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The Effect of Aloe Vera on *Staphylococcus aureus*

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Abstract

In recent years, interest in Aloe vera-based herbal products has quickly increased, especially in medicine due to their antibacterial capabilities, which included many active ingredients such saponin and anthraquinone. The first priority due to their resistance, methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as one of the most significant human pathogens causing nosocomial infections. The goal of this study is to establish the lowest concentrations of ethanol extract from Aloe vera gel necessary to inhibit MRSA. Using the serial dilution method with nine repetitions to test MRSA for sensitivity to Aloe vera gel extract, the results were statistically assessed using the t-test method. The outcome demonstrates that the Aloe Vera gel extract's Minimum Inhibitory Concentration (MIC) cannot be observed. There is no difference in the absence of antibacterial efficacy blocking MRSA at different concentrations (10, 25, 50, and 100%).

Keywords: Aloe Vera Extract Gel, MRSA, Antibacterial Activity, MIC, DDM

Introduction

Staphylococcus aureus is one of the most frequent human pathogens that can cause a variety of diseases. Although initial *Staphylococcus aureus* infections are uncommon, cross-infection from patient to patient in hospitals and other institutional settings causes a significant amount of pathogenicity (Arch et al., 2006). Most healthy

persons have *Staphylococcus aureus* on their skin and mucous membranes, as it is common in the environment and in ordinary human flora. These bacteria do not usually cause infection on healthy skin, but they can cause a range of potentially fatal disorders if they reach the bloodstream or cause internal difficulties. (Taylor & Unakal, 2001).

Medicinal herbs have long been recognized and used throughout Asia to alleviate a variety of symptoms. Since the time of Western colonization, they have been pushed aside to make way for Western medicine. People are beginning to recognize that, while western medicine has a lot of benefits, it also has some drawbacks, according to recent studies. The risks of side effects can outweigh the potential advantages for patients. Despite the fact that each person is unique, contemporary medicine nevertheless offers a one-size-fits-all answer to the problem. Traditional herbal therapy likewise provides a one-size-fits-all treatment, but its negative effects are less noticeable due to the plants' organic nature. Plants that produce natural compounds such as flavonoids, terpenoids, and steroids, for example, have attracted a lot of attention in recent years due to their diverse pharmacological properties, including antioxidant and anti-tumor potential. (Karthikumar et al., 2007).

There are various possibilities to consider while looking for alternatives to traditional medicine, which can be learned more about through research papers and then put into practice. The World Health Organization (WHO) advises using herbal ingredients as an alternate treatment for bacterial resistance to some antibiotics (Pratiwi et al., 2015). Traditional herbs have been used for medicinal purposes in Asia for thousands of years. Although India and China have taken the lead, several other Asian countries are following suit. In the Middle East, traditional Arabic medicine is also practiced. Similar species of medicinal plants are more abundant in countries with similar climates. This means that a similar herb could be found in China, India, Malaysia, and other countries, however the name would be different (Rodrigues et al., 2008).

Research Methodology

This research is a laboratory study, designed to be in a lab control environment. Collect sample of Aloe vera (Babardensis, 8-12 months old) on January and July 2022 from Chonburi province. The size of this rush green Aloe Vera (L.) Burm is approximately 45cm in length and weight at 2.5kg. The widest width is approximately 15cm and taper up to the top.

1. Preparation of Aloe vera

1) Clean Aloe vera thoroughly and then chop them into smaller pieces. (It can be furthered chopped using Blender: Philips HR201: 250w, 50Hz.)

2) Dry them via sunshine and wind, using petri dish (15cm in diameter).

3) Soak 500 grams of Aloe Vera gel into 95% ethanol in a closed lid container (250ml) for 7 days.

4) Filter what we have with Whatman paper filter (No. 1001-070, 70mm in diameter). Use only the solution and vaporize it with Rotary Evaporator (Buchi R-210), and get the rough extraction 1.6% of the dry weight.

5) Keep them in the refrigerator (Panasonic: SBC-P2DB) at 4°c for further actions.

2. Preparation of Bacteria

Culturing *Staphylococcus aureus* ATCC6538 in Nutrient Broth (NB) at 35°c for 24 hours, at Faculty of Science and Technology, Huachiew Chalermprakiet University, and National Science and Technology Development Agency:

1) Wear a protective laboratory coat, eye googles, and disposable latex gloves while working in a biosafety cabinet. Using the sterile loop, scrape a little amount of microorganisms from a frozen stock (keep frozen stocks on ice or in a cooler to minimize alterations in temperature, which otherwise may affect the viability of the frozen stock).

2) To obtain isolated colonies, use the loop to transfer the frozen aliquot of *Staphylococcus aureus* onto an agar plate, streaking across the plate from left to right and top to bottom.

3) Invert the plates and incubate at 35°C overnight.

After drying up to 500 grams of Aloe vera gel, it must first be smoothed before being macerated in 95 percent ethanol so that the ethanol completely covers the simplicia powder in the macerator. The macerated were recovered and remaserated with brand-new 95 percent ethanol solutions twice every 24 hours. By concentrating the obtained macerate using a rotary evaporator, a thicker extract is created. A vaporizer cup is then filled with the extract, which is then allowed to evaporate over a water bath until a thick extract is created. Later, thick extract is weighed. For a 100 percent concentration, weigh 100 grams of extract and then add 100 ml of purified water.

1. An ethanol extract of aloe vera gel was diluted to create solutions with concentrations of 10, 25, 50, and 100% using the series dilution method.

2. There are six sterile test tubes required, numbered 1-6. Following the pipetting of up to 1 ml of an ethanol extract of aloe vera leaves from a standard solution, which was then uniformly spun to produce a concentration of 100 percent, the first tube was filled with 1 ml of glucose bulb.

3. After adding 1.25 ml of glucose bulb to the second tube, 0.75 ml of an ethanol extract of aloe vera leaves was pipetted from a standard solution and uniformly spun to reach a concentration of 50%.

4. The third tube was filled with 1.5 ml of glucose bulb and consistently spun to achieve a concentration of 25% after being pipetted with a standard solution of ethanol extract from Aloe vera plants.

5. In order to obtain a concentration of 10%, 1.75 ml of glucose bulb was added to the fourth tube, along with a standard solution of ethanol extract from Aloe vera leaves.

6. The suspension of methicillin-resistant *Staphylococcus aureus* (MRSA) in the made-up ball is comparable to the turbidity of Mc. Farland's 0.5 pipelines of 0.1 ml each into tubes 1 to 4.

7. The entire tube was placed in the applicator and incubated for 24 hours at 35 °C. The tube used to test for sterility was incubated as the sixth tube using only the test material, while the tube used to test for bacterial growth was incubated as the fifth tube using only a bacterial culture (control +). - (control).

It can be established whether or not there is microbial growth by contrasting positive controls. Minimum inhibitory values are determined in order to observe a tube that does not support bacterial growth at the lowest concentration. Cultures clearly visible in the tubes demonstrate the test substance's ability to halt microbial growth. Mixed cultures, on the other hand, show this since the test material cannot stop the test microorganisms from growing.

Up to one solution from each tube was removed, planted in LAB, and then re-incubated using an excavator at 35°c for 24 hours to further confirm the earlier findings. For that treatment, aloe vera gel infused with ethanol extract is also employed. The minimal inhibitory concentration (MIC) is the lowest concentration that can prevent the growth of microorganisms. Every sample was tested twice using the same procedure.

The results of the studies were repeated 9 times (n=9) by using the lowest concentration that inhibit the growth of *Staphylococcus aureus,* which would be considered as the Minimum Inhibition Concentration (MIC) value, and then review the Zone of Inhibition. For comparison purpose with the typical treatment of *Staphylococcus aureus,* there was positive control of penicillin group antibiotic; Ampicillin - concentration of 10 µg/disc, Chloramphenicol - concentration of 30 µg/disc, and Vancomycin - concentration of 5 µg/disc. The difference was statistically using t-test one sample and the level of statistical significance was determined at p-value < 0.05.

Results

Based on standardized tests, the Disc Diffusion Method was used to test the susceptibility of 10, 25, 50, and 100 percent concentrations of Aloe Vera gel against *Staphylococcus aureus*. The Clinical and Laboratory Standards Institute (CLSI) observed the antimicrobial droplet cycle (clean zone), and the Independent-Sample T test was used to assess it. The findings demonstrated that no amount of aloe vera gel could stop *Staphylococcus aureus* from growing. The gram-positive bacterium *Staphylococcus aureus* was clearly inhibited by the antibiotics ampicillin (10 g/disc), chloramphenicol (30 g/disc), and vancomycin (5 g/disc) during the allotted

inoculation time, with respective Zones of Inhibition widths of 44 mm, 31 mm, and 13.47±0.21mm.

As a consequence of the test results, it can be said that Aloe Vera gel at concentrations of 10, 25, and 100% cannot prevent the growth of *Staphylococcus aureus*, respectively, within the allotted inoculation period.

The Result as per Huachiew Chalermprakiet University (January 2022)

Table 1 Huachiew Chalermprakiet University's Test Result

Number	Extract Herb	Concentration (mg/ml)	Diameter of Inhibition Zone (mm)
1	Aloe vera	100 mg/ml	-
		50 mg/ml	-
		25 mg/ml	-
	Negative Control: Dimethyl Sulfoxide (DMSO) at 20% Concentration		
	Positive Control:Disc Antibiotic		
	Ampicillin	10 µg/disc	44
	Chloramphenicol	30 µg/disc	31

Note Using 6 mm diameter of paper disc, drop the testing chemical 10 µg/l

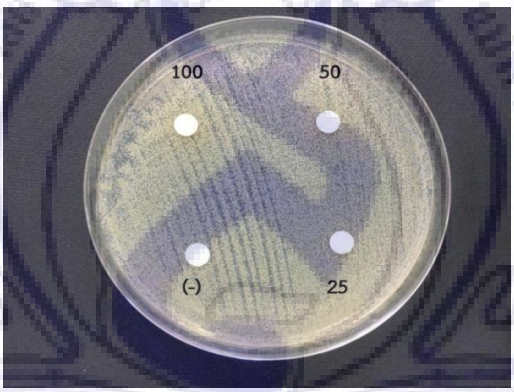

Figure 1 Sample of Aloe Vera Gel in the petri dish

Figure 2 Positive Control 1: Ampicillin - concentration of 10 µg/disc

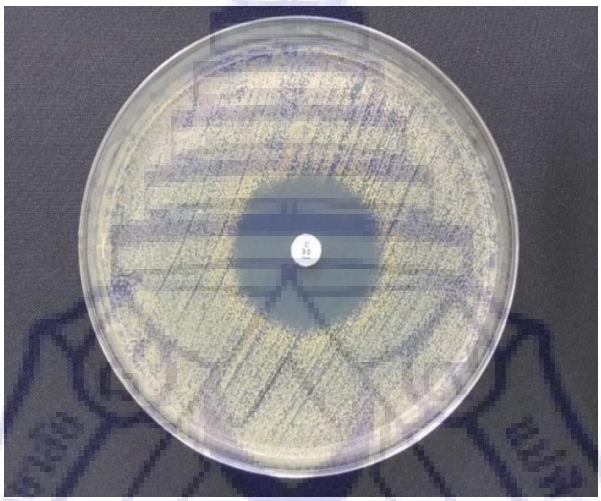

Figure 3 Positive Control 2: Chloramphenicol - concentration of 30 µg/disc

It has clearly shown that with the concentration of 25, 50, and 100%, there has been no effect in inhibiting the growth of *Staphylococcus aureus.* In comparison to Ampicillin at concentration of 10 µg/disc, and Chloramphenicol at concentration of 30 µg/disc, the results have clearly shown the Zone of inhibition at 44mm and 31mm respectively, as show in pictures above.

Figure 4 Sample extracts from aloe Vaea

The Result as per National Science and Technology Development Agency NSTD (July 7, 2022):

The result as per NSTD shows that Aloe Vera concentration at 10, 50, and 100% has no ability to inhibit the growth of *Staphylococcus aureus.* However, Vancomycin 5 µg has the Zone of inhibition at 13.47±0.21 mm as shown in Table 1 below.

Table 2 Size of clear zone ± Standard Deviation (Mean ± SD) from 9 replicates in millimeter of test samples and positive control disks against *Straphylococcus aureus* ATCC 6538

	Zone of inhibition (millimeter)*
	Straphylococcus aureus
Test Sample	
1. Aloe Vera (10%)	- (Viable bacteria were observed under and around the tested disk of the specimen)
2. Aloe Vera (50%)	- (Viable bacteria were observed under and around the tested disk of the specimen)
3. Aloe Vera (100%)	- (Viable bacteria were observed under and around the tested disk of the specimen)
Positive control	
Vancomycin 5 µg	13.47 ± 0.21

Note
1. Observed clear zone (millimeter) = diameter of the zones of complete inhibition, including the diameter of the disk.
2. Dash (−) indicates zone of inhibition was not observed
3. Diameter of the disks used was 6 mm
4. * size of zone of inhibition calculated from 9 replicates
5. Zone of inhibition calculated from zone of inhibition = Diameter of the zones–Diameter of the disks

The Result as per National Science and Technology Development Agency NSTD (July 7, 2022):

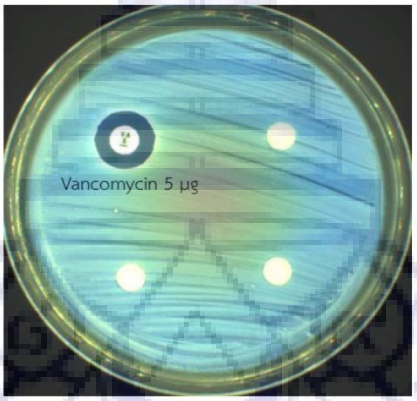

Figure 5 Disk diffusion of Aloe Vera (10%) sample against *Staphylococcus aureus ATTCC 6538*

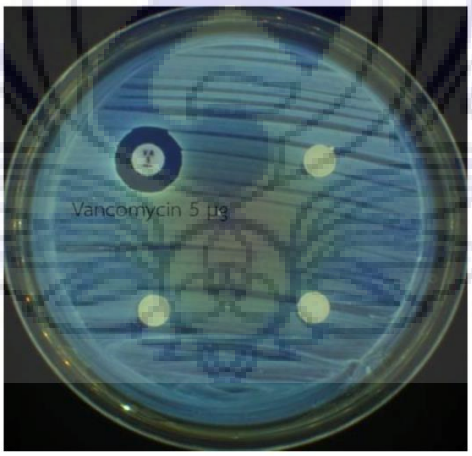

Figure 6 Disk diffusion of Aloe Vera (50%) sample against *Staphylococcus aureus ATTCC 6538*

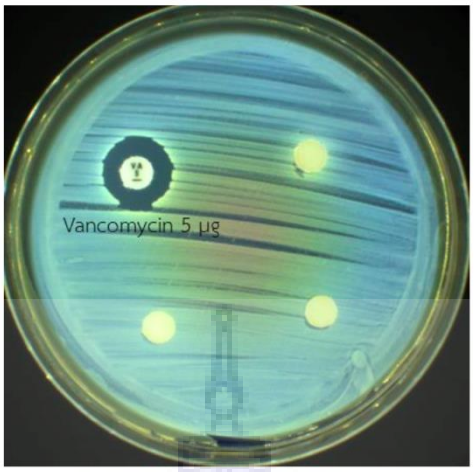

Figure 7 Disk diffusion of Aloe Vera (100%) sample against *Staphylococcus aureus ATTCC 6538*

Discussion and Suggestion

The purpose of this test technique was to determine the susceptibility of gram-positive bacteria, *Staphylococcus aureus*, to 10, 25, 50, and 100% concentrated Aloe vera by determining the susceptibility of bacteria to Aloe vera using the examination standards. The Clinical and Laboratory Standards Institute (CLSI) observed and assessed the antimicrobial droplet cycle (clean zone) using an Independent T test. The findings of the disc diffusion test demonstrated that Aloe vera could not stop the growth of gram-positive bacteria, *Staphylococcus aureus*, with a zone of inhibition of 0.00±0.00 mm over the designated incubation period, even at its greatest concentration of 100%.

While the earlier studies has shown that Aloe vera has the effectiveness in inhibiting the growth of *Staphylococcus aureus*, my study does not have the same result. There are some factors that could have influence the outcome of the test, which deviated from the earlier studies. They could be as follows:

Birth Place of Aloe vera

All of the studies that have been used as references were conducted usage in India. The type of Aloe vera that I am using Babardensis, grew in Chonburi province. The weather, soil, and amount of water could effect how Aloe Vera in different countries obtain their minerals and active component. The difference in the results could be attributed to the plant from geographical locations with variation in their

chemical composition, different processing and isolation techniques that were applied to extract the gel and gel components from the Aloe leaf.

Organic Control Environment

The Aloe Vera that was used in this study was randomly purchased from the market seller. I do not have the full information how the seller and/or farmer raised the plants. They may not been raised in a controlled environment and degree of pesticide usage is unknown.

Different Part of Aloe Vera has different effect

As per Eka Pratiwi mentioned in his study that, between leaf and gel extract, the strength on the inhibiting of *Staphylococcus aureus* is different. Not every study reveals the part of Aloe Vera that they were using. My study focuses only on the leaf while others focused on skin or flower.

Transport Contamination

The Aloe Vera that I used was transport directly from Chonburi to both labs via Thai Post. Though I have asked both labs to keep the fresh Aloe Vera refrigerated prior testing, they could be the case where they left it outside too long, the temperature of the labs' refrigerator was not stable.

Suggestions

The findings of this study demonstrated that the Aloe Vera leave gel has been demonstrated to lack of inhibitory capabilities against MRSA based on 10, 25, 50, and 100% in vitro. After undergoing clinical trials, the qualities of the extract must be further investigated in order to demonstrate its antibacterial efficacy and advocate its usage as an alternative treatment for treating infections brought on by MRSA. Given the various advantages of the aloe vera plant, more research is absolutely necessary before it can be utilized as an alternative medicine to stop the spread of bacterial resistance.

Increase concentration of Aloe Vera gel - To test with the greater concentration beyond 100% to possibly see the inhibition result of *Staphylococcus aureus*.

To extract key active antibacterial compounds from different part of Aloe Vera, which may posses better strength in fighting with this gram positive bacteria.

To further study the benefits of Aloe Vera on other areas of health and wellness, especially in the field of skin and wound healing.

Limitation

The study took place during the first 6 months of 2022, which COVID-19 was still in effect. The labs set the rule by not allowing anyone outside the lab to enter and participate in the study. They could only communicate via phone and e-mail to update the status. Thus, this limit my ability to closely monitored the process.

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