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Antiseptic Activity Examination of *Moringa oleifera* Leaf Extract Against Anaerobic Bacteria *Cutibacterium acnes*

Conny Riana Tjampakasari[1*], Stivan Junan Navidad[2], Ariyani Kiranasari[1]

[1]Department of Microbiology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
[2]Medical Education Study Program, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
*Corresponding Author: connyrianat@yahoo.com

ABSTRACT

Moringa leaves (M. oleifera) contain chemicals beneficial as antibacterials for Gram-positive and Gram-negative bacteria. This content can damage DNA and cell membranes, penetrate bacterial cell walls, disrupting bacterial metabolism and resulting in death. The bacteria used in this study were Cutibacterium acnes (C. acnes) an aerotolerant, anaerobic, Gram-positive bacteria. This bacteria causes Acne vulgaris (acne), a skin disorder caused by blockage of the fat glands. This research was conducted to test the activity of Moringa oleifera leaf extract as an antiseptic against C. acnes. The Percentage Kill test is used to determine the potential antiseptic activity of the extract. The comparison of growth of bacterial colonies on control and extract that grew on solid media was calculated according to the specified contact time. Kill test result are said to meet the criteria if the results obtained in each contact time are ≥90%. The Percentage Kill test results at 1, 2, and 5 minutes of contact with C. acnes bacteria are 59.7%, 72%, and 91.8%, respectively. These results indicate that at the 5th minute, moringa leaf extract can eradicate C. acnes bacteria effectively. This experiment demonstrates that the Percentage Kill test was ineffective in the first and second minutes but became effective in the fifth minute.

Keywords: *M. oleifera, Cutibacterium acnes, Percentage kill, Antiseptic*

INTRODUCTION

Cutibacterium acnes (*C. acnes*) is a Gram-positive, rod-shaped bacterium that lives anaerobically, facultatively, and does not form spores. These bacteria are part of the normal flora of the skin, oral cavity, gastrointestinal, and genitourinary coating [1,2].

Although *C. acnes* is well-known for its role in causing swollen legs, it has also been shown to be involved in a variety of infections, such as bone and prosthesis infections (BPIs), *Cerebrospinal fluid* CSF infections, endocarditis pathogenesis, septic arthritis, endophthalmitis, osteomyelitis, chronic prostatitis, sarcoidosis, synovitis, pustulosis, hyperostosis, and osteitis syndrome [3,4].

Acne (*Acnes vulgaris*) is the main clinical manifestation of the presence of *C. acnes* bacteria. This normal flora bacteria is found on the skin with sebaceous glands on the scalp and face. This rod-shaped Gram-positive bacteria is the main cause of acne compared to other bacteria (Figure 1) [2].

Acne is a chronic inflammatory disease that originates from the pylosebaseus unit and is characterized by the appearance of blackheads, pustules, papules, nodules, cysts, and scartes which generally occur in the skin, face, neck, chest, and back. Generally, this is not dangerous, but the most impact is a decrease in a person's confidence, especially for someone who pays great attention to the beauty of his body. Acne is caused by many things (multifactorial), including genetics, dietary factors, endocrine, activity of the sebaceous glands, psychological factors, bacterial infections, seasons, cosmetics, and chemicals [5,6].

Acne vulgaris itself attacks a lot in adolescence, which can reduce confidence levels. This skin disease has been experienced by almost

everyone, especially at a young age with an incidence rate of 85%. The most significant prevalence occurs in women aged 14-17 years, as much as 83-85%, and men 16-19 years old as much as 95-100% [5,6].

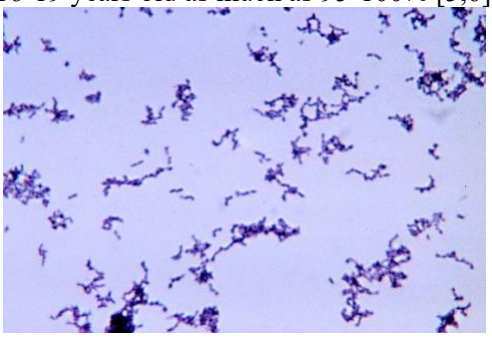

Figure 1. *Cutibacterium acnes* on Gram staining [2]

Based on survey results in Southeast Asia, there are 40-80% of acne vulgaris cases. In Indonesia itself, based on the Indonesian Aesthetic Dermatology Research, the number of cases was 60% in 2006, 80% in 2007, 90% in 2009 suffering from Acne vulgaris. It is stated that 80% of teenagers have experienced this disease with typical clinical characteristics in the form of blackheads, pustules, papules, nodules, scar tissue, etc [7,8].

Triggering factors that can cause this condition include changes in keratinization patterns, the formation of free fatty acid fractions, increased sebum, increased androgen hormones, stress, and an increased bacteria. The main triggers are age, race, diet, and weather [5]. Ways Acne vulgaris is formed especially during puberty [9,10].

Management can be topical using retinoids, clindamycin 1-2%, nadifloxacin 1%, and azithromycin 1%. Systemically, therapy can be done with doxycycline 100mg twice daily, minoxcycline 50-100 mg once daily, and other antibiotics, such as amoxicycline, erythromycin, and trimethoprim. In addition, treatment can also use herbal plants from tropical and subtropical regions in southern Asia, namely moringa leaves or *Moringa oleifera* (*M. oleifera*) [11,12].

With its diversity of plant types, Indonesia has *Moringa* leaves (*M. oleifera*) as an alternative treatment that is considered capable of treating various diseases. The compounds contained can function as an antiseptic by preventing various inflammations, one of which is the appearance of acne [13,14].

Various parts of the moringa plant can be utilized with its properties as a heart and circulatory stimulant, antitumor, antipyretic, antidiabetic, antioxidant, cholesterol, antibacterial, and antifungal. In addition, according to research, moringa leaves contain 36 anti-inflammatory substances, and their powder contains a lot of cytokinins. Cytokinin contains zeatin, a high anti-oxidant compound that is anti-aging and anti-inflammatory. This compound can help to replace body cells so that it can prevent aging and acne. Therefore, in this study, it will be assessed how effective the content contained in the moringa plant is to kill *C. acnes* bacteria [15,16,17].

METHOD

Extract preparation

The extract was obtained from the Department of Medical Pharmacy, Faculty of Medicine, University of Indonesia, weighing 120.8495 grams with a water concentration of 15.30% so that the purity of the extract was obtained 84.70%.

Bacterial preparation

Ten milliliters of Tripsoy Broth were inoculated with 1 loop of test bacteria, which had been standardized to McFarland 0.5. The suspension underwent serial dilution to achieve a 10^5 dilution, and the resulting dilution served as the test bacterium [18].

Antiseptic test of the extract against *C. acnes* using the Percentage Kill Test

This step was divided into two groups, namely treatment and control. In the treatment, 0.5 ml of microorganisms obtained from the calculation of the Colony Forming Unit (CFU) were added to 4.5 ml of extract. After 1 minute, 1 ml of the mixture is transferred into 9 ml of sterile aquades. Likewise, for contact times of 2 and 5 minutes, 1 ml of each dilution is transferred to three sets of blood agar media. Then, it is flattened using a disposable curved spatula on the entire surface of the blood agar medium. Next, it is placed in an anaerobic jar equipped with an anaerobe gas pack system and incubated in an incubator for 24-48 hours at a temperature of 35ºC. Colony counting is carried out using an Electronic colony counter to calculate the number of colonies in each blood vessel [19].

In the control, 0.5 ml of microorganisms obtained from the calculation of the Colony Forming Unit (CFU) were added to 4.5 sterile aquades. The next step is done in the same way as the treatment of the

extract. Both, the test of the extract and control was carried out under the same conditions and time [19].

Percentage Kill Calculation

The Percentage Kill result is calculated by calculating the difference between the number of colonies growing in the control and the number of colonies in the extract, then dividing by the number of colonies in the control and multiplying it by 100%. This calculation is done for each contact time. The result is declared effective if the value obtained is ≥90% [19].

RESULT AND DISCUSSION

The analysis was carried out by collecting data through experiments conducted by triplo in the control group and treatment. The control group in the first minute obtained results of 161, 169, and 187 with an average of 172.33; in the second minute 156, 157, and 160 with an average of 157.67, and in the fifth minute 140.136, and 127 produced an average of 134.33. Furthermore, the treatment group obtained results at the first minute 80, 71 and 57 with an average of 69.33, in the second minute the results were obtained 58, 44, and 30 with an average of 44, and at the fifth minute the bacteria that grew obtained results of 11, 12, and 10 with an average of 11 (Table 1).

The results showed that in the first minute, there was no significant reduction in the number of bacteria in the extract group. However, by the second minute, the bacteria had been eradicated quite a lot, even up to half of the control group. Furthermore, at the 5th minute, the treatment group was drastically reduced compared to the control group, with a difference of up to 123.33. These results show that the length of contact time between the extract and the bacteria affects the number of bacterial colonies that grow (Figure 2).

Table 1. Growth of *Cutibacterium acnes* on controls and extracts

Contact time	Colonies (C)			A Average (C)	Colonies (X)			Average (X)
	I	II	III	Average ±SD	I	II	III	Average ±SD
1 minute	161	169	187	172,33 ± 10,87	80	71	57	69,33 ± 9,46
2 minutes	156	157	160	157,67 ± 1,69	58	44	30	44 ± 11,43
5 minutes	136	136	127	134,33 ± 4,24	11	12	10	11 ± 0,81

I,II. III : Repetation, C : Control , X: Extract , SD: Standard Deviation

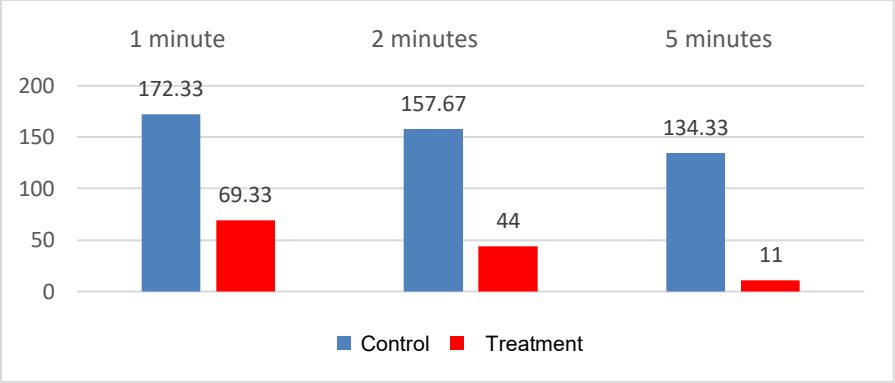

Figure 2. Average growth of C. acnes colonies in Control and Treatment (extract) for each contact time in percentage.

Table 2. The result of Percentage Kill Test

Contact time	Number of colonies		Percentage kill (%)
	Average C ±SD	Average X±SD	
1 menit	172,33 ± 10,87	69,33 ± 9,46	59,7
2 menit	157,67 ± 1,69	44 ± 11,43	72
5 menit	134,33 ± 4,24	11 ± 0,81	91,8

C: Control, X: Ekstrak *M.oleifera*

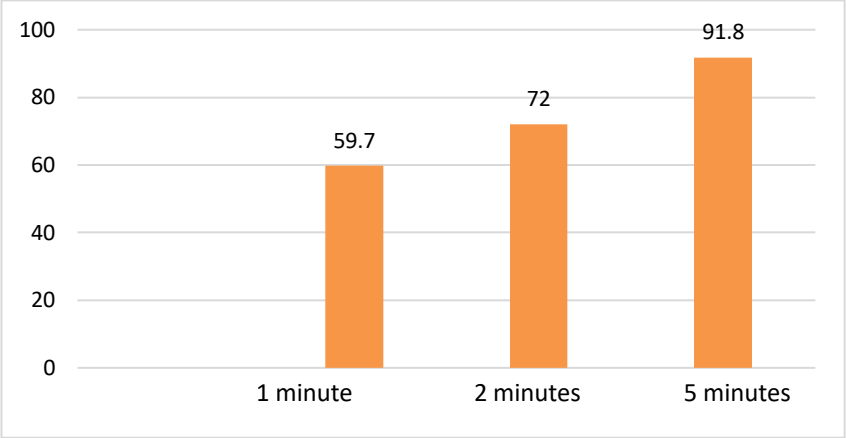

Figure 3. Percentage kill test results (%)

From this data, the results of the antiseptic test of the extract against C. acnes were obtained as follows: at the 1st minute by 59.7%, at the 2nd minute and at the 3rd minute 72% and 91.8%, respectively (Table 2).

The average growth of bacterial colonies in the control and treatment groups can be compared between the two groups. In the first minute of the comparison of the two groups of 1:0.40, the percentage kill result of this minute was only 59.7%, which showed that it was almost completely meaningless because the average in the control group and the treatment was only slightly different in the number of colonies. Then, in the second minute, the comparison of the two control and treatment groups was 1:0.27 with a percentage kill result of 72%, this result was quite good but still could not be included in the effective category, while in the fifth minute, the comparison of the two groups was 1:0.08 with a percentage kill result of 91.8% which made this result very good and even almost effective.

This study shows how *M. oleifera* leaf extract can inhibit the growth of Cutibacterium acne bacterial colonies. This result can be seen from comparing the number of bacteria growing in the control group and treatment. It was found that the number of colonies in the treatment group was a considerable distance from the control group, where the influence of moringa leaf extract worked in inhibiting the growth of bacteria. The results can be seen in the calculation of percentage kill where in the first minute the results obtained are only 59.7%, then in the second and fifth minutes, the percentage kill results are 72% and 91.8% (Figure 3), which means that the longer the extract is in contact with microorganisms, the more inhibited the growth of bacteria will be [20,21].

This study can be compared with other studies conducted using moringa leaf extract concentrations, which are measured based on the diameter of the inhibition zone. Fitriani et al, 2023 stated that moringa leaf extract has the potential to inhibit the growth of *C. acnes* bacteria at a concentration of 10% at 16 mm, a concentration of 20% at 17.5 mm, and a

concentration of 30% at 18.5 mm where below 5 mm is categorized as weak, the diameter of the inhibition zone of 5-10 mm is categorized as moderate, and the diameter of the inhibition zone of 10-20 mm is categorized as strong [22,23,24].

Furthermore, a similar study by Wahyuningsih et al, 2021 showed that moringa leaf extract at 5% could make 15.58 mm in diameter of the inhibition zone, a concentration of 10% of 17.25 mm, and a concentration of 20% of 26.45 mm. These results show that moringa leaf extract can potentially strong and very strong in inhibiting the growth of *C. acnes* bacteria. Therefore, the study also supports this research in determining antiseptic ingredients, namely moringa leaves as a natural ingredient that is very useful strongly to radiate *C. acnes* bacteria [25,26].

The results of another comparative study by Boonchaya et al, 2022, used Benzoyl peroxide (BPO) as a therapy for *C. acnes.* The bactericidal effectiveness of BPO can be felt instantly in just 0.5 minutes with the use of 5% or more BPO. Meanwhile, when using 2.5%, the bactericidal effect of 100% can be seen when the contact time is more than 15 minutes. This result is proof of the effectiveness of bactericidal ingredients that can effectively kill *C. acnes* with different contact times in each given concentration. This previous study also supports the researcher's research in seeing how minimal contact time can affect the eradication of *C. acnes* bacteria with various concentrations used [27].

Another study that also used percentage kill with contact time as an independent variable by Tjampakasari, et al.[19] This study discusses the test of moringa leaf extract against *E. coli* bacteria and *Streptococcus pyogenes* (*S. pyogenes*) bacteria. The test was carried out using percentage kill, measured after bacterial contact with moringa leaf extract at minutes 1, 2, and 5. The test can be categorized as meeting the standard if the mortality percentage of ≥90% is achieved [28].

The results obtained in this study on *E. coli* bacteria minutes 1, 2, and 5 were 93.41%, 94.14%, and 96.87%, respectively, while *S. pyogenes* bacteria had results of 73.27%, 83.15%, and 94.19%, respectively. This means that measurements using *E. coli* bacteria as Gram-negative bacteria that are facultative anaerobic showed the results of *Moringa oleifera* extract which is effective in radiating bacteria. This is shown because the entire percentage of death values for each contact time is ≥90%. Meanwhile, *S. pyogenes* bacteria as a facultative anaerobic Gram-positive bacteria on the activity of moringa leaf extract only showed effectiveness at the fifth minute with a mortality percentage of 94.19% [29].

This study shows how effective the antiseptic ingredient is *Moringa* leaf extract, which can kill facultative anaerobic Gram-negative bacteria and facultative anaerobic Gram-positive bacteria. Of course, these results show the possibility that *Moringa* leaf extract can also radiate other bacteria, such as in this study *C. acnes* as an anaerobic positive Gram-positive bacterium aerotolerant [30].

The activity test against *C. acnes* bacteria can be assessed from the magnitude of the Percentage Kill calculated from comparing of the average difference between the control group minus the treatment group for the control group itself. As a comparison from the previous study that has been presented, the growth in this study resulted in the average number of colonies growing in the treatment group at minutes 1, 2 and 5 were 69.33; 44; 11. This extract has an antibacterial effect that produces a percentage kill of 59.7%, 72%, 91.8% [31].

These results indicate that *Moringa* leaf extract has not been able to effectively radiate *C. acnes* bacteria in less than 5 minutes. This happened because the results obtained did not meet the minimum criteria for effective antiseptics, namely a percentage kill of ≥90%. However, there is an increase in the percentage kill from 1.2 to 5, minutes which indicates that this antiseptic ingredient has an antibacterial function. Increasing the number of kills every minute is also a marker of antimicrobial efficacy related to contact duration. These results can occur due to bacteria that are eradicated by *Moringa* leaf plant extracts [32,33].

CONCLUSION

The Percentage Kill value in the first minute was 59.7%, in the second minute it was 72% and in the fifth minute it was 91.8%. *M. oleifera* leaf extract against *C. acnes* is effective as an antiseptic in the fifth minute.

CONFLICT OF INTEREST

This article does not have any conflict of interest. This research has passed the ethical review in accordance with the Official Memorandum from the

FKUI Ethics Commission No. 1275/UN2. F1/ETIK/PPM.00.02/2020.

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