

Antibacterial Activity Test of Leaf Ethl Acetate Extract Distance of Fence (*Jatropha curcas L.*) Against Bacteria *Propionibacterium Acnes* and *Staphylococcus Aureus*

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Abstract

Skin diseases are very easy to found in countries with a tropical climate. The climate that facilitate the development of bacteria, parasites, and fungi. Acne is a skin disease that occurs due to inflammation of the follicles polisebasea characterized by the presence of comedones, papules, pustules and nodules. *Propionibacterium acnes* and *Staphylococcus aureus* is a bacterium that play a role in the pathogenesis of acne that will cause inflammation. The study was to determine the concentration of ethyl acetate extract of leaves of *Jatropha* effectively inhibits the growth of bacteria *Propionibacterium acnes* and *Staphylococcus aureus*. This study was experimental research that includes plant identification, manufacture of crude dru, ethyl acetate extract of *Jatropha*, examination of the phytochemical screening, and test the antibacterial activity of extracts of *Jatropha* leaves by the method of pitting using MHA media, the manufacture of the extract with maceration using solvent ethyl acetate. The results of phytochemical screening of *Jatropha* showed the presence of flavonoid compounds, alkaloids, saponins and tannins. The results of the antibacterial activity test showed that each concentration had an inhibitory response that was categorized as moderate to strong on *Propionibacterium acnes* bacteria with clear zone diameters of 10% (7.50mm), 20% (9.18mm), 30% (12.10mm), and 40% (15.16mm) in *Staphylococcus aureus* bacteria with a clear zone diameter of 10% (8.13mm), 20% (10.21mm), 30% (12.13mm), and 40% (15.53mm). The conclusion showed that the ethyl acetate extract of *Jatropha* leaves (*Jatropha curcas L.*) has antibacterial activity against *Propionibacterium acnes* and *Staphylococcus aureus*.

Keywords: *Jatropha* Leaves (*Jatropha Curcas L.*), Flavonoids, *Propionibacterium Acnes*, *Staphylococcus Aureus*

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Introduction

Skin diseases are very easy to find in countries with tropical climates. This climate facilitates the development of bacteria, parasites, and fungi (Wardani, 2020). Acne is a skin disease that occurs due to chronic inflammation of polysebaceous follicles which is characterized by the presence of comedones, papules, pustules and nodules (S. Handayani, 2021). Acne can be caused by overactivity of the oil glands and exacerbated by bacterial infections. The bacteria that cause acne consist of *Propionibacterium acnes* and *Staphylococcus aureus* (Djuanda et al., 2007).

Propionibacterium acnes is a bacterium that plays a role in the pathogenesis of acne which can cause inflammation. *Propionibacterium acnes* uses glycerol in sebum as a source of nutrition,

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forms free fatty acids from sebum which causes neutrophil cells to release enzymes that can damage hair follicle walls and inflammation occurs resulting in pustules and papules on the skin (Radji, 2009).

Staphylococcus aureus is a gram-positive bacterium, which can live aerobically or facultatively anaerobically in the form of balls or cocci in irregular groups, does not form spores and does not move, the colonies are yellow, these bacteria are found on the skin (Jawetz et al., 2010).

Ethyl acetate solvent is classified as a solvent with low toxicity which is semipolar so that it is expected to be able to attract polar and non-polar compounds, the semipolar nature of ethyl acetate causes ethyl acetate extract to have two solubility properties, namely hydrophilic and lipophilic. Hydrophilic and lipophilic groups are required for the action of antimicrobial compounds. The hydrophilic group is needed so that the antimicrobial substance can dissolve in water where the microbes grow, while the lipophilic group is needed so that the substance reacts with the membrane of the microbes (Yasni, 2013).

Treatment of acne is done by reducing sebum production, reducing inflammation in the skin, repairing follicular abnormalities and reducing the number of bacterial colonies and/or their metabolic products. The way to treat acne that is needed by today's youth, many products and ways to treat acne have appeared, ranging from the use of expensive anti-acne drugs and treatments to beauty salons (Retnaningsih et al., 2019).

One of the plants that can be used as efficacious medicine is *Jatropha curcas* (*Jatropha curcas* L.). *Jatropha curcas* plant has been widely used by the community to be used as a nutritious plant for health and empirically, the sap of this plant has been used by the community as a treatment for acne, canker sores, wound medicine and blood styptic. Apart from that, the leaves of the *Jatropha* plant are also used as a medicine for fever, and for treating rheumatism (Yulianto & Sunarmi, 2018).

Previous research on *Jatropha curcas* leaves was identified as containing flavonoids, alkaloids, steroids, tannins, terpenoids and saponins (Nzubechukwu et al., 2015). In the research to test the effectiveness of *Jatropha curcas* as an anti-microorganism on *Escherichia coli* bacteria at concentrations of 20%, 40%, 60%, 80%, namely with an inhibition of 12.00 mm, 14.25 mm, 20.25 mm and 24.25 mm (Guranda & Maulanza, 2016).

Methods

Research design

This study used experimental methods, including identification of plants, manufacture of *simplicia*, manufacture of ethyl acetate extract of *Jatropha* leaves, phytochemical screening examination, and antibacterial activity test of *Jatropha curcas* L. leaf extract by means of wells using MHA media.

Place and time of research

This research was conducted at the Phytochemical Laboratory of the Helvetia Institute of Health and at the Herbarium Medanense FMIPA, University of North Sumatra. This research was conducted in July-September 2021.

Research Tools

The tools used in this study were Autoclave, petri dish, steel joiner, incubator, caliper, ose wire, analytical scales, waterbath, oven, micropipette, rotary evaporator, porcelain dish and blender.

Research Materials

The ingredients used in this study were pirdot leaves, *Propionibacterium acnes* bacteria and *Staphylococcus aureus* media MHA (Mueller Hinton Agar), ethyl acetate, DMSO (Dimethyl Sulfoxide), clindamycin, NaCl 0.9%, Dragendorff reagent, Mayer reagent, Bouchardic reagent, HCl 2N, HCl(p), FeCl₃ 1%, anhydrous acetic acid, Mg powder, H₂SO₄(p), and aquadest.

Creation of Simplicia

Making simplicia is by collecting fresh *Jatropha* leaves and then sorting them wet to separate impurities or foreign materials from the simplicia. then washed with running water until clean to remove soil, microbes and other impurities attached to the simplicia. The dried simplicia is sorted to separate foreign objects such as unwanted plant parts and other impurities that are still left in the dried simplicia. Then weigh the dry weight. The dried simplicia was then crushed using a blender, the simplicia powder was stored in a glass container to prevent the effects of moisture and other contaminants (Ministry of Health, 1989).

Manufacture of *Jatropha* Leaf Extract

Jatropha leaf extract is made by maceration. Comparison of 1:10, namely as much as 500 g of sample with 5 liters of solvent. *Jatropha* leaves were put into a glass container, then soaked with 75 parts of ethyl acetate solvent as much as 3750 ml then the container was covered with aluminum foil and left for 5 days protected from sunlight while stirring occasionally then filtered with filter paper so that the first filtrate was obtained and the residue was soaked again using 25 parts of the remaining 1250 ml of ethyl acetate solvent then the container covered with aluminum foil was left for 2 days while occasionally stirring. After 2 days the sample was filtered using filter paper and a second filtrate was obtained, then the first filtrate and second filtrate were combined then the filtrate obtained was evaporated with a rotary evaporator at 40°C and concentrated using a water bath at 40°C to obtain a thick extract then a thick extract obtained were weighed and stored at room temperature (Ministry of Health, 1979).

Phytochemical Screening

Examination of flavonoids, namely 0.5 g of sample added to 10 ml of aquadest, heated for 5 minutes and filtered, into 5 ml of the filtrate added 0.1 g of Mg powder and 10 drops of HCl(p) and 1 ml of amyl alcohol allowed to separate if a color is formed red, yellow or orange on the amyl alcohol layer is positive for flavonoids.

Examination of alkaloids, namely as much as 0.5 g of sample added 1 ml of HCl 2 N and 9 ml of water, then heated over a water bath for 2 minutes, then cooled and filtered. The filtrate was transferred 3 drops each into a test tube, then 2 drops of Mayer's, Bouchardat's and Dragendorff's reagent solution were added to each test tube. If alkaloids are present, the Mayer reagent forms a white or yellowish white precipitate, with the Bouchardat reagent a brown, reddish brown to blackish brown precipitate is formed, and with the Dragendorff reagent a red or orange precipitate is formed.

Examination of saponins, namely as much as 0.5 g of sample is put in a test tube, added 10 ml of hot water, cooled and shaken for 10 minutes until foam forms, then dripped with HCl 2 N if the foam does not disappear with the addition of HCl 2 N then it is positive for saponins.

Examination of tannins, namely as much as 0.5 g of sample added to 5 ml of distilled water and then heated for 5 minutes. Then filtered, taken 2 ml of filtrate, added 3 drops of 1% FeCl₃ solution. If a dark blue or green-black color is formed, it indicates the presence of tannins.

Steroid examination as much as 0.5 g sample added to ether or n-hexane, then allowed to stand for 2 hours, filtered then the filtrate was evaporated. To the remainder, anhydrous acetic acid was added, then dripped with H₂SO₄(p) (Liebermann-Burchard reagent). Appearance of purple and red or turning blue-green indicates the presence of triterpenes/steroids (Lubis et al., 2014).

Media Creation

The preparation of MHA media was carried out by weighing 9.5 g of MHA powder into an Erlenmeyer then dissolving it with 250 ml of distilled water and heating it over a water bath until it boils and dissolves completely, covered with cotton. The media was then sterilized using an autoclave at 121°C for 15 minutes after which it was allowed to cool, the media was ready to be used for making media for bacterial culture and bacterial growth (F. Handayani et al., 2018).

Bacterial Inoculation of the Medium to Be Oblique

Propionibacterium acnes bacteria were taken with a sterile loop needle, then instilled in oblique agar media by scraping and then incubated at 37°C for 24 hours. The same work was carried out on staphylococcus aureus bacteria (F. Handayani et al., 2018).

Preparation of Solution Turbidity Standards

9.5 ml of 0.36 N H₂SO₄ solution mixed with 0.5 ml of 1.175% BaCl₂H₂O solution in Erlenmeyer. Then shaken until a cloudy solution is formed. This turbidity is used as a standard for the turbidity of the test bacterial suspension (F. Handayani et al., 2018).

Preparation of Test Bacterial Suspensions

Propionibacterium acnes bacteria that had been inoculated were taken with sterile wire loops and then suspended in a tube containing 10 ml of 0.9% NaCl solution until a turbidity equal to the standard McFarland solution was obtained. The same work was carried out on Staphylococcus aureus bacteria (F. Handayani et al., 2018).

Preparation of Extract Dilution

Weigh the 50% concentrated stock extract solution by dissolving 2.5 g of the concentrated extract in 5 ml of DMSO. For a 40% concentration, 1.6 ml of 50% extract was taken and then added up to 2 ml of DMSO, for a 30% concentration, 1.2 ml of 50% extract was taken and then added up to 2 ml of DMSO, for a 20% concentration, 0.8 ml was taken from extract 50% then add up to 2 ml of DMSO, for a concentration of 10% take 0.4 ml of the extract 50% then add up to 2 ml of DMSO. Clindamycin powder with a concentration of 15% by dissolving 0.75 g of powder in 5 ml of DMSO. For a 1% concentration, 0.13 ml of 15% clindamycin was taken and then added up to 2 ml of DMSO where DMSO (Dimethyl Sulfoxide) served as a negative control.

Antibacterial Activity Test

The base layer was prepared by pouring 10 ml of MHA media into a petri dish and then allowing it to solidify. After the base layer has solidified, steel scrapers are planted which are spaced so that the observation area is not supported. As much as 0.1 ml of the bacterial suspension was

put into the MHA medium, then 15 ml of MHA media was poured as the second layer. After the second layer has solidified, the scraper is removed using tweezers to form wells. This procedure was performed on two petri dishes. Ethyl acetate extract of *Jatropha* leaves with a concentration of 10%, 20%, 30%, 40%, was put in each well in a petri dish 1 as much as 50 μ l, then positive control (clindamycin) and negative control (DMSO) were put into each -each well in 2 petri dishes of 50 μ l, incubated at 37°C for 18-24 hours. This test was carried out 3 repetitions (Ngajow et al., 2013).

Data analysis

The data used is quantitative data obtained from data analysis on antibacterial activity tests using the One Way Anova statistical method (F. Handayani et al., 2018).

Results and Discussion

In this study, extraction was carried out by maceration method and the solvent used was ethyl acetate. The wet weight of the simplicia was 3 kg, then it was dried to obtain a dry weight of 1400 g. Then taken as much as 500 g for extraction. The yield of simplicia was obtained at around 16.67% and yielded a viscous extract of 23.50 g so that a viscous extract yield of 4.7% was obtained.

Then carried out phytochemical screening of *Jatropha* leaf extract to determine the class of secondary metabolites contained therein. Phytochemical screening examination consists of examining the class of flavonoids, alkaloids, saponins, tannins, and steroids. The results of the phytochemical screening examination of *Jatropha* leaf extract are shown in Table 1.

Table 1. Results of Phytochemical Screening of *Jatropha Curcas* (*Jatropha curcas* L.) Leaf Extract

Compound Group	Reagent Name	Result
Alkaloids	Mayer	-
	Dragendorff	-
	Bouchardat	+
Flavonoids	Sebuk Mg + HCl _(p)	+
Saponins	Hot water + HCl 2N	+
Tannins	Aquadest + FeCl 1%	+
Steroids	Liebermann Burchard	-

Based on Table 1, it is known that *Jatropha curcas* L. leaf extract contains secondary metabolites of flavonoids, alkaloids, saponins and tannins.

Furthermore, the antibacterial activity test in this study used the well method and used *Propionibacterium acnes* and *Staphylococcus aureus* bacteria. Antibacterial activity was indicated by the diameter of the clear zone formed around the wells. Based on the research that has been done, the results of the bacterial inhibition test of the ethyl acetate extract of *Jatropha curcas* L. leaves (*Jatropha curcas* L.) were obtained which can be seen in Table 2.

Table 2. Data on the diameter of the inhibition zone of *Propionibacterium acnes* and *Staphylococcus aureus*

Concentration	Diameter of the Inhibitory Zone		Categories of Inhibition Zones
	<i>Propionibacterium acnes</i>	<i>Staphylococcus aureus</i>	
10%	7,50	8,13	Keep

20%	9,18	10,21	keep
30%	12,10	12,13	Strong
40%	15,16	15,53	strong
Control +	30,68	28,66	Very Strong
Control-	0	0	None

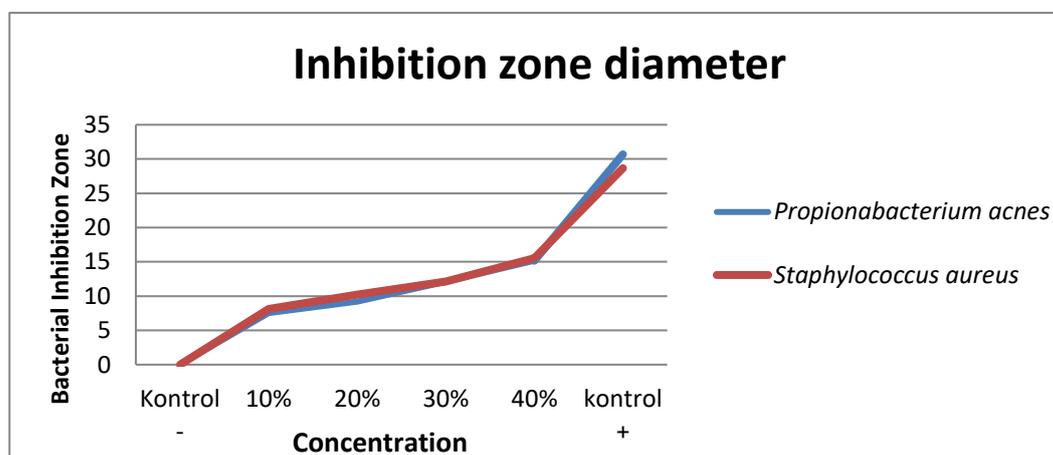


Figure 1. Graph of the diameter of the inhibition zone of *Propionibacterium acnes* and *Staphylococcus aureus* bacteria

Based on the test results in table 2, it shows that the ethyl acetate extract of *Jatropha curcas* L. leaves has antibacterial activity against *Propionibacterium acnes* and *Staphylococcus aureus* bacteria. Marked by the results from the table above which shows that there is a difference to the negative control group.

The sample used in this study was *Jatropha curcas* L. leaves. 500 g of dry simplicia powder used. The sample is mashed into powder aiming to speed up the maceration time. The powder is macerated using ethyl acetate solvent with a ratio of 1:10 Extraction of *Jatropha* leaves is carried out using the maceration method because this method is easy to do, the process is simple and the tools used are easy to obtain. Maceration was carried out for five days and then filtered, then remaceration was carried out for two days to obtain more extract. Then it was evaporated using a rotary evaporator at a temperature of 40°C to obtain an almost viscous ethyl acetate extract and concentrated with a water bath at a temperature of 40°C to obtain a thick extract of *Jatropha* leaves as much as 23.50 g.

The phytochemical test in this study was carried out only up to the qualitative stage. The qualitative phytochemical test of *Jatropha curcas* aims to see the content of secondary metabolites contained therein. The flavonoid test showed a positive result with a yellow color change. Flavonoid test was carried out by adding Mg powder and HCl(p) to *Jatropha curcas* leaf samples. Reduction with Mg and HCl(p) can produce red, yellow or orange complex compounds in flavone. Flavonoids in the phenolic compound group have many –OH groups with a high electronegativity difference, so they are polar in nature and in the presence of hydroxyl groups so that hydrogen bonds can form (Nuraini & Widada, 2016).

In the alkaloid test, the sample was dissolved in HCl. Alkaloids are basic, so the addition of HCl will cause the formation of salts. The addition of distilled water aims to dissolve the alkaloid salts and heating is carried out to break down the alkaloids with hydrochloric acid, so that the alkaloids are obtained which are not from their salts. Identification of alkaloids was

carried out using three reagents, namely Mayer, Dragendorff, Bouchardat (Nuraini & Widada, 2016). The results of the identification of the sample which was reacted with Mayer's reagent did not have a white precipitate so the result was negative. In the Bouchardat reagent, a brown precipitate appears, so the result is positive. In the Dragendorff reagent, a red precipitate appears, so the result is positive.

In the saponin test, the sample was dissolved with distilled water and then shaken until foam formed and then dripped with 2 N HCl to see the stability of the foam. The results of the identification showed positive for containing saponins where after shaking the foam stabilized. Formation of foam / froth because saponin compounds have properties that dissolve easily in water and will cause foam when shaken. The appearance of foam in this test indicates the presence of glycosides which have the ability to form foam in water which hydrolyzes into glucose and other compounds (Nuraini & Widada, 2016).

In the tannin test identification using FeCl₃ reagent. The results of the identification showed positive for containing tannins by giving a black-green color. The formation of this green color is due to the formation of complex compounds between Fe metal and tannins. Complex compounds are formed due to coordinate covalent bonds between metal ions or atoms and metal non atoms. On the addition of FeCl₃, it is estimated that this solution will react with one of the hydroxyl groups present in the tannin compound (Nuraini & Widada, 2016).

In testing the antibacterial activity of the ethyl acetate extract of *Jatropha* leaves in inhibiting the growth of *Propionibacterium acnes* and *Staphylococcus aureus* bacteria using the well diffusion method. This method is used because of the simplicity of the technique, easy to measure the area of the inhibition zone and the antibacterial activity using the well method is higher (Nurhayati et al., 2020).

Table 2 shows that the ethyl acetate extract of *Jatropha* leaves with a concentration of 10%, 20%, 30% and 40% each has an inhibition zone against the test bacteria. In *Propionibacterium acnes* bacteria the diameter of the clear zone formed around the wells at each concentration was: 10% (7.50 mm), 20% (9.18 mm), 30% (12.10 mm), and 40% (15.16 mm) at each concentration of 10%, 20%, 30%, and 40% had an inhibitory response that was categorized as moderate to strong. The smallest inhibition zone was found in the extract with a concentration of 10% then the inhibition increased at a concentration of 40%. The positive control showed an inhibition zone and was classified as very strong with a diameter of (30.68 mm) and the negative control showed no inhibition zone response to the bacteria.

In *Staphylococcus aureus* bacteria the diameter of the clear zone formed around the wells at each concentration was: 10% (8.13 mm), 20% (10.21 mm), 30% (12.13 mm), and 40% (15.53 mm) at each concentration of 10%, 20%, 30%, and 40% had an inhibitory response that was categorized as moderate to strong. The positive control showed an inhibition zone and was classified as very strong with a diameter of (28.66 mm) and the negative control showed no inhibition zone response to these bacteria.

In a previous study on the activity test of the ethyl acetate extract of *Jatropha* leaves against *Shigella flexneri* with the highest inhibition diameter of 7.7 mm. while the research that I did, ethyl acetate extract of *Jatropha* leaves on the growth of *Propionibacterium acnes* and *Staphylococcus aureus* bacteria with the highest diameter of 15.16 mm and 15.53 mm respectively from the two studies. which was higher in inhibiting *Propionibacterium acnes* and *Staphylococcus aureus* bacteria than ethyl acetate extract of *Jatropha* leaves in inhibiting *Shigella flexneri* bacteria. The difference in the diameter of the inhibition zone in the two

studies was due to the different test bacteria used. In my research, *Propionibacterium acnes* and *Staphylococcus aureus* are gram-positive bacteria, while *Shigella flexneri* is gram-negative bacteria. So it can be said that the ethyl acetate extract is better at inhibiting gram-positive bacteria.

The appearance of microbial inhibition activity by a compound is strongly influenced by the concentration of the test material and microbes. The resistance of a bacterium to antibacterial compounds is closely related to the structure of its cell wall. Active compounds derived from plants often show better activity against gram-positive bacteria than gram-negative bacteria because gram-positive bacteria have a simpler cell wall structure than gram-negative cell wall structures so that antibacterial compounds more easily enter gram-positive bacterial cells (Nuraini & Widada, 2016).

Inhibited bacterial growth or bacterial death due to an antibacterial agent can be caused by inhibition of cell wall synthesis, inhibition of cell membrane function, inhibition of protein synthesis, or inhibition of nucleic acid synthesis (Jawetz et al., 2001).

The existence of antibacterial activity against *Propionibacterium acnes* and *Staphylococcus aureus* bacteria is caused by the presence of secondary metabolites contained in *Jatropha curcas*, the most important of which are flavonoids which are secondary metabolites in plants that have antibacterial properties. The mechanism of action of flavonoids as antibacterials is by interacting with bacterial cells through an absorption mechanism involving hydrogen bonds with phenol groups. The H atom in the protein complex found in the bacterial cell wall binds to the phenol group on the flavanoid. Then the protein undergoes decomposition followed by the penetration of flavonoids into the cells which causes denaturation of plasma proteins (Idroes et al., 2019).

Antibacterial activity is influenced by several factors, namely the content of bacterial compounds, the concentration of extracts and the type of bacteria that are inhibited. Based on this study, the higher the concentration, the higher the inhibition of bacterial growth (Pelczar & Chan, 2008).

In this study, the data obtained were analyzed statistically. The statistical test that was carried out was One-way ANOVA. The One-way ANOVA test was chosen because there is only one variable to be tested. Before carrying out the ANOVA test, there are 2 conditions that must be met, namely the data tested must be normally distributed with $p > 0.05$ and the data must have the same (homogeneous) data variation. From the results of the normality test on *Propionibacterium acnes* and *Staphylococcus aureus* bacteria, a sig value was obtained for each concentration > 0.05 , which means that the data was normally distributed. Based on the homogeneity test, the sig value obtained was 0.060 (> 0.05) for *Propionibacterium acnes* bacteria and 0.061 (> 0.05) for *Staphylococcus aureus*, which means that the data has homogeneous variations.

Conclusion

Ethyl acetate extract of *Jatropha curcas* (*Jatropha curcas* L.) leaves has antibacterial activity against *Propionibacterium acnes* and *Staphylococcus aureus* bacteria.

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