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Antibacterial Activity Test of Ethanol Extract Pineapple (*Ananas comosus* (L.) Merr.) Peel against Growth of *Propionibacterium acnes*

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Abstract

Pineapple peel (*Ananas comosus* (L.) Merr.) is waste from the pineapple fruit. The purpose of this study was to determine the content of pineapple peel extract and to antibacterial activity in various concentrations effective in inhibiting the growth of *Propionibacterium acnes*. The pineapple peel extract is made by using the extraction method in the form of maceration. The method used in the inhibitory test using the three replication samples in each treatment group. The sample consisted of 10 treatment groups ie pineapple peel extract concentration 12.5%, 25%, 37.5%, 50%, 62.5%, 75%, 87.5%, and 100%, as well as positive and negative control. The results obtained from pineapple peel screening contain flavonoid and saponin compounds. The data analysis shows that pineapple peel extract concentrations of 50%, 62.5%, 75%, 87.5%, and 100% had inhibitory zones with the medium-strong category while clindamycin as the control has a strong inhibition zone. The conclusion of this research proves that pineapple peel extract has antibacterial power to *P. acnes* with effective concentration is 100% with the strong category.

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INTRODUCTION

Acne is a skin disease that attacks more than 85% of adolescents throughout the world (Lynn *et al*., 2016). Acne is characterized by the growth of blackheads, inflammation, and nodular cystic acne. Acne can grow based on several factors, namely, excess sebum production, abnormal growth of keratin in the follicles, and the growth of *Propionibacterium acnes* (Fox *et al*., 2016; Bergler-Czop & Brzezińska-Wcisło, 2013).

Propionibacterium acnes belongs to the Corynebacterial group of bacteria. *Propionibacterium acnes* is the first organism that generally contributes to the occurrence of acne. *Propionibacterium acnes* belongs to a group of Gram-positive, rod-shaped, and non-sporous (McLaughin *et al*., 2019). *Propionibacterium acnes* plays a role in the formation of acne by producing lipases that break down free fatty acids from skin lipids. These fatty acids can cause tissue inflammation when related to the immune system and support the occurrence of acne. Treatment of infected acne can be done by reducing the bacterial population by using an antibiotic such as tetracycline, erythromycin, and clindamycin (Lely *et al*., 2016; Daud *et al*., 2018).

The use of antibiotics in a long time, the higher the resistant microorganisms (Ventola, 2015). An alternative method that can be taken is by utilizing natural antibacterial, which has the potential to inhibit or kill bacteria, including the causes of acne like *P. acnes* (Sinha *et al*., 2014). One of them is pineapple (*Ananas comosus* (L.) Merr.), which is one of the fruits that are consumed by many people, but only the flesh of the fruit is utilized, while the tubers and the peel only become waste, which

will eventually become an environmental polluter (Deng *et al*., 2012).

Previous research revealed that pineapple fruit peel could inhibit the bacteria *Streptococcus mutant* with concentrations of 25%, 50%, 75%, and 100% (Bahtiyar *et al*., 2017). Also, pineapple fruit peel extract shows antibacterial activity against *Enterococcus faecalis* (Putri *et al*., 2016). The purpose of this study was to determine the content of pineapple peel extract and to antibacterial activity in various concentrations effective in inhibiting the growth of *P. acnes*.

MATERIALS AND METHODS

Materials

Pineapple peel that has been extracted by maceration, 96% ethanol, NA media (Nutrient Agar), MHA (Mueller Hinton Agar) media, aquadest, *P. acnes* bacterial culture provided by the Laboratory of Pharmaceutical Microbiology, University of North Sumatra, Medan, clindamycin antibiotic disk, Na-CMC, concentrated sulfuric acid, Mayer reagent, Dragendorff reagent, Wagner reagent, HCL, Mg powder, $FeCl_3$, and chloroform.

Sample preparations

Pineapple peel samples were obtained from pineapple fruit merchant waste in Banjarbaru, South Kalimantan, which had been previously washed. The pineapple peel is then dried in an oven at 40°C and then chopped to a small size and blended to a powder. Pineapple peel powder was then transferred to maceration vessels and extracted using 96% ethanol solvent in a ratio of 1 : 7.5 (Damogalad *et al*., 2013), then let stand for three days (stirred every 24 hours) and then filtered using filter paper. Then the extraction was repeated three times for nine days. The macerate is collected and then concentrated with a rotary evaporator with a temperature of 40°C. After obtaining concentrated extracts with a fixed weight, then the yield obtained from the extraction process is calculated.

Phytochemistry tests

Alkaloid test

Pineapple peel extract 0.5 ml of 2% HCl and the solution was divided into two tubes. The tube I added 2-3 drops of Dragendorff reagent, tube II added 2-3 drops of Mayer reagent. Orange precipitates indicate the presence of alkaloids in the tube I and white deposits in tube II (Ngibad, 2019).

Flavonoid test

The extract was weighed as much as 0.5 g. As much as 0.1 g of Mg powder and 2 ml of 2N HCl were added to 2 ml of extract solution. Flavonoid compounds will show orange to red (Nurhasnawati *et al*., 2019).

Saponin test

As much as 0.5 g of the extract, then added with 2 ml of water until all parts of the extract were submerged and then shaken vigorously. There is foam after shaking, and the foam is waited for ten minutes to remain constant, then the positive extract contains saponin compounds (Mir *et al*., 2016).

Tannin test

As much as 0.5 g of extract added 3 ml of warm water. The extract was tested with 1-2 drops of $FeCl_3$ 1%, formed dark blue or blackish green, indicating the presence of tannin compounds.

Steroid test

As much as 0.5 g of extract was put into a test tube, dissolved in 0.5 ml chloroform and then added with 0.5 ml anhydrous acetic acid. This mixture is then added to 1-2 ml of concentrated sulfuric acid through the tube wall. The presence of a triterpenoid is indicated by the presence of a brownish or violet ring at the border of two solvents, while the presence of steroids is indicated by the presence of a bluish-green color (Mandal *et al*., 2013).

Antibacterial test

Three Petri dishes were prepared, where two plates were used for samples, each divided into four sections, while the remaining one dish was divided into two sections for positive and negative control. As a positive control, clindamycin was used, while negative control was used MHA media. The 20 µl *P. acnes* culture inoculate suspension was then transferred into each petri dish by micropipette using the spread plate method. (Sanders, 2012).

In each petri dish, a well is made and then filled with samples of pineapple peel extract of various concentrations as well as the positive and negative control in laminar air flow. Each petri dish is then transferred to the refrigerator at 4°C for 24 hours until the sample diffuses into the media, then incubated at 37°C for 24 hours (Debalke *et al*., 2018). Furthermore, the diameter of the inhibition zone (bright or clear zone) is measured using a ruler. The whole process was repeated three times, and then the inhibition zone calculations were performed for each sample concentration.

RESULTS AND DISCUSSION

Phytochemical screening is carried out to identify the content of secondary metabolites contained in 96% ethanol extract of pineapple fruit peel so that it can be known what secondary metabolites are likely to have antibacterial activity. The results of screening can be seen in **Table I**. Pineapple peel containing saponins and flavonoids. This is according to research from Yeragamreddy *et al*. (2013) that pineapple peel contains saponins and flavonoids. The mechanism of action of saponins as an antibacterial is to reduce surface tension resulting in cell leakage and cause intracellular fluid to come out (Nuria *et al*., 2009).

Furthermore, pineapple peel also contains flavonoids. Flavonoid compounds are disinfecting and play a role in inhibiting the growth of Gram-positive bacteria. Flavonoids are polar, so it is easier to penetrate the peptidoglycan layer, which is also polar in Gram-positive bacteria than it is in the non-polar lipid layer. On the walls of Gram-positive bacterial cells contain polysaccharides, which are a water-soluble polymer, which functions as a transfer of positive ions to get in and out. It is this solubility which shows that the Gram-positive cell wall is more polar than Gram-negative cell. After entering, the flavonoids work to destroy bacteria by denaturing proteins that can cause metabolic activity. Bacterial cells stop because all bacterial cell metabolic activities are catalyzed by an enzyme, which is a protein. Cessation of metabolic activity will result in bacterial cell death (Osonga *et al*., 2019; Echeverria *et al*., 2017).

Table I. Phytochemical screening of pineapple peel extract

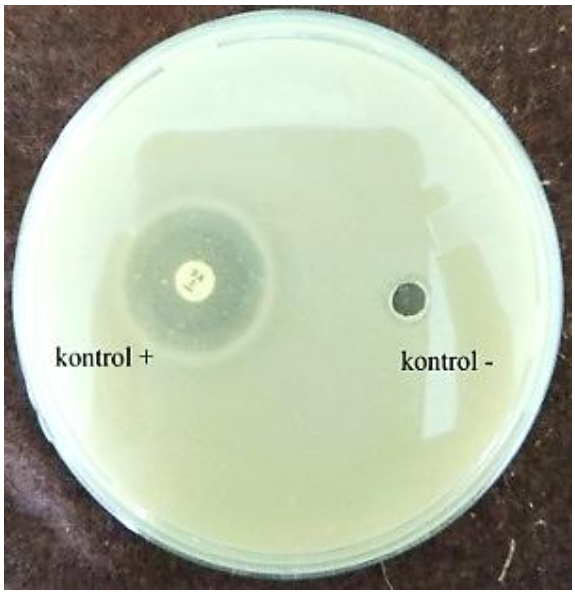
Test	Reagent	Result
Alkaloid	Dragendorff	-
	Mayer	-
Flavonoid	Mg and HCl 2N	+
Saponin	Aquadest	+
Tannin	$FeCl_3$	-
Steroid	Liebermann-Burchard	-

Various inhibition zone diameters were obtained, which were formed from each concentration of pineapple peel extract solution used in the study. The increasing concentration of pineapple peel extract solution shows that there is an increase in the inhibition zone diameter. The result of the inhibition zone can be observed in **Table II** and **Figure 1.** Based on the test results of data analysis with SPSS shows there are differences in inhibition zone values in negative control and concentrations of 12.5%, 25%, and 37.5%, while between concentrations of 50%, 62.5%, 75%, 87, 5% and 100% and positive controls were significantly different. The average value of the inhibition zone in the negative control and composition 12.5%, 25%, and 37.5% determine the lowest value of 0 mm while the average value of the inhibition zone in the positive control shows the highest value of 19.33 mm. **Table II**

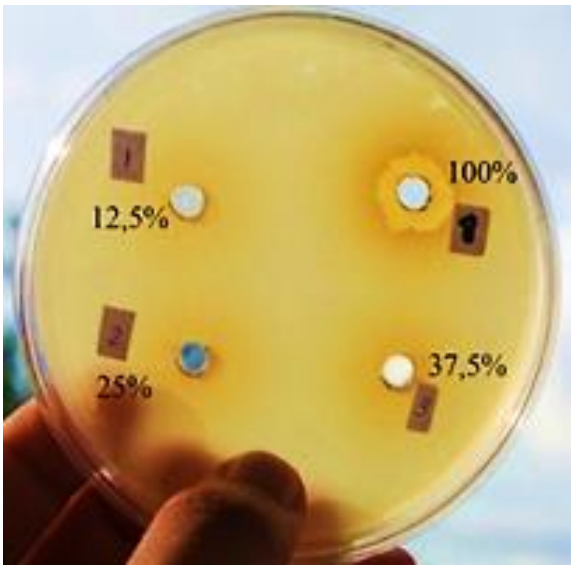
also shows a significant increase in inhibition zones each period after the concentration of pineapple peel extract by 37.5% to 100%. The categorizing of the results was based on Davis & Stout (1971), who mentioned that the antibacterial inhibition zone with diameter > 20 mm means very strong, 10-20 mm means strong, 5-10 mm means medium, and < 5 mm indicates weak inhibition. Based on these categories, the pineapple extract with a concentration of 100% is in a strong category, while at a concentration of 50 to 87.5 is in the medium category.

Table II. Inhibition zone of pineapple peel extract for *P. acnes*

Sample	Inhibition zone diameter (mm)	± Standard deviation (mm)
Negative control	0	0
Positive control	19.33	0.57
Extract 12.5%	0	0
Extract 25%	0	0
Extract 37.5%	0	0
Extract 50%	5.17	0.29
Extract 62.5%	6.67	0.29
Extract 75%	7.33	0.29
Extract 87.5%	8.17	0.29
Extract 100%	10.17	0.76



a



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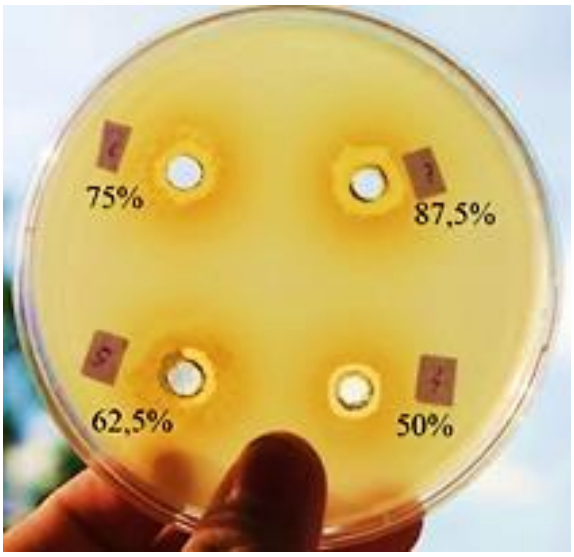

c

Figure 1. The antibacterial test results of positive and negative control (**a**), 96% ethanol extract of pineapple peels with a concentration of 12.5; 25; 37.5; and 100% (**b**) as well as and 50; 62.5; 75; and 87.5% (**c**) against *P. acnes*

CONCLUSION

The results of the research can be concluded that: pineapple peel extract contains compounds flavonoids and saponins, which have an antibacterial effect on the growth of *P. acnes*. Ethanol extract of pineapple peel has a strong category at a concentration of 100% on *P. acnes* growth with an average inhibition zone diameter of 10.16 mm.

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