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Antibacterial Activity Test of Ethanol Extract of Palm *Leaves (Catharantus roseus)* Against *Staphylococcus aureus* and *Escherichia coli Bacteria*

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Abstract: Ethanol extract of virgin palm *leaves (Catharantus roseus)* contains active compounds that have potential as natural antibacterial. This study aims to test the antibacterial activity of the extract against Staphylococcus aureus *and* Escherichia coli *bacteria*. The leaves of the tread are extracted using the maceration method with a 70% ethanol solvent. The extraction results showed a yield of 6.1% and the identification of compound content showed the presence of alkaloids, flavonoids, saponins, tannins, and phenols. The antibacterial test was carried out by disc diffusion method using concentrations of 60%, 80%, and 100%. Antibacterial activity was tested by calculating the resulting inhibition zones against both types of bacteria. The results showed that in *Staphylococcus aureus* bacteria, the concentration of 100% resulted in an inhibition zone of 11.66 mm which was classified as strong. In contrast, in *Escherichia coli* bacteria, the same concentration resulted in an inhibition zone of 2.66 mm which was in the weak category. This difference in effectiveness is due to the cell wall structure of gram-positive and gram-negative bacteria. The results of this study identified that ethanol extract of virgin leaves has a greater potential for gram-positive bacteria than gram-negative. The ethanol extract of virgin leaves demonstrated antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli*, with stronger effects observed against *S. aureus.*

Keywords: Antibacterial; Inhibition zone; Virgin leaf

Introduction

Indonesia is famous for its biodiversity and abundant natural resource potential. One type of diversity that exists is plant diversity. Plants are a source of various types of chemical compounds that are useful as medicines and are a long ancestral heritage in almost all parts of the world. In addition, plants are also an alternative in the prevention and treatment of diseases, as they are considered to have minimal side effects and can reduce the level of resistance to antibiotics (Savitri & Harris, 2018).

One of the plants that is used as herbal medicine is the virgin palm plant *(Catharantus roseus)*. Tapak virgin is known as an ornamental plant that has uses to relieve muscle pain, antidepressants, treatment of various conditions to overcome swelling due to insect stings, nosebleeds and strep throat), antidote to poisons, antibacterial, and lowering blood pressure in humans. The potential efficacy comes from the content of secondary metabolites of virgin plants, namely 150 types of alkaloids produced from roots, stems, leaves, flowers, and seeds. Tapak Dara leaves contain more than 70 types of alkaloids, including vinkristin and vinblastin which have antineoplastic properties In addition, these leaves contain inorelbin and vincadioline, as well as several other compounds such as aquaamine, ß– Sitosterol and kaemferol in the whole plant as well as saponins, flavonoids and tannins in the leaf section (Anatje J. Pattipeilohy et al., 2022).

The increase in bacterial resistance to antibiotics opens up a great opportunity to discover new

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antibacterial compounds through the utilization of bioactive compounds produced as secondary metabolites of biodiversity richness. Various plant extracts have been shown to have an important role in inhibiting the growth of pathogens, even extracts with antibacterial activity can help control infections (Savitri & Harris, 2018).

Diseases due to infection are one of the health problems that need special attention. *Eschericia coli* is a gram-negative bacterium that naturally resides in the human digestive tract as normal flora. But under certain circumstances, these bacteria can turn into pathogens and cause various health problems, such as diarrhea, urinary tract infections, pneumonia, infectious wounds, especially in the abdominal cavity, as well as inflammation of the brain membranes. Meanwhile, *Staphylococcus aureus* is a gram-positive bacterium that usually lives as normal flora in the human body, covering the surface of the skin, sweat droplets, and digestive tract. However, under certain conditions, these bacteria can act as a disease (Maharani et al., 2017). (Anatje J. Pattipeilohy et al., 2022) In his research, he used diffusion so that the plumbing technique was used to find out whether the extract of virgin palm leaves showed antibacterial activity against Gram Positive bacteria, namely bacteria *Staphylococcus aureu* which is characterized by the formation of an inhibition zone in the bacterial media (Khairani et al., 2022).

Based on the above background, a study will be conducted on "Antibacterial Activity Test of Ethanol Extract of Palm Leaves *(Catharantus roseus)* Against *Staphylococcus aureus* and *Eschericia coli* Bacteria", whether it can produce an effective inhibitor or not using the disc diffusion method.

Method

This research is a laboratory experimental research with a cross-sectional approach. Samples in the form of Catharanthus roseus leaves were obtained from BTN Olat Rarang Hamlet, Labuhan Badas District, Sumbawa Besar Regency. The leaves used are green, ovate, and have been sorted before further processing. The preparation of ingredients is carried out by washing, drying using an oven at 60°C, grinding, and sifting until simplicia powder is obtained. The extraction and testing process is carried out using various laboratory equipment such as ovens, blenders, strainers, erlenmeyers, measuring pipettes, petri dishes, autoclaves, incubators, micropipettes, and rotary evaporators. The ingredients used include virgin leaf powder, 70% ethanol, aquadest, Nutrient Agar, 0.9% NaCl solution, McFarland standard, 1% H_2SO_4 solution, 1% $BaCl_2$ solution , and chloramphenicol antibiotics as positive control. All tools and materials are prepared in sterile conditions to ensure the validity of the test results.

The prepared powder is weighed as much as 500 grams and then extracted by the maceration method using 70% ethanol in a glass jar, ensuring that the entire powder is completely submerged. The remaceration process is carried out three times by changing the solvent every 24 hours. After the process is complete, the maceration results are filtered using a funnel lined with filter paper. The results of the remaceration (mafiber) were then thickened using a rotary evaporator vacuum device at a temperature of 45 0-500C, so that a thick extract was obtained. Then the yield calculation is carried out with the Formula 1 (Tamrin, 2022).

$$\%\text{Yields} = \frac{\textbf{berat ekstrak yang didapat (gram)}}{\textbf{berat serbuk simplisia (gram)}} \boldsymbol{x100}\%$$

Phytochemical screening is carried out qualitatively to identify the content of secondary metabolite compounds, such as alkaloids, flavonoids, saponins, tannins, phenols, as well as steroids and terpenoids. Each compound was tested using a specific reagent. A positive reaction is characterized by discoloration or the formation of deposits according to the test indicators (Nurhaliza, 2023). A total of 4 grams of NA (Nutrient Agar) was dissolved in 200 ml of aquadest using erlenmeyer. Heating is carried out on a hotplate with the help of a magnetic stirrer until a clear solution is obtained. Next, the media is sterilized using an autoclave at 1210C for 15 minutes. The sterile media is then poured into a 20 ml petri dish and left to solidify (Dwijayanti & Pamungkas, 2023).

Bacterial rejuvenation is carried out using NA (Sodium Agar) for Staphylococcus aureus and Eschericia coli. This process begins by taking one bacterial oose using a sterile ose, then scratching it on the surface of the agar with a cross pattern (zig-zag). After that, the media was incubated for 24 hours at a temperature of 37^{0C} (Dwijayanti & Pamungkas, 2023). The McFarland standard is made by mixing 9.95 mL of 1% H_2SO_4 solution with 0.05 mL of 1% $BaCl_2$ solution in a test tube. This mixture is shaken to produce a cloudy solution that is used as a reference for the turbidity of the bacterial suspension (Khairani et al., 2022). The 24-hour-old bacteria were taken from the medium to use the colony ose and then mixed into 5 mL of sterile 0.9% NaCl solution in a sterile test tube. The suspension is homogenized with a vortex, then the turbidity level is adjusted to the McFarland standard of 0.5 (Khairani et al., 2022).

The antibacterial activity test was carried out by the disc diffusion method. Sterile paper discs are soaked in virgin palm leaf extract at concentrations of 60%, 80%, and 100% for ±15 minutes. A total of 20 µL of bacterial

suspension is evenly dripped onto the surface of the medium. The test disc, as well as the positive (chloramphenicol 30 µL/mL) and negative control (aquades) control discs, are then placed on the media and lightly pressed. Each treatment is carried out three times. The cups are incubated at 37°C for 24 hours.According to (Harmini, 2023) zThe ONA inhibition is observed as a clear area around the disc and is measured using calipers in two directions, i.e. horizontal diameter (D_1) and vertical (d_2), then the average is calculated with Formula 2.

$$\textbf{Average diameter of the buffer zone} = \frac{(d1-d3)+(d2-d3)}{2} \quad (2)$$

Information:
d1 = horizontal line diameter in the inhibition zone
d2 = vertical line diameter in the inhibition zone
d3 = overall clear zone media diameter

The inhibition zone is categorized as very strong if >20 mm, strong (11–20 mm), medium (6–10 mm), and weak (<5 mm). The measurement results were analyzed descriptively based on the average value of the inhibition zone of each treatment.

Result and Discussion

Extraction Results of Tapak Dara Leaves (Chataranthus roseus)

Extraction is the process of separating components in plants to obtain pure active compounds. The method used in this study is maceration, which in principle involves soaking simplicia with a solvent whose polarity corresponds to the desired secondary metabolite compound. This process takes advantage of the pressure difference between the inside and outside of the cell, so that the active compounds that are in the simplicia dissolve in the solvent. The solvent used is 70% ethanol because it has the ability to dissolve active compounds in solvent-soluble simplicia. The solvent used is 70% ethanol because it has the ability to dissolve chemical compounds, kill microbes, is polar, universal and easy to obtain (Anatje J. Pattipeilohy et al., 2022). The results of the extraction of virgin tapak leaves are summarized in Table 1.

Table 1. Yield Yield of Tapak Dara Leaf Extract

Parts used	Simplicia weight (g)	Weight of thick extract (g)	Yields (%)
Virgin Palm Leaves	500	30,50	6.1

The maceration process is carried out by soaking 500 grams of virgin sole powder in a solvent in a ratio of 1:2 for three days. Remaceration was carried out three times with a total solvent use of 3 liters. The solution is changed every 24 as the solvent becomes soggy, which is characterized by the discoloration of the extract to saturation, which is characterized by the discoloration of the extract to dark brown. At each rotation each time the maserrate replacement is separated through filtration to ensure that the pulp is not mixed in the solution (Dewi et al., 2023).The filtration process uses filter paper, and the fibers are accommodated in the erlenmeyer. Stirring during immersion aims to even out the distribution of solvent fluid so that the concentration remains optimal. In addition, the extract is evaporated using a rotary evaporator at 500C and followed by additional evaporation with a waterbath at 600C (Avitananda, 2019). The yield is calculated as the ratio between the weight of the extracted obtained and the weight of the initial simplisia, multiplied by 100%. The yield obtained is 6.1%. Factors such as solvent polarity, simplicia particle size, concentration, and duration of immersion can affect the amount of yield (Walid et al., 2020).

Phytochemical Screening Results

Phytochemical screening is performed to identify groups of compounds in the leaves of the virgin leaf that have antibacterial activity. The principle observes the change in color when the extract is reacted by reacting to certain reagents. inside the test glass. The color that appears is compared to the standard to determine a positive outcome. This process is carried out in the test glass, and the color change indicates the presence of certain groups of compounds (Khoiriyah, 2014). Based on the results of observations, the results of the phytochemical test of virgin palm leaves were obtained as in Table 2.

Table 2. Phytochemical Screening Results

Compound Groups	Reagents	Result
Alkaloids	Bouchardat	+
	Mayer	+
	Dragendrof	+
Flavonoids	Mg+HCL concentrate	+
Saponins	Aquades Heat+ HCL	+
Phenolic	HCL 2N	+
Tannins	FeCl3	+
Steroids/Terpenoids	Chloroform+Bouchardat	-

Ket : (+) contains compounds

Table 2 above shows that the results of qualitative phytochemical screening of thick extracts of virgin palm leaves contain alkaloids, flavonoids, tannins, saponins, and phenols. As for this, it can be caused by several factors that affect the content of secondary metabolite

compounds in plants, including plant age, geographical location, and extraction process. (Walid et al., 2020).

Results and Discussion of Antibacterial Activity Test

The antibacterial activity test of ethanol extract from virgin palm leaves aims to assess the ability of the extract to inhibit bacterial growth *Staphylococcus aureus* and *Eschericia coli*. This study applied the disc diffusion method, which is used to measure antibacterial activity by observing the inhibition zones formed around the disc paper that has been soaked in an extract solution. The disc paper is then placed on a medium that has been inoculated with bacteria. The inhibition of bacteria can be seen from the presence of an inhibition zone that appears on the surface of the media so that (Intan et al., 2021). The results of measuring the diameter of the barrier zone or clear zone from this study can be seen in Table 3.

Table 3. Observation Results of Antibacterial Activity Test of Ethanol Extract of Virgin Leaves against *Staphylococcus aureus Bacteria*

Test Sample	Resistance Zone Diameter (mm)			Average Diameter of Barrier Zone (mm)	Category
	R1	R2	R3		
60%	7.45	7.70	7.50	7.55	Keep
79%	8.35	8.39	8.60	8.61	Keep
80%	11.95	11.90	11.20	11.68	Strong
K+	22.50	20.10	20.90	21.16	Very powerful
K-	-	-	-	-	-

Information: K+: Positive (chloramphenicol); K- : Negative control (aquades); R1 : Replication 1; R2 : Replication 2; R3 : Replication 3

Palm leaf extract was divided into three concentrations, namely 60%, 80%, and 100%, with a comparison of positive controls using chloramphenicol antibiotics and negative controls using aquades. According to Harmini (2023), the inhibition zone is categorized as very strong if the diameter is > 20 mm, strong if it is in the range of 11-20 mm, medium if the diameter is 6-10 mm, and weak if the diameter of the inhibition zone is < 5 mm. On bacteria *Staphylococcus aureus*, the average diameter of the inhibition zone for the extruder at a concentration of 60% is 7.55 mm (medium category), at a concentration of 80% is 8.61 mm (medium category), and the concentration of 100% is 11.68 mm (strong category). The highest inhibition zone diameter was found at a concentration of 100% which is 11.68 mm, which belongs to the strong category. This is due to the greater content of antibacterial extracts at 100% concentration, The higher the antibacterial concentration used, the wider the inhibition zone formed (Saputera, Marpaung, 2019).

Table 4. Observation Results of Antibacterial Activity Test of Ethanol Extract of Tapak Dara Leaves against *Eschericia coli Bacteria.*

Test Sample	Resistance Zone Diameter (mm)			Average Diameter of Barrier Zone (mm)	Category
	R1	R2	R3		
60%	1.10	1.00	1.00	1.03	Weak
79%	1.70	1.60	1.60	1.63	Weak
80%	2.70	2.60	2.60	2.66	Weak
K+	24.70	24.20	24.8	24.56	Very powerful
K-	-	-	-	-	-

Information: K+: Positive (chloramphenicol); K- : Negative control (aquades); R1 : Replication 1; R2 : Replication 2; R3 : Replication 3

The results of this study are not in line with the research Pattipeilohy et al, (2022) which uses the method of diffusion of oil and ethanol solvents. In the study, the diameter of the barrier zone to *Staphylococcus aureus* With a concentration of 60% is 20 mm (strong category), 80% concentration is 21 mm (very strong category). In a study conducted by (Dwijayanti & Pamungkas, 2016) About the Influence of Virgin Tapak Leaf Extract on Bacterial Growth *Staphylococcus aureus* which uses ethanol solvents with the wells diffusion method at a concentration of 100% up to 26.67 mm (very strong category). Some factors that affect antibacterial activity include the concentration level of the extract, the content of antibacterial substances, the diffusion ability of the extract, and the type of bacteria tested (Saputera, Marpaung, 2019). On bacteria *Escherichia coli*, the average diameter of the inhibition zone at 60% concentration is 1.03 mm (weak category), 80% concentration is 1.63 mm (weak category), and 100% concentration is 2.66 mm (weak category). The diameter of the inhibition zone is highest at a concentration of 100% (2.66 mm). Antibacterial activity against *Escherichia coli* lower, compared to *Staphylococcus aureus* due to the difference in cell wall structure between gram-negative and gram-positive bacteria. Gram-negative bacteria have a more complex cell wall with layers of peptidoglycan, lipoproteins, and polysaccharides, making it difficult for antibacterial compounds to penetrate (Sofyana et al., 2024).

While *Staphylococcus aureus* Gram-positive bacteria have a simpler cell wall, without an outer membrane, so that antibacterial materials penetrate the cell more easily. Other factors that affect antibacterial activity

include the diffusion rate of the active compound, the interaction of the active ingredient with the medium, the incubation temperature, the level of bacterial sensitivity, the pH of the environment, and the condition of the microbial metabolites (Baharun, Rukmi, Lunggani, 2019)

The positive control group in this study used chloramphenicol, which was chosen because of the broad spectrum effective against gram-positive and gram-negative bacteria, including aerobic and nonaerobic organisms. In contrast, compounds that are only able to inhibit one type of bacteria, such as gram-positive or gram-negative, are grouped as narrow-spectrum-only compounds. Based on the results of the study, ethanol extract of virgin palm leaves is more effective against gram-positive bacteria *Staphylococcus aureus* compared with *Escherichia coli* gram-negative bacteria. Therefore, ethanol extract of virgin leaves *(Catharanthus roseus)* are categorized as compounds with a narrow spectrum. Its effectiveness is compared to chloramphenicol to determine the level of its antibacterial activity (Anatje J. Pattipeilohy et al., 2022).

Average diameter Inhibition zones produced by chloramphenicol in bacteria *Staphylococcus aureus* and *Escherichia coli* The 21.16 mm and 24.56 mm respectively are both in the very strong category. In comparison, the extract of virgin palm leaves with a concentration of 100% produced an inhibition zone of 11.68 mm in the *Staphylococcus aureus* (strong category) and 2.66 mm on *Escherichia coli* (weak category). This difference occurs because the mechanism of action of chloramphenicol inhibits the activity of bacterial girase DNA. While virgin palm leaf extract works through a combination of various antibacterial active compounds (Sofyana et al., 2024). In negative control using aquades did not produce an inhibition zone on average, with an average diameter of 0 mm. Aquades was chosen because it is neutral and does not affect the growth of bacteria. The inability of aquades to produce an inhibition zone suggests that the antibacterial activity comes from the compounds in the extract of the virgin leaf, not from its dissolution (Henaulu & Kaihena, 2020).

The inhibition zones observed in NAP (Sodium Agar Plate) media showed that ethanol extract of virgin leaves had antibacterial potential against *Staphylococcus aureus* and *Escherichia coli*. This ability is due to the active compounds found in the leaves, such as alkaloids, flavonoids, phenols, tannins, and saponins. This is in line with research (Dewi et al., 2023) Secondary metabolites in the leaves of the virgin site serve as antibacterial agents.Putri & Nasution, (2022) states that alkaloids inhibit the formation of cell walls into lysis, and cause cell death. The flavonoids in the leaves of the soleum inactivate proteins on the cell membrane, damage the protein structure, and cause the cell to lose its shape and lysis (Dewi et al., 2023). Saponins involve the formation of complexes with bacterial cell membranes, which then destroy the permeability of the cell wall. This causes bacterial cells to break down (lysis) (Anatje J. Pattipeilohy et al., 2022).

Phenol as an antibacterial, destroys lipids contained in the plasma membrane of microorganisms, cell contents come out. Phenols can also damage the cell walls, proteins, and DNA of bacteria, thus effectively inhibiting bacterial growth (Abigail Jonathan et al., 2020). Tannins involve the inactivation of microbial adhesions that are present on the cell surface. This disrupts metabolism and causes so that the cell wall polypeptide will cause damage to the bacterial cell wall, which ultimately results in the cell being denatured and unable to survive, especially in severe conditions (Walid et al., 2020).

Conclusion

Ethanol extract of virgin leaves showed antibacterial activity against *Staphylococcus aureus* and *Escherichia coli.* Antibacterial activity against *Staphylococcus aureus* is higher than *that of Escherichia coli*. At concentrations of 60%, 80%, and 100%, the inhibition zones for *Staphylococcus aureus* were 7.55 (moderate category), 8.61 mm (moderate category), and 11.68 mm (strong category), respectively. Meanwhile in *Escherichia coli* at the same concentration, the inhibition zones are 1.03 mm, 1.63 mm, 2.66 mm, which fall into the weak category.

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The author states that there is no conflict of interest in this study.

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