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Antibacterial Effectiveness Test of Mulberry Leaf Extract (Mours Alba .L) Against Propionibacterium Acnes Using Agar Diffusion Method

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Abstract. Propionibacterium acnes bacteria are gram-positive bacteria that cause acne. This study aims to determine the antibacterial activity of mulberry leaf extract (Mours Alba L) against Propionibacterium acnes bacteria using the agar diffusion test method. Based on the results of phytochemical screening of mulberry leaf powder containing flavonoids, saponins, tannins, steroids, and triterpenoids which function as antibacterials. Based on the antibacterial activity test, mulberry leaf extract at concentrations of 20%, 30%, 40%, and 50% is effective in inhibiting the growth of Propionibacterium acnes bacteria. This can be proven from the results of the inhibition zone diameter, there is an increase in antibacterial effectiveness. Along with the increase in the concentration of mulberry leaf extract in inhibiting the growth of Propionibacterium acnes bacteria , namely at a concentration of 20%, the average diameter of the inhibition zone was 11.94 mm, then at a concentration of 30% the average diameter of the inhibition zone was 13.3 mm at a concentration of 40% the average diameter of the inhibition zone was 12.5 mm and at a maximum concentration of 50% the average diameter of the inhibition zone was 11.84 mm. The results of the study concluded that the most effective mulberry leaf extract inhibited the growth of Propionibacterium acnes bacteria at a concentration of 30% with a strong category.

Keywords : Antibacterial, Mulberry leaf extract, Proponibacterium acne, Agar diffusion

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Introduction

Acne or in medical terms acne vulgaris is one of the problems that is often experienced by everyone. Propionibacterium acnes is a microorganism that causes acne on the mucous membranes of human skin [1]. In the results of the Global Burden of Disease (GBD) study, the prevalence of acne in 2019 occurred in the 15-49 age group as much as 95% [2]. Data on the prevalence of acne in Indonesia in 2020 in adolescents was 80% to 85% with the highest incidence in the 15 to 18 age group. In women over 25 years old it was 12% and in the 35-44 age group it was around 35% [3]. Data on the prevalence of acne vulgaris In East Java, the male population is 41.46%, while in women it is 58.54%, with the highest prevalence occurring at the age of 16-19 years [4].

The main bacteria that causes acne is Propionibacterium acnes. This bacteria is a gram-positive bacteria that can infect the skin and gastrointestinal tract. Propionibacterium acnes bacteria can cause opportunistic infections in the form of acne, especially during puberty because increased androgen activity during puberty triggers the growth of sebaceous glands and increased sebum production [5]. Acne can be treated with antibacterial drugs, there are two types of treatment, namely topical and oral. One example of a treatment that can be used is antibiotics [6]. However, excessive use of antibiotics without medical supervision will result in antibiotic resistance in the body. This has encouraged researchers to look for various alternative efforts that use traditional medicines derived from plants containing many active substances for use in treatment, one of which is mulberry leaves which can function as an antibacterial[7].

Mulberry leaves are plants that have been proven to have antibacterial properties. Compounds in mulberry leaves are generally alkaloids, polyphenols and flavonoids that function as antibacterials by forming complex compounds against extracellular proteins that disrupt membrane integration bacterial cells[8]. Flavonoid compounds work by inhibiting the formation of bacterial cell walls [9]. Mulberry leaves are currently used as traditional medicine and cosmetics. In research [10] Mulberry leaf extract can inhibit Streptococcus mutans bacteria very strongly in dental caries. The results of the study [11] showed that mulberry leaf extract has the potential for antibacterial activity against Escherichia coli bacteria and the one that has good potential as antibacterial activity is the ethanol extract with a concentration of 10% with an inhibition zone diameter of 9.89 mm. The results of the antibacterial effectiveness test Ethanol extract of mulberry leaves (Morus alba L.) in the study [12] The most effective inhibition zone for Staphylococcus aureus bacteria at a concentration of 100% obtained an inhibition zone of 16 mm. For the most effective inhibition of Escherichia coli bacteria at a concentration of 100% obtained an inhibition zone of 16.3 mm.

In previous studies, most of them tested the antibacterial effectiveness of mulberry leaf extract against Streptococcus and Escherichia coli bacteria. This time, researchers will test mulberry leaf extract on Propionibacterium acne bacteria which function as anti-acne.

Experimental

Tool

The tools used in this study were blender, analytical balance, 120 mesh sieve, filter paper, oven, spoon, petri dish, beaker glass, measuring cup, incubator, ose, vernier caliper, measuring flask, autoclave, test tube, pH meter, Erlenmeyer flask, tweezers, ose needle, test tube, vacuum, vortex, hot plate.

Material

The materials used in this study were 96% ethanol, mulberry leaves, corn starch, and oatmeal, pure culture of Propionabacterium acne, paper disk, nutrient agar, and distilled water.

Sample Preparation

Mulberry leaf samples were washed with running water until clean and then dried in an oven at 50°C for the best drying time of 96 hours [13]. Then the mulberry leaves were ground with a blender and then sieved to obtain powder from the mulberry leaves.

Mulberry Leaf Extraction

A total of 500 grams of mulberry leaf powder was put into a beaker glass, 1000 mL of 96% ethanol was added and stirred. After 3 days, the solvent was replaced with 1000 mL of new 96% ethanol, then the sample was stirred. The solvent was replaced once a day with the same amount of sol-

vent, namely 1000 mL. The fluid was replaced 6 times. The extract obtained was then collected and the solvent was evaporated using a rotary evaporator at a temperature of 70°C until a thick extract was obtained [8].

Mulberry Leaf Extract Concentration Variations

This study used varying concentrations (Table 1) of mulberry leaf extract to see the effect of mulberry leaf extract on the antibacterial inhibitory power produced.

Preparation of nutrient agar media

Nutrient agar media is made by weighing 6.6 grams of nutrient agar , then dissolving it in 100 mL of distilled water in an Erlenmeyer flask and heating it until it dissolves, then sterilizing it in an autoclave at a temperature of 121°C, at a pressure of 1 atm for 15 minutes [8].

Preparation of test bacteria

The test bacteria used were *Propionabacterium acne* bacteria obtained from the Laboratory Microbiology, University of Muhammadiyah Malang.

Pure Culture Rejuvenation of *Propionabacterium acne* bacteria

Pure culture, *Propionabacterium acnes* was scraped and inoculated aseptically by streaking. on a slant of nutrient agar medium, then incubated anaerobically at 27°C for 24 hours.

Preparation of Test Bacteria

A pure suspension of *Propionabacterium acnes* was scraped and placed into a test tube containing distilled water and homogenized, then applied to a petri dish containing nutrient agar medium.

Antibacterial Test

Antibacterial testing of the mask formula was carried out using the agar diffusion method using disc paper. The disc paper was dipped into the mask formula, left for approximately 3 minutes, then lifted and left to dry slightly for approximately 3 minutes, then the disc paper placed on the surface of the media using tweezers and pressed slightly. Incubation for 1x24 hours using an incubator with a temperature of 27°C. After incubation for 1x24 hours, it is then observed whether there is inhibition zone around the disc paper. Measure the clear zone formed using a vernier caliper.

Result and Discussion

Sample Preparation Results

Sample preparation was done by sorting mulberry leaves, cleaning with running water and then drying in an oven at a temperature of 50°C for 96 hours. Low temperature drying was done to avoid chemical changes in the active compound content of mulberry leaves.

Drying in the oven serves to accelerate the removal of water and obtain samples with low water content, so that they are not easily rotten during storage. The dried samples are then ground and sieved with a 120 mesh sieve.

Table 1. Variation of Murberry Leaf Extract Concentration

Material	Concentration of Materials					
	K-	20 (%)	30 (%)	40 (%)	50 (%)	K+
Murberry Leaf Extract	-	2g	3g	4g	5g	-
DMSO	10ml	10ml	10ml	10ml	10ml	10ml
Dohixat	-	-	-	-	-	1mg

Figure 1. Dried Mulberry Leaves and Mulberry Leaf Powder

Figure 2. Results of Mulberry Leaf Extract

Mulberry Leaf Extraction Results (see Figure 2)

The extraction method used in this study is maceration extraction. Maceration is a process of extracting simple drugs using solvents with several stirrings at room temperature. In this study, the solvent used in the maceration process was 96% ethanol. Ethanol is used as a solvent because it is polar, universal and easy to obtain. In addition, ethanol is also a solvent for organic and inorganic substances.

The lowest purity of ethanol solvent that can dissolve a secondary metabolite compound is 96%, so 96% ethanol is expected to be able to extract more secondary metabolite compounds. Because the higher the concentration of ethanol, the easier it will be in the process of separating secondary metabolite compounds from the sample. Ethanol 96% is also able to extract compounds needed for the activity test of powdered leaves, namely alkaloids, flavonoids, phenols, and terpenoids. The maceration process during 24 hours and the extract obtained was then separated from the solvent using a rotary evaporator at a temperature of 70°C until a thick green ethanol extract was obtained.

Antibacterial Test

In the inhibition test, variations in the concentration of mulberry leaf extract were carried out with the aim of finding the most effective concentration in inhibiting the growth of Propionibacterium bacteria acne.

Based on the hypothesis, the higher the concentration of the extract used, the higher the inhibition of bacterial growth. This is due to the increasing number of active compounds that interact with bacteria at higher concentrations, thereby inhibiting bacterial growth more effectively. The diameter of the inhibition zone (**Table 2**), which indicates how wide the clear zone is around the disc paper (**Figure 3**).

The results of the antibacterial activity test showed that mulberry leaf extract was able to inhibit the growth of Propionibacterium acnes. Variations in the concentration of mulberry leaf extract provided the most effective inhibition zone against Propionibacterium acnes bacteria, namely 30% with an inhibition diameter of 13.3 mm, in the strong category. At concentrations of 40% and 50% the results were not as large as at a concentration of 30%, this was caused by the depth of the agar media which was too deep which could affect the results of the bacterial inhibition test using the disc method. Where too great a depth can inhibit the diffusion of active substances into the bacterial growth medium [15]. This shows that mulberry leaf extract has a flavonoid content that has strong antibacterial potential in inhibiting the growth of proponibacterium acne bacteria. This flavonoid compound has a way of working to inhibit the formation of bacterial cell walls [9].

Based on the results of this study, the mulberry leaf extract preparation has a smaller inhibi-

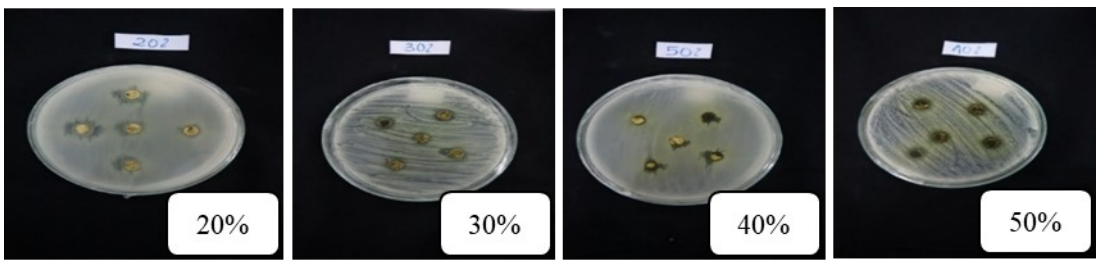

Figure 3. Antibacterial Test Results

Table 2. Results of the Inhibitory Power Test of Mulberry Leaf Extract

Treatment Extract Formulation Mulberry Leaves	Inhibition Zone Diameter (mm)						Inhibition zone category
	U1	U2	U3	U4	U5	Average	
20%	14,5	14,5	9,5	11,7	9,5	11,94	Strong
30%	10,5	16	11	15	14	13,3	Strong
40%	15,5	11	11,5	10,5	14	12,5	Strong
50%	11,5	13	13,5	9,5	11,7	11,84	Strong
K+	52,50	52,25	52,20	52,30	52,27	52,304	Very Strong
K-	0	0	0	0	0	0	There isn't any

Inhibition zone category: Weak: < 5 mm, Medium: 5-10 mm, Strong: 11-20 mm, Very strong: >20 mm [14]

tion zone compared to the control (+) in the form of dohixate. This can be caused because the mulberry leaf extract as an active substance in the mulberry leaf extract preparation does not contain pure compounds while the preparation used as a positive control is a pure chemical compound.

Conclusion

Based on the research that has been conducted, it can be concluded that mulberry leaf extract at concentrations of 20%, 30%, 40% and 50% can inhibit Propionibacterium acnes bacteria with inhibition zone diameters of 11.94 mm, 13.3 mm, 12.5 mm and 11.84 mm respectively with a strong category. Variations in the concentration of mulberry leaf extract provided the most effective inhibition zone against Propionibacterium acnes bacteria, namely 30% with an inhibition diameter of 13.3 mm, in the strong category.

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