Antibacterial activity testing of ethanolic extract of aloe vera leaf and gel against methicillin-resistant *Staphylococcus aureus*

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ABSTRACT

Introduction: The interest on Aloe vera based herbal products is fast growing during recent years, particularly in medication regarding their antibacterial properties which had a lot of active components such as saponin and anthraquinone. Methicillin-resistant Staphylococcus aureus has emerged as one of the most important human pathogens causing nosocomial infections and became the first priority as a consequence of their resistancy. The purpose of this study is to determine the minimum levels of ethanol extract of leaves and Aloe Vera gel that can inhibit MRSA and find out whether there are differences in antibacterial power between of ethanol extract of leaves and Aloe Vera gel. **Methods:** Ten isolates of MRSA were investigated for their sensitivity to Aloe vera leaf and gel extract using the serial dilution method by doing two times repetition and statistically analyzed according to t-test method. **Result**: The result shows that the Minimum Inhibitory Concentration of Aloe vera leaf and gel extract each located at a concentration of 25% and 12,5%. There is a significant difference in antibacterial power between the ethanol extract of leaves and Aloe Vera gel in inhibiting MRSA with MIC values of 25% and 12.5% **Conclusion:** There was a difference between Aloe vera leaf and gel extract antibacterial activity, where the gel extract is more effective than leaf extract.

Keywords: Aloe vera ethanolic extract leaf, Aloe vera ethanolic extract gel, MRSA, antibacterial activity

INTRODUCTION

Staphylococcus aureus is the leading cause of most bacterial infections that are toxic to humans, and this bacterium is found in many parts skin and nasopharynx where it can cause local infections of the skin, nose, urethra, vagina and gastro-ducts intestinal.[1]

Efforts to overcome Staphylococcus aureus infection initially was by using penicillin group antibiotics. Still, there was a change in the pattern the sensitivity of this bacterium to penicillin, so methicillin antibiotic was developed.[2] Methicillin is known to inhibit the growth of Staphylococcus aureus. Still, after two years of usage, these bacteria become resistant to methicillin so-called methicillin-resistant Staphylococcus aureus (MRSA).

MRSA was first identified in a hospital.[3] Research in the United States has managed to classify MRSA into two types, including *hospital-acquired* MRSA (HA-MRSA) which have proven to be resistant to many antibiotics and *community-associated* MRSA (CA-MRSA) which have proven to

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be resistant only to class antibiotics ß-lactam.[4] In 2006 the prevalence of infection methicillin-resistant Staphylococcus aureus in Indonesia reached 23.5%.[5]

World Health Organization (WHO) recommends the use of herbal ingredients as an alternative treatment to prevent bacterial resistance to certain types of antibiotics. One of the herbal ingredients that have been widely used to treat diseases is Aloe Vera.

Aloe Vera contains 75 ingredients that have benefits in the medical field, including lignin, saponin, and *anthraquinone*. Lignin is a cellulose substance found in Aloe Vera gel and can penetrate human skin. Saponin has been widely used as a detergent and contains antiseptic ingredients. *Anthraquinone* is located in the sap of Aloe Vera, and this ingredient can function as a painkiller and has antibacterial, antifungal, and antiviral abilities. Another component that is no less important in Aloe Vera is plant sterols. Sterols are anti-inflammatory, antiseptic, and analgesic.[6]

The results of the study stated that the antibacterial activity of Aloe Vera leaf extract with Aloe vera gel against Staphylococcus aureus at a concentration of 25mg / ml resulted in inhibition zones of 18.0 mm each for gel extracts and 4.0 mm for leaf extracts.[7] Similar things also occur in some gram-bacteria other positives, such as Streptococcus mutans which were successfully inhibited at a concentration of 12.5 µg / ml with an inhibition zone of 10 mm with ethanol gel extract,[8] whereas Bacillus subtilis and Bacillus cereus with inhibition zones were 28 mm and 26.7 mm respectively.[9]

The purpose of this study is to determine the minimum levels of ethanol extract of leaves and Aloe Vera gel that can inhibit MRSA and find out whether there are differences in antibacterial power of of ethanol extract of leaves and Aloe Vera gel.

METHODS

This research is an experimental laboratory study. The population in this study is the Aloe Vera plantation found in Sekeloa and Dago areas, Bandung City. The study was conducted in May 2012 using the serial dilution method and statistically analyzed using the t-test method.

The variables in this study are Methicillin-resistant Staphylococcus aureus, and ethanol extract of leaves and Aloe vera gels are divided into individual levels. The tools and materials used in this study are others, methylated lamps, sliding tubes, tweezers, pipettes, test tubes and racks, and other devices commonly used in the Microbiology Laboratory.

Making Aloe Vera leaf extract and Aloe vera gel was carried out at the Unpad Chemistry Laboratory. The initial step is to smooth the simplicia leaves and Aloe vera gel which has dried as much as 600 and 800 grams respectively then macerated using 70% ethanol so that all the simplicia powder is submerged by ethanol in the macerator. Maceration was carried out for 2x24 hours and every 24 hours, and the macerated were collected and remaserated with a new 70% ethanol solution. The macerate obtained is concentrated with a rotary evaporator until a thicker extract is obtained. The extract is then poured into a vaporizer cup, then evaporated on a water bath until an extract is obtained thick. Thick extract later is weighed. To achieve 100% concentration, weigh 100 grams of extract then add 100 ml of distilled water.

The ethanol extract of the leaf and Aloe Vera gel was diluted to obtain a solution with a concentration of 50, 37.5, 25, and 12.5% with the series dilution method. Provide six sterile test tubes, each numbered 1-6. The first tube was filled with 1 ml of glucose bulb, then from a standard solution of ethanol extract of Aloe vera leaves pipetted as much as 1 ml, then stirred evenly to get a concentration of 50%. The second tube was filled with 1.25 ml of glucose bulb, then from a standard solution of ethanol extract of Aloe vera leaves pipette as much as 0.75 ml, then stirred evenly to get a concentration of 37.5%. The third tube was filled with 1.5 ml of glucose bulb, then from a standard solution of ethanol extract of Aloe vera leaves pipette 0.5 ml, then stirred evenly to get a concentration of 25%. The fourth tube was filled with 1.75 ml of glucose bulb, then from a standard solution of ethanol extract of Aloe vera leaves pipette as much as 0.25 ml, then stirred evenly to get a concentration of 12.5%. The MRSA suspension in the ball that was made is equivalent to the turbidity of Mc. Farland 0.5 pipelines of 0.1 ml each into tubes 1 to 4. Next, the entire tube

incubated using the applicator at 37oC for 24 hours. The tube as a control for bacterial growth was also incubated as the fifth tube containing only a bacterial suspension (control +), and the tube as a sterility control material was incubated as the sixth tube containing only the test material (control -).

Observe the presence or absence of microbial growth by comparing positive controls. Minimum inhibitory levels are obtained to observe a tube that does not show bacterial growth at the lowest concentration. Tubes that display evident cultures indicate that the test material can inhibit the growth of microorganisms. Conversely, mixed cultures show the growth of test microorganisms because the test material is not able to hinder it.

Furthermore, as much as one solution from each tube was taken and then planted in LAB and re-incubated using an excavator at 37oC for 24 hours to confirm the previous results. That treatment the same is also applied using ethanol extract Aloe vera gel. MIC is indicated by concentration the smallest that can inhibit the growth of microorganisms. Each sample was tested twice with the same method.

RESULTS

The MIC test of ethanol extract of leaves and Aloe vera gel against MRSA isolates gave results as seen in table 1.

Based on calculations, the Aloe Vera gel extract concentration of 50% there is no bacterial growth. Still, at concentrations of 37.5, 25, and 12.5%, there began to be bacterial growth with

Table 1. Shows the results of sectoral tests to determine minimum concentration inhibition

Sample	Repeatation	Gel 50	Gel 37,5	gel 25	Gel 12,5	Leaf 50	Leaf 37,5	Leaf 25	Leaf 12,5	Control (+)	Control (-)
1	I	0	0	0	89	0	0	336	525	934	0
	II	0	0	0	66	0	0	320	390		
2	I	0	0	0	4	0	0	41	628		
	II	0	0	0	3	0	0	20	134		
3	I	0	2	328	0	0	0	76	668		
	II	0	1	449	1	7	1	102	355		
4	I	0	0	0	10	1	3	294	176		
	II	0	0	1	4	1	0	272	69		
5	I	0	0	1	0	1	1	37	94		
	II	0	0	0	2	0	0	13	62		
6	I	0	0	116	1	0	1	60	736		
	II	0	0	5	1	0	0	88	69		
7	I	0	0	1	0	0	0	214	307		
	II	0	0	0	1	0	0	288	250		
8	I	0	0	0	12	0	0	5	90		
	II	0	0	0	9	0	0	8	63		
9	I	0	0	1	0	22	36	42	353		
	II	0	0	0	2	21	22	13	423		
10	I	0	0	0	1	1	2	49	600		
	II	0	0	0	1	1	0	43	594		
Rata-rata		0	0.15	45.1	10.35	2.75	3.3	116.05	329. 3	934	0

Table 2. Shows that the average MRSA growth in gel extracts with a concentration of 12.5% lower from leaf extract with a concentration of 25%.

Sample	N	Mean	Std. Deviation	T	Df	Sig.	Kesimpulan
Gel 12.5%	10	10.350	23.830	-2.690	18	0.015	MRSA dengan metode difusi agar Berbeda
Daun 25%	10	116.050	121.958				

an average number of bacterial colonies of 0.15, 45.1, and 10.35, respectively. The researchers determined that the minimum inhibitory concentration of Aloe vera gel extract, which could still inhibit MRSA colony growth was at a concentration of 12.5%. In contrast to gel extracts, Aloe Vera leaf extract test results have begun to show bacterial growth at concentrations of 50 and 37.5%. More and more colonies are growing at levels of 25 and 12.5% with each average number of bacterial colonies 2.75, 3.3, 116.05, and 329.3. The researcher determined that the minimum inhibitory concentration of Aloe Vera leaf extract, which could still inhibit MRSA colony growth was at a level of 25%.

Table 2 shows that the average MRSA growth in gel extracts with a concentration of 12.5% lower from leaf extract with a concentration of 25%. The test results are independent t-test can be concluded that the significance value of 0.015 is smaller than the p-value (sig.) of 0.05, meaning that Aloe Vera gel extract 12.5% has a significant difference with leaf ethanol extract 25% in inhibiting the growth of MRSA.

DISCUSSION

Antibacterial power test of ethanol extract Aloe vera leaves and gels with concentrations of 50, 37.5, 25, and 12.5% of MRSA in this study were carried out using the broth dilution method or series dilution. The advantage of this method is that the results obtained are qualitative in the form of Minimum Inhibitory Concentration (MIC), but the weakness of this method is that it requires a lot of material and specialised training in making serial dilutions so that the possibility of errors can be avoided.[10]

The results of the preliminary study were in the form of antibacterial effectiveness test of Aloe Vera leaf extract and gel against(wells) prove that Aloe Vera. The amount of extract concentration is proportional inversely with the number of growth bacteria. Test with this method is required as a preliminary test, so you can know whether the bacteria is still classified sensitive or resistant to test material. If the bacteria are still classified as sensitive, then proceed with serial dilutions to obtain the MIC material of the test material against bacteria.

Testing KHM gel extracts and Aloe Vera leaves as seen from the results of the planting sector (table 1) gives the result that there are differences extract concentration which can inhibit MRSA growth. Following Lethal Dose 50 (LD50), the concentration of ethanol extract of leaves and gels that showed the most minimal MRSA growth was 25% and 12.5%, because at that concentration 50% of the total bacterial colonies could be inhibited. This is in line with research conducted by Agarry et al. (2005) which concluded that the ethanol extract of leaves and Aloe vera gel against Staphylococcus aureus was effective at a concentration of 25% with ethanol gel extract more effective than leaves.[7]

Based on the results of statistical calculations with the t-test method in table 2, it can be seen that there is a significant difference between the ethanol gel extract of 12.5% and the aloe vera leaf extract 25% in inhibiting the growth of MRSA when viewed from the MIC value owned, with an average MRSA growth in gel extract with a concentration of 12.5% is lower than leaf extract with concentration of 25%. This proves that ethanol gel extract is still more effective in inhibiting MRSA growth when compared to leaf extract.

Differences in MICs obtained between ethanol extracts of leaves and gels may occur due to differences in active substances contained in each part of the Aloe Vera. Based on phytochemical tests, Park and Jo[11] state that Aloe vera has more than 75 nutrients and 200 active substances, including vitamins, enzymes, minerals, sugars, lignin, *anthraquinone*, saponins, salicylic acids, and amino acids. This active ingredient causes Aloe Vera to have an analgesic effect, and the wound heals antifungal and antibacterial.[11]

The most active substance found in leaves is an *anthraquinone*. Pellizoni et al.[12] state that this substance has antibacterial power by inactivating the work of enzymes needed by bacteria.[12] Enzymes play a role in the process of protein synthesis in the metabolic process. If the enzyme is inactivated, it will disrupt the metabolic process causing bacterial death. Another way of working with *anthraquinone* is to produce Reactive Oxygen Species (ROS), such as oxygen molecules. MRSA is known to live in Susana anaerobic facultative, which requires only a little

air. Therefore *anthraquinone* works in a way increase oxygen levels so that bacterial growth can be inhibited.[13]

Aloe vera gel is also known contains various active substances, including saponin, *acemannan*, *aloin* and *aloe-emodin*.[8] Saponin can cause saponification reactions and will cause damage to the bacterial membrane fat structure so that the bacterial cell wall will rupture and lysis and then die.[14] *Aloin* and *aloe-emodin* are derivatives the main *anthraquinone* which has a polyphenol structure and can inhibit protein synthesis from bacterial cells disrupt metabolic processes.[8]

Acemannan is the most active complex carbohydrate compound, composed of glucose and manosa chains.[12] this substance is thought to have an indirect antibacterial effect as an activating agent of the human immune system, related with its ability to increase the number of monocytes in the tissue and stimulate macrophages causing phagocytosis.[15]

The content of other active substances such as vitamins, enzymes, minerals, salicylic acid and amino acids, can interact with active antibacterial compounds that have been mentioned earlier and might influence the effectiveness of antibacterial in inhibiting the growth of MRSA.

The results of this study prove that the ethanol extract of leaves and Aloe Vera gel in vitro has been shown to have inhibitory properties against MRSA. The properties possessed by the two extracts in vitro are proven to have antibacterial power so it can be recommended to be used as alternative medicine in handling infections caused by MRSA after going through clinical trials. Further research is fundamental to do considering the many benefits of the Aloe Vera plant so that it can be used as an alternative treatment in the medical world to prevent further bacterial resistance.

CONCLUSION

There was a difference between Aloe vera leaf and gel extract antibacterial activity, where the gel extract is more effective than leaf extract.

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