin compounds

The identification of saponin compounds is carried out by taking a thick extract with a spatula and dissolving it in distilled water, then shaking it for 10 seconds, marked positive for saponin if it forms a froth as high as 1 to 10 cm for 10 minutes (Afifah Rukmini, 2020).

Identification of alkaloid compounds

Identification of alkaloid compounds is done by taking a thick extract with a spatula and dissolving it in distilled water. Then, dripped with Mayer's reagent, if there is a yellow or white precipitate, it tested positive for alkaloids (Sambode, Simbala, and Rumondor, 2022).

Preparation of Test Solution

Negative control uses aqua pro injection; positive control is made by grinding 500 mg amoxicillin tablets weighing as much as 65 mg and then dissolved in 50 mL of distilled water (Fijriati et al., 2022). Take 1 mL of the solution and add distilled water to 10 mL. The preparation of 10% and 15% concentration extract solutions was made by weighing 1 gram and 1.5 grams of wrap leaf extract (*S. rotundifolia*), which was then dissolved with aqua pro injection in 10 mL and then shaken until homogeneous.

Antibacterial Effectiveness Test

Tool Sterilization

First, the glassware was washed and dried. Then, the equipment was wrapped using aluminium foil and tied firmly. The sterilization process was done using an autoclave at 121°C for 15 minutes (Wulandari et al., 2021).

Preparation of NA media

The media was made by dissolving 7 g of Nutrient agar into 250 mL of distilled water in an Erlenmeyer. Next, it was heated to boiling, covered with cotton wrapped in aluminum foil, and sterilized. The sterilization process used an autoclave for 15 minutes at 121°C (Susanti, Khalimatusa'diah, and Rasyid, 2022).

Rejuvenation of Bacteria

Bacterial rejuvenation was performed via the scratch method. One piece of pure *P. acnes* culture was collected and inoculated aseptically by scraping it onto NA media. The culture was then incubated for 24 hours at 37°C (Helmi et al., 2023).

Preparation of test bacteria

A bacterial suspension solution is made by taking 1-2 ose of bacteria from rejuvenation and then suspending it in 0.9% physiological NaCl until the turbidity level is under Mcfarland turbidity (Wardaniati and Gusmawarni, 2021).

Antibacterial activity test

Pour 20 ml of Nutrient Agar (NA) into a sterilized Petri dish and allow it to harden. After that, streak the *P. acnes* bacteria suspension onto the NA media. Next, make four holes in the media, and each hole is filled with 50 µl of ethanol extract of wrap leaves at various concentrations, namely 10% and 15%. For the negative control, aqua pro injection was used, while for the positive control, the conventional drug amoxicillin was used. All containers were placed in an incubator and incubated at 37°C for 24 hours. After incubation, measurements were taken using a caliper by measuring the clear zone formed on the medium.

Data Analysis

The data used in this study is the inhibition of bacterial growth. Data analysis was done using normal distribution using Kolmogorov Smirnov and homogeneity data using Levene. If the resulting data is normal and homogeneous, it is analyzed using the SPSS application with the One Way ANOVA (Analysis of Variance) method. Then, to see the mean difference (average) data, we continued with the LSD (Least Significance Difference) test. If the resulting data is not normal and homogeneous, the Mann-Whitney test will be used to see the difference between the groups.

Result and Discussion

Phytochemical analysis

The results of the phytochemical screening test show that the ethanol extract of wrapper leaves contains secondary metabolite compounds such as flavonoids, alkaloids, and saponins. Table 1 shows the results of the phytochemical test.

Table 1. Phytochemical test results of wrap leaf extract

Compounds	Reagent	Observation	Results
Flavonoids	Pb2 acetate	Change to a yellow-brown color	+
Saponins	Aquades	Formation of foam	+
Alkaloids	Mayer	White precipitate forms	+

Table 1. shows positive results by identifying three different compounds using the appropriate reagent, so the ethanol extract of wrapper leaves (*S. rotundifolia*) is identified as containing secondary metabolite compounds such as flavonoids, saponins, and alkaloids. The flavonoid test using Pb2 acetate reagent produces a yellow-brown colour if the sample contains flavonoid compounds due to bond breaking at the C3 atom (Musiam et al., 2022). The identification of saponins using water tested positive for the formation of foam because saponins are compounds with hydrophilic and hydrophobic groups. When shaken, the hydrophilic group binds to water, while the hydrophobic group binds to air to form froth (Suleman et al., 2022). The alkaloid test using Mayer reagent is positive if a white precipitate is formed; this is because mercury ions are heavy metal ions that can precipitate alkaline alkaloid compounds (Rizky et al., 2024).

Propionibacterium acnes Bacterial Inhibition Test

Table 2. Diameter of the inhibition zone of ethanol extract of wrap leaves against *P. acnes* bacteria

Replication	Concentration		(+)	(-)
	10%	15%	(mm)	(mm)
1	5,75	7,20	7,32	0
2	5,10	6,22	7,60	0
3	5,10	5,85	6,87	0
Average (mm)	5,32	6,42	7,26	0
Growth inhibitory response	Medium	Medium	Medium	-

Description:
* (+) : Positive control (Amoxicillin)
  (-) : Negative control (aqua pro injection)
* Inhibition criteria
  ≤5 mm : Weak
  5-10 mm : Medium
  11-20 mm : Strong
  ≥20 mm : Very strong

Table 2. shows that the results of bacterial inhibition tests carried out using ethanol extracts from wrapper leaves (*S. rotundifolia*) against *P. acnes* bacteria show significant variations at each concentration. At a concentration of 15%, the results obtained were almost equivalent to the positive control, with an average bacterial inhibition zone of 6.42 mm and 7.26 mm. The clear zone in the media shows the antibacterial effectiveness of the wrapper leaf extract (*S. rotundifolia*) in inhibiting the growth of *P. acnes* bacteria. The effectiveness of the extract increases with its concentration, the greater the inhibition against bacterial growth.

The content of active compounds contained in the wrapper leaf extract (*S. rotundifolia*) plays a role in inhibiting bacterial growth. Flavonoid compounds in wrapper leaf plants (*S. rotundifolia*) have effective antibacterial properties. The mechanism of action of flavonoid compounds as antibacterials is by damaging the structure of the bacterial cell wall, which occurs through the formation of complexes with extracellular proteins and blocks the movement of bacteria. When the bacterial cell wall of amino acids and lipids is disintegrated, flavonoid compounds can enter the bacterial cell nucleus thanks to its interaction with its alcohol group. After entering the cell nucleus, flavonoids interact with bacterial DNA. This process is triggered by the difference in polarity between lipids and alcohol groups on DNA, which can cause lysis or damage bacterial cells (Sadiah et al., 2022).

The mechanism of inhibition of saponin compounds as antibacterials can be understood by reducing cell wall surface tension. Saponins work by binding to lipopolysaccharides found in bacterial cell walls, which can increase cell wall permeability. As a result, when saponins interact with the cell wall, damage or lysis can occur, making it easier for antibacterial substances to enter the cell and disrupt metabolic processes. This can result in the death of the bacteria (Dwicahyani & , Sumardianto, 2018). Alkaloid compounds work by damaging peptidoglycan components in bacterial cells. This peptidoglycan component is important in maintaining bacterial survival, especially in a hypotonic environment. When the layer is damaged, the bacterial cell wall becomes rigid, and this results in cell death (Sadiah et al., 2022).

Data analysis was then conducted using SPSS with normal distribution and homogeneity tests based on the data obtained as anti-bacterial inhibition zones. The normal distribution test results show a Sig.<0.05 value, meaning the data is not normally distributed. Because the normality test results showed a Sig.<0.05 value, it did not continue with the homogeneity test but continued with the Mann-Whitney method test.

The Mann-Whitney test is a non-parametric test determining the median difference between two independent samples because the data does not meet normality assumption. The results of the Mann-Whitney test were obtained by comparing the negative control with a concentration of 10% and 15% and the positive control with a concentration of 10%. There is a difference between the concentration comparisons where the Sig.<0.05 value. Then, comparing the positive control with a 15% concentration resulted in a Sig.>0.05 value, which means there is no difference between the two. Based on the results of the Mann-Whitney test, it can be concluded that the most effective concentration is a concentration of 15%.

Escherichia coli Bacterial Inhibition Test

Table 3. Diameter of the inhibition zone of ethanol extract of wrap leaves against *E. coli* bacteria

Replication	Concentration		(+)	(-)
	10%	15%	(mm)	(mm)
1	0	0	31.60	0
2	0	0	30.62	0
3	0	0	31.75	0
Average (mm)	0	0	31.32	0
Growth inhibitory response	-	-	Very strong	-

Description:
* (+) : Positive control (Amoxicillin)
  (-) : Negative control (aqua pro injection)
* Inhibition criteria
  ≤5 mm : Weak
  5-10 mm : Medium
  11-20 mm : Strong
  ≥20 mm : Very strong

Table 3 shows that ethanol extract of wrapped leaves at various concentrations has no antibacterial effectiveness against *E. coli* bacteria. This can be seen from the absence of inhibition zones around the wells. Even after repetition three times, there was no inhibition zone around the wells. The negative control using aqua pro injections could not inhibit bacterial growth. In contrast, the positive control using Amoxicillin inhibited bacterial growth, with an average inhibition zone diameter of 31.32 mm.

The results of the antibacterial effectiveness test against *E. coli* bacteria differ from *P. acnes* bacteria. The collection environment and sample extraction method were the same, and the working procedures were carried out simultaneously. The concentration of ethanol extract of wrapper leaves may affect the results of the antibacterial effectiveness test because the levels of secondary metabolite compounds contained by wrapper leaves usually correlate with the inhibition of bacterial growth (Arni et al., 2024). The results of this study are also expected to be used as a study material and guide for students on how to utilize microbiology-based laboratories, data analysis, experimental tests, and the use of biodiversity, especially endemic Papuan plants, as antibacterials.

Conclusion

Based on the results of this study, it is concluded that wrap leaves (*S. rotundifolia*) can inhibit the growth of *P. acnes* bacteria. The best results were at a concentration of 15%, followed by a concentration of 10% with each diameter of the inhibition zone at a concentration of 10% of 5.32 mm and a concentration of 15% of 6.42 mm. However, ethanol extract from wrapper leaves (*S. rotundifolia*) did not have antibacterial effectiveness against *E. coli* bacteria. Further research is recommended by testing the antibacterial effectiveness using higher extract concentrations.

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Conflicts of Interest
The authors declare no conflict of interest.

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