EFFECTIVITY IN VITRO OF *Averrhoa bilimbi L* ETHANOLIC EXTRACT AGAINTS *Escherichia coli* AND
Staphylococcus aureus GROWTH

Erwin Kukuh Widhianto*, Ransidelenta Vistaprila Elmarda*, Maya Dian Rakhmawatie**

* Undergraduate Student of Medical Faculty, Muhammadiyah University of Semarang
** Pharmacology Department, Medical Faculty , Muhammadiyah University of Semarang

Abstract

Averrhoa bilimbi L, or in Indonesian named belimbing wuluh has been widely used by the community as a complement to cuisine. From many studies, fruit or leaves of *Averrhoa bilimbi L* declared to have antibacterial activity. The activity is caused by the antibacterial content in *Averrhoa bilimbi L*, such as flavonoids, saponins, and tannins. This research is conducted to test the effectiveness of ethanolic extract of *Averrhoa bilimbi L* as antibacterial. The bacteria used to tested are Gram + *Staphylococcus aureus* and Gram- *Escherichia coli*, both of which are pathogenic bacteria in humans. This research was a pure experimental design with posttest only control group design. *Averrhoa bilimbi L* was extracted using maceration method with 96% ethanol solvent. The thick extract of the *Averrhoa bilimbi L* was diluted using 2% DMSO solvent to concentration range 0,19%; 0,39%; 0,78%; 1,0%; 1,56%; and 3,12% (v/v). Extracts with various concentrations were then tested to *S. aureus* and *E. coli* bacteria, and each concentration was repeated four times. MIC and MBC test were conducted by dilution method using Mueller Hinton Broth and Mueller Hinton Agar. MIC values were minimal concentration of no bacterial test growth , as measured by observing the difference of absorbance before and after incubation of treatment solution using spectrophotometer λ 625 nm. The MBC values are minimal concentration there is no bacterial colony growth after treatment.The statistical test showed that there was significant difference of absorbance value at each concentration of *Averrhoa bilimbi L* ethanolic extract test on both bacteria. Based on ANOVA analysis, p value was 0,001. In linear regression analysis, the relationship between extract concentration and the absorbance of *S. aureus* bacteria, is generated with regression value y = 0,081x-0,181 and R^2 = 0,93 (p value 0,001). For the relationship between extract concentration and the log colony number of *S. aureus,* obtained regression value y = 2,250x- 1,521 and R^2 = 0,72 (p value 0,001). In statistic test of *E. coli*, there was significant difference from six *Averrhoa bilimbi L* ethanolic concentration groups. Based on test Kruskal-Wallis analysis, p value was 0,003. In linear regression analysis, the relationship between extract concentration and the absorbance of *E. coli* bacteria, is generated with regression value y = 0,089x-0,212 and R^2 = 0,89 (p value 0,001). For the relationship between extract concentration and the log colony number of *E. coli*, obtained regression value y = 0,526x + 6,998 value R^2 = 0,79 (p value 0,001).Ethanolic extract of *Averrhoa bilimbi L* fruit was effective against *S. aureus* and *E. coli* with MIC and MBC concentration of 1.56% (v/v). Increased concentration of *Averrhoa bilimbi L* ethanolic extract will increase the inhibition growth of *S. aureus* and *E. coli* bacteria

Keywords : *Averrhoa bilimbi L*, belimbing wuluh, antibacterial, *S. aureus*, *E. coli*.

INTRODUCTION

Indonesia has the abundant potential of medicinal plant resources used for the development of traditional medicine for the benefit of health.[1] From various types of medicinal plants owned by Indonesia one of the plants that can be used is *Averrhoa bilimbi L* (belimbing wuluh). *Averrhoa bilimbi L* is a plant that can be found in the yard of the house. This plant is often used by the community for ingredients for cooking, sprue medicine, and as a beverage. Results from several studies showed that fruit or leaf extract of *Averrhoa bilimbi L* has an antibacterial effect. [2,3]

The content of compounds owned by *Averrhoa bilimbi L* are flavonoids, alkaloids, saponins, terpen, and tannin.[4] The

mechanism action of such compounds is to form complexes with extracellular proteins of microorganisms and inhibit the cell cycle of bacteria.[5] In detail, flavonoids can interfere with bacterial metabolism[6], tannins inhibit bacterial cell wall synthesis[7,8], and saponins can decrease the surface tension of bacterial cells.[9]

The most common bacterial infection in Indonesia is *Staphylococcus aureus*.[10] *S. aureus* are the main pathogenic bacteria in humans. Almost everyone has had *S. aureus* infections during their lifetime. *S. aureus* live colonize in the skin and mucosal surface of the human body. In addition to *S. aureus*, other bacteria *Escherichia coli* can also be pathogenic bacteria in humans. *E. coli* is actually a normal flora in the intestine that responsible for helping the decay of food waste from digestion.[11] *E. coli* can spread easily through contaminated food or water. The level of food contamination is quite high in some cities in Indonesia, especially Jakarta, which is 65,5% with the prevalence of diarrhea as much as 116,075 case.[12]

Gram-positive bacteria have different thickness and cell wall layers when compared with Gram-negative bacteria, thus causing differences in response to antibacterial compounds from *Averrhoa bilimbi L*. *S. aureus* is a Gram-positive bacteria that has a single-celled cell wall composed of peptidoglycan and has a cell wall thickness of 15-80 nm. Meanwhile, *E. coli* is a Gram-negative bacteria, which has a cell wall consisting of three layers. The outermost layer is lipopolysaccharide, the middle layer is protein, and the inner layer is peptidoglycan with a thickness of 10-15 nm.[13]

The water and chloroform extracts of the *Averrhoa bilimbi L* are declared effective against Gram-positive bacteria such as *S. aureus, S. epidermis*, and *B. cereus* and also against Gram negative bacteria such as *S. typhi*. While ethanol extract of *Averrhoa bilimbi L* leaves was effective against Gram positive bacteria such as *B. cereus* and Gram negative such as *E. coli*.[4] Based on the above description, we want to compare the effectiveness of ethanolic extract of *Averrhoa bilimbi L* against Gram positive *S. aureus* and Gram negative *E. coli*.

RESEARCH METHODS

a. Research design and the bacteria test.

This research is an experimental research with post test only control group design. The test bacteria used were *S. aureus* and *E. coli* ATCC (wild type strains). The test bacterial density used was 5 x 10^5 cfu/mL.[14]

b. Preparation of ethanolic extract of *Averrhoa bilimbi L.*

Averrhoa bilimbi L that has been washed and sliced are dried using a temperature oven 50°C for 4 hours. Purpose of drying is to reduce water content and prevent damage of extract by stopping the enzymatic reaction. *Averrhoa bilimbi L* extract was obtained from the extraction using maceration method with 96% ethanol solvent for 2 days. The maceration method is chosen with the consideration of not damaging the thermolabil compound.[15,16] 96% ethanol solvent was chosen to extracting flavonoids, saponins, and tannins due to their polarity properties. In addition, ethanol is safer than methanol or toluene.[17,18,19] The extract obtained was then concentrated and solvent evaporated using a rotary evaporator device with temperature 50°C.

c. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) test of ethanolic *Averrhoa bilimbi L* extracts againts *S. aureus* and *E. coli*

The thick extract of *Averrhoa bilimbi L* was then diluted to several concentrations (% v/v) using 2% dymethylsulfoxide (DMSO). Initial concentration of *Averrhoa bilimbi L* extract used for antibacterial test was 1,56%; 3,12%; 6,25%; 12,5%; 25%; and 50% (v/v). In the preliminary test results showed no bacterial growth at all these concentrations, so the concentration was reduced to 0,19%; 0,39%; 0,78%; 1%; 1,56%; 3,12% (v/v).

In addition to the treatment group, we added a negative control group that is a mixture between Mueller Hinton Broth (MHB) and *Averrhoa bilimbi L* extract. Negative controls were performed to determine the sterility of the medium and the extract used in the treatment. The positive control group is a mixture of Mueller Hinton Broth (MHB) and bacteria, used to determine whether the media used in the treatment can grow the test bacteria.

The solvent control group is a mixture of 2% DMSO, bacteria, and MHB media. Solvent control is used to ascertain whether the DMSO solvent used can not kill the test bacteria. All treatment groups and control groups were performed 4 repetitions.

The effectiveness test of ethanolic *Averrhoa bilimbi L* extract was done by assessing MIC and MBC againts *S. aureus* and *E. coli*. The MIC test was performed by dilution method using MHB media. Each concentration of ethanolic *Averrhoa bilimbi L* extract which have been added media and suspension of the test bacteria was then performed pre-incubation absorbance reading, then continued with post-incubation reading after incubation at 37°C for 16-20 hours. Pre and post-incubation absorbance readings were performed using a spectrophotometer with λ 625 nm. After that, MBC values were determined by determined at minimal concentrations of no bacterial colony growth after treatment and incubation.

d. Result analysis

The research data was tested using descriptive analysis to describe the effectiveness of ethanolic *Averrhoa bilimbi L* extract against *S. aureus* and *E. coli* bacteria. Effectiveness results were assessed with MIC and MBC values after treatment using various concentrations of ethanolic *Averrhoa bilimbi L* extract. Statistical analysis was performed using a 95% confidence level. The normality test of the data was done by using Kolmogorov - smirnov, while the homogeneity test was done by Levene's test. For *S. aureus*, because the test results are normally distributed, parametric tests are performed using one way ANOVA test. For *E. coli*, because of normal distributed but not homogenous data, a non-parametric test was performed using Kruskal Wallis statistic test. To determine whether the independent variables (ethanolic *Averrhoa bilimbi L* extract concentration) significantly influence the dependent variable (growth of test bacteria), then using simple regression test coefficient.

RESULTS

E. Minimum inhibitory concentration of *Averrhoa bilimbi L* ethanolic extract againts *S. aureus* and *E. coli*

Based on the results in table 1, the MIC value of *Averrhoa bilimbi L* ethanolic extract against *S. aureus* and *E. coli* bacteria was 1,56% (v/v). The absorbance data was then analyzed statistically to test the normality of the data with Kolmogorov-Smirnov, yielding *p value* of successively for *S. aureus* and *E. coli* was 0,299 and 0,201 (> α 0,05), so it can be concluded that absorbance data are normally distributed. For homogeneity test the data was analyzed with levene's test, yielding *p value* of successively for *S. aureus* and *E. coli* was 0,903 and 0,271 (> α 0,05), so it can be concluded that absorbance data are homogeneous distributed.

Because the absorbance data is normally distributed and homogeneous, then it is continued with ANOVA test. In the ANOVA test results obtained *p value* for both bacteria is 0,001 (<α 0,05). This suggests that there is a significant difference between the post-incubation and pre-incubation values on each concentration *Averrhoa bilimbi L* ethanolic extract.

Simple linear regression test was done to see the correlation between *Averrhoa bilimbi L* ethanolic extract concentration with the antibacterial activity. The antibacterial activity are seen from the difference value between post-incubation and pre-incubation of treatment solution. The correlation value shows strength and pattern of relationship between variables.

Based on the results of simple linear regression test, obtained that correlation coefficient value for *S. aureus* is 0,96 and *E. coli* 0,94. That value means there is a strong relationship closeness between *Averrhoa bilimbi L* ethanolic extract concentration and their antibacterial activity. The coefficient of determination (r2) was obtained for *S. aureus (0,96)* or 93% and for *E. coli* (0,94) or 89%. This shows that the increase of antibacterial activity can be explained by the increase of *Averrhoa bilimbi L* ethanolic extract concentration by 93% for *S. aureus* and 89% for *E. coli*.

The result of linear regression test of *S. aureus* was obtained *p value* 0,001 (<α 0,05), so the result is significant with regression line equation y = 0,081x-0,181.

The value of y is a prediction of antibacterial activity, while the value of x is the concentration of *Averrhoa bilimbi L* ethanolic extract. Graph 1 showed the relationship between the concentration of *Averrhoa bilimbi L* ethanolic extract with the difference in absorbance value post- and pre-incubation of *S. aureus* treatment. The result of regression test of *E. coli* treatment obtained *p value* is 0,001 (<α 0,05) with regression line equation is y = 0,089x-0,212. Graph 2 showed the relationship between the concentration of *Averrhoa bilimbi L* ethanolic extract with the difference in absorbance value post- and pre-incubation of *E. coli* treatment.

F. Minimum bactericidal concentration of *Averrhoa bilimbi L* ethanolic extract againts *S. aureus* and *E. coli*

Based on the results in table 2, the value of MBC *Averrhoa bilimbi L* ethanolic extracts againts *S. aureus* and *E. coli* bacteria was 1.56% (v/v). Result of normality test by using Kolmogorov-Smirnov got *p value* of successively for *S. aureus* and *E. coli* is 0,268 and 0,118 (> α 0,05), so it can be concluded log colony data are normally distributed. Result of homogeneity test with levene's test got *p value* of *S. aureus* is 0,053 (> α 0,05), so it can be concluded that log colony data is homogeneous distributed. For homogeneity test results of *E. coli* data with Levene's test obtained *p value* 0,040 (<α 0,05) which means that log colony data is not homogeneous distributed.

Result analysis on bacterial treatment of *S. aureus* test was continued with ANOVA test and obtained *p value* 0,001 (<α 0,05). This shows that there is a significant difference from log of colony count of *S. aureus* bacteria at each concentration of *Averrhoa bilimbi L* ethanolic extract. For the treatment of *E. coli* bacteria because the data is normally distributed and not homogeneous, then the test is continued with non-parametric statistic Kruskall-Wallis. Result of Kruskal-Wallis test obtained *p value* 0,003 (<α 0,05). So it can be concluded there is a significant difference of log of colony count of *E. coli* bacteria at each concentration of *Averrhoa bilimbi L* ethanolic extract.

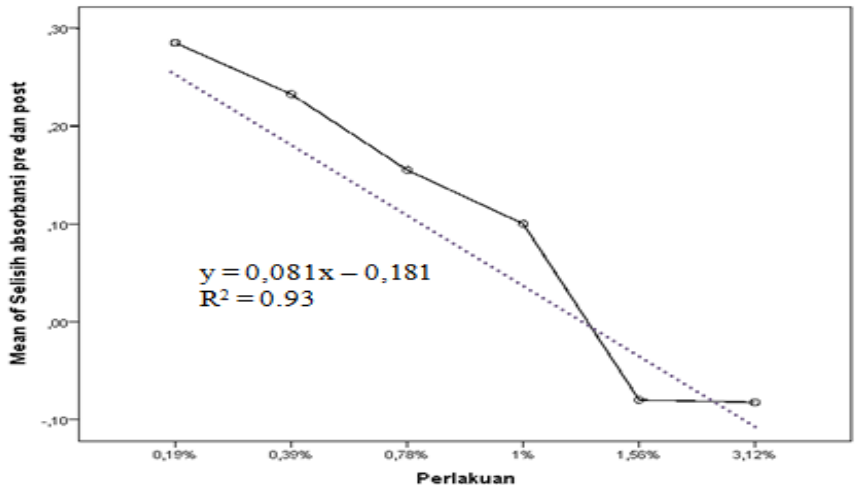
From the results of the correlation test obtained correlation coefficient value is 0,854 for *S. aureus* and 0,893 in *E. coli* which means there is a close relationship between the concentration of *Averrhoa bilimbi L* ethanolic extract with changes in the number of log colony of *S. aureus* and *E. coli* after treatment. The coefficient of determination (r2) was obtained (0.85) or 72% in *S. aureus* and (0,89) or 79% in *E. coli*. This shows that the change in log colony activity can be explained by the increase of *Averrhoa bilimbi L* ethanolic extract concentration by 93% for *S. aureus* and 89% for *E. coli*.

The result of regression test of *S. aureus* data obtained *p value* 0,001 (<α 0,05) with regression line equation y = 2,250x-1,521. Graph 3 shows a decrease in the growth of log colony *S. aureus* bacteria along with increasing concentration of *Averrhoa bilimbi L* ethanolic extract. For *E. coli*, regression test result got *p value* 0,001 (<α 0,05) with regression line equation y = 0,526x + 6,998. The value of y is the predicted prediction of the number of loh colony bacteria while the value of x is the concentration of *Averrhoa bilimbi L* ethanolic extract. Graph 4 is a graph of the relation of *Averrhoa bilimbi L* ethanolic extract concentration with decreasing log colony growth of *E. coli* bacteria.

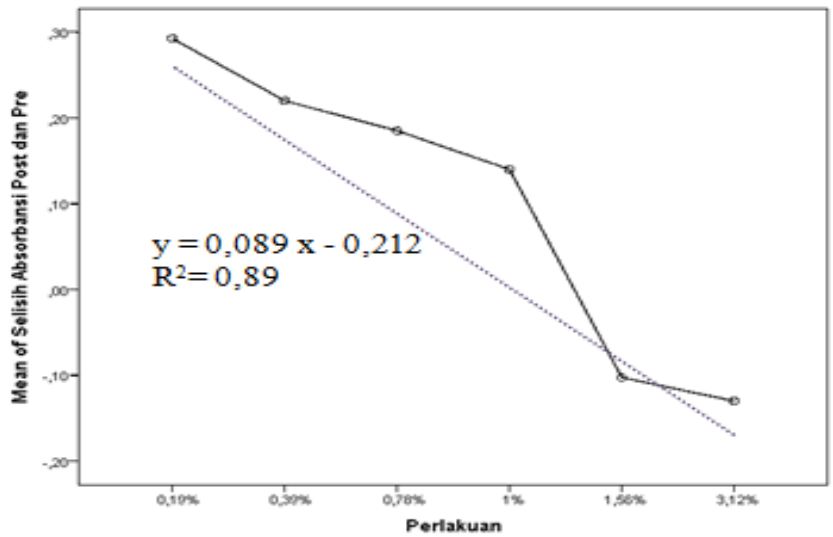
Table 1. Mean ± SD Reduction Value Of Absorption Post- And Pre-Incubation Treatment Of *S. Aureus* And *E. Coli* Bacteria On Various Concentrations of *Averrhoa bilimbi L* ethanolic Extract For Determination Of Minimum Inhibitory Concentration (MIC)

Concentration	Mean ± SD difference absorbance of *S. aureus*	Mean ± SD difference aborbance of *E. coli*
3,12%	-0,08±0,02	-0,13±0,03
1,56%	-0,08±0,02	-0,10±0,01
1%	0,10±0,02	0,14±0,02
0,78%	0,15±0,02	0,18±0,02
0,39%	0,23±0,01	0,22±0,01

0,19%	0,28±0,02	0,29±0,01



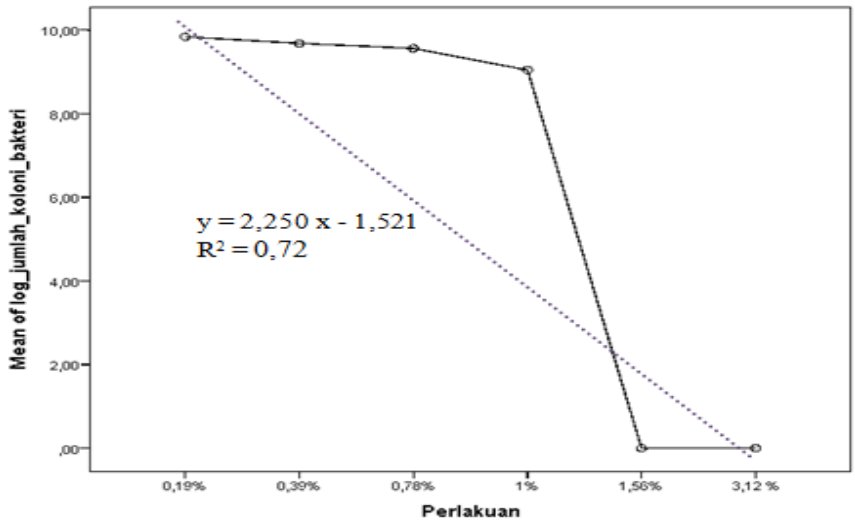
Graph 1. The Linear Regression Chart Of The Relationship Between The Concentration Of *Averrhoa bilimbi L* ethanolic extract With The Absorbance Value Post-And Pre-Incubation Of *S. Aureus*



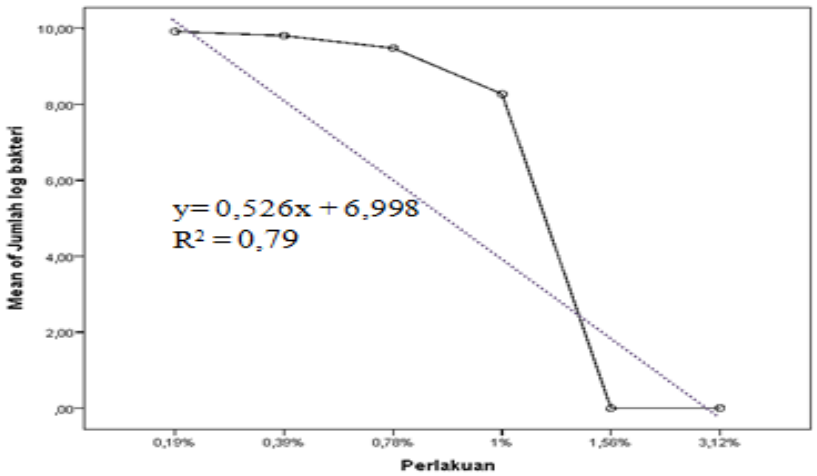
Graph 2. The Linear Regression Chart Of The Relationship Between The Concentration of *Averrhoa bilimbi L* Ethanolic Extract With The Absorbance Value Post-And Pre-Incubation of *E. coli*

Table 2. Mean ± SD Of The Number Of Bacterial Colonies of *S. aureus* and *E. coli* After Treatment And Incubation 37°C For 18-20 Hours *, For Determination Of Minimum Bactericidal Concentration (MBC)

Concentration	Mean ± SD Log Colony of *S. aureus*	Mean±SD Log colony of *E. coli*
3,12%	-	-
1,56%	-	-
1%	9,04±0,14	8,26±0,19
0,78%	9,56±0,02	9,47±0,02
0,39%	9,68±0,05	9,80±0,02
0,19%	9,83±0,01	9,91±0,01



Graph 3. Graph Of Linear Regression Relationship Between Concentration of *Averrhoa bilimbi L* Ethanolic Extract With Log Of Colony of *S.aureus* after treatment



Graph 4. Graph Of Linear Regression Relationship Between Concentration of *Averrhoa bilimbi L* Ethanolic Extract With Log Of Colony of *E. coli* after treatment

DISCUSSION

At concentration of *Averrhoa bilimbi L* ethanolic extract 1,56%, 3,12%, 6,25%,

12,5%, 25%, 50% (v/v) did not get the growth of bacteria either *S. aureus* or *E. coli* , so the concentration is lowered to 0.19%, 0.39%, 0.78%, 1%, 1.56%, 3.12% (v/v). Determination of MIC values can't be determined from visual observations, because of the viscosity visually due to concentrated *Averrhoa bilimbi L* ethanolic extract. The determination of MIC value was done by reading absorbance value using spectrophotometer with wavelength 625 nm by calculating difference absorbance post- and pre-incubation. *Averrhoa bilimbi L* ethanolic extract is declared effective against *S. aureus* or *E. coli* bacteria if absorbance value after treatment/incubation is less than absorbance value before treatment/incubation.

The principle of absorbance readings with this tool is by spreading the light fired on the cuvette containing the microorganism inside. The more light that is disseminated the more microorganisms in it.[20] The determination of MIC is done by incubating the treatment for 16-20 hours with temperature 37°C. The value of MIC *Averrhoa bilimbi L* ethanolic extract on *S. aureus* and *E. coli* bacteria was 1,56% (*p value* 0,001).

MBC value is determined by counting the number of bacterial colonies that grow after the second incubation, i.e. 16-20 hours incubation at temperature 37°C after determination of MIC. In concentrations of 3.12% and 1.56% there is no growth of either *S. aureus* or *E. coli* bacteria, so it can be said that *Averrhoa bilimbi L* ethanolic extract ata concentration 1.56% can kill both *S. aureus* and *E. coli*.

This study used 3 controls, positive control, negative control, and 2% DMSO control. The minimal inhibitory test on positive controls showed turbidity and increased absorbance after 16 - 20 hours of incubation. This indicates that Mueller Hinton Broth (MHB) media can grow test bacteria. Positive control contains test bacteria and Mueller Hinton Broth (MHB) media. On the negative control showed no turbidity, it showed that there were no bacteria and no increase in absorbance after incubation 16 - 20 hours. This indicates that the negative control consisting of *Averrhoa bilimbi L* ethanolic extract at concentration 0.39% and medium Mueller Hinton Broth (MHB) are sterile or not in a contaminated state. 2% DMSO control containing 2% DMSO, Mueller Hinton Broth (MHB) media, and test bacteria showed turbidity and increased absorbance after 16 to 20 hours of incubation. This shows that 2% DMSO control does not affect the growth of test bacteria.

Dymethylsulfoxide (DMSO) can be used to determine minimal inhibitory levels as a solvent of either natural or artificial antibacterial compounds. Previous studies have suggested that DMSOs with small concentrations have the best results compared to ethanol and methanol because they do not affect bacterial growth. DMSO concentration used is 2% because it does not affect bacterial growth significantly. This is to ensure that the determination of the minimum inhibitory content is not affected by the organic solvent concentration.[21]

Averrhoa bilimbi L extract can contains flavonoids, tannins, and saponins. These components have the ability to affect bacterial activity that is interfering with the formation of bacterial cells. Flavonoids can alter the cell protein composition, resulting from changes in cell membrane function; an increase in cell permeability may result in the death of bacterial cells. Tannin affects the synthesis of bacterial cell walls and the synthesis of bacterial proteins so that bacterial cell walls can melt and damage cell membranes.[6,22] Saponins are attractive water (hydrophilic) and lipid-soluble (lipophilic) molecules, through these properties saponins can form foam and damage the bacterial cell membrane due to the formation of lipid bond from the cell membrane so membrane tension becomes decreased and membrane cell damage occurs.[9,23] In this study the *Averrhoa bilimbi L* ethanolic extract content such as flavonoids, tannins and saponins that have antibacterial ability against *S. aureus* (Gram positive) or against *E. coli* (Gram negative) bacteria.

The effectiveness of *Averrhoa bilimbi L* ethanolic extract as antibacterial has been reported from several studies, such as extract of *Averrhoa bilimbi L* able to inhibit *Corynebacterium diphteriae* at concentration 10%[3], and effective against *Streptococcus sanguis* bacteria at concentration 2,5%.[24] Then, research of Dina

(2012) states that the extract of *Averrhoa bilimbi L* can inhibit the growth of *Bacillus sp*.[25] Based on some of these studies, it can be concluded that the *Averrhoa bilimbi L* extract has the ability to inhibit and kill the growth of Gram positive bacteria.

Some research results on the effectiveness of *Averrhoa bilimbi L* and leaf extract against Gram negative bacteria, among others, *Averrhoa bilimbi L* extract can inhibit and kill *Salmonella Sp* at 4,5% concentrations.[26] Meanwhile, Hasdiana *et al* (2012) also report the effectiveness of the *Averrhoa bilimbi L* leaf extract *S. typhi* at doses 0,3-0,5 g/mL.[27] Another study on the effectiveness of *Averrhoa bilimbi L* extract against Gram negative bacteria is that the extract able to inhibit the growth of *Shigella dysenteriae* at concentration 3,2 %[28], able to inhibit the growth of *Pseudomonas aeruginosa* bacteria at concentration 4,3%.[29] Based on this, *Averrhoa bilimbi L* and leaves extract have the ability to inhibit and kill the growth of Gram negative bacteria.

However, differences in the extraction methods used, the type of extract solvent, and the time of soaking simplicia during the extraction process can also affect the amount of extract content and then affect the antibacterial effectiveness of the *Averrhoa bilimbi L* and leaves extract.[30] For example, the following research, that has been done the determination of MIC test of chloroform and methanol *Averrhoa bilimbi L* extract using disc diffusion method to some Gram positive and negative bacteria. In that study there was no difference in effectiveness against Gram positive bacteria (*S. aureus* and *B. subtilis*) or Gram negative (*K. pneumonia* and *S. marcescens*). However chloroform extract has greater effectiveness compared to methanol extract.[31]

Meanwhile, from the screening of phytochemical compounds from *Averrhoa bilimbi L* and leaves ethanolic extract found alkaloid compounds, glycosides, saponins, tannins, steroids, and reducing sugars. The results of the MIC test using disc diffusion method, *Averrhoa bilimbi L* ethanolic extract at concentration 200 µg have medium activity against Gram positive bacteria *B. cereus* and Gram negative *E. coli*.[32] Several extracts of *Averrhoa bilimbi L* and leaves extract with various solvents are also reported by Aziz (2016). Although the majority of extracts have activity as antibacterial agent both Gram positive and negative, but one study stated that the *Averrhoa bilimbi L* methanolic extract at concentration 102,4 mg/mL have no activity against *E. coli*.[33]

CONCLUSION

This research can be concluded that the *Averrhoa bilimbi L* ethanolic extract effective as antibacterial with MIC and MBC value againts growth of *S.aureus* and *E. coli* bacteria at concentration 1,56% (*p value* 0,001).

ACKNOWLEDGEMENT

Acknowledgments are giving to the Biomedical Laboratory Faculty Medicine of UNIMUS and Laboratory of Biology UNNES.

REFERENCES

1. Kurdi A. Tanaman herbal indonesia: cara mengelola dan manfaat bagi kesehatan. Tanjung, 2010, p 2–5.
2. Mokhtar SI dan Aziz NAA. Antimicrobial properties of Averrhoa bilimbi extracts at different maturity stages. *J. Med Microb Diagn*, 2016, 5(3); p 1-3
3. Zakaria AZ, Zaiton H, Henie EFP, Jais AMM, Zainudin ENH. In vitro antibacterial activity of averrhoa bilimbi L leaves and fruit extracts. *International Journal of Tropical Medicine*, 2007, 2; p 96–100.
4. Alhassan AM dan Ahmed QU. Averrhoa bilimbi Linn: a review of its ethnomedicinal uses, phytochemistry, and pharmacology. *J. Pharm Bioallied Sci*, 2016, 8(4); p 265-271
5. Dhaliamartha S. Atlas tumbuhan obat Indonesia. Jilid 2. Jakarta: Trumbus Agriwidya, 2006, p 54–115
6. Prabu GR, Gnanamani A, Sadulla S. A plant flavonoid as potential antiplaque agent againt Streptococcus mutans. *Journal of Applied Microbiology*, 2006, 101(2); p 478-95
7. Ummah MK. Ekstraksi dan pengujian aktivitas antibakteri senyawa tanin pada daun belimbing (Averrhoa bilimbi L). Malang: Universitas Islam Negeri Malik Ibrahim, 2010, p 1–12
8. Mailoa MM, Mahendradatta M, Laga A, Djide N. Antimicrobial activities of tannins extract from guava leaves (Psidium Guajava L) on pathogens microbial. *International Journal*

Of Scientific & Technology Research, 2014, 3(1); p 236–41
9. Burger I, Burger VB, Albercht CF, Hendrik SC, Peter S. Triterpenoid saponin from Becium gradiflorum. *Phytochemistry*, 1998, 49; p 2087-2095
10. Rasyid R, Suheimi K. Prevalensi infeksi nasokomial pada pasien post sectio sesaria pada bagian kebidanan & penyakit kandungan RSUP DR. M. Djamil. Padang: *Majalah Kedokteran Andalas*, 2000, 2(24); p 1–29
11. Brook GF, Butel JS, Morse SA. Mikrobiologi kedokteran. Alih Bahasa. Mudihardi EK, Wasito EB. Jakarta: EGC, 2005, p 225–232
12. Firizki F. Pattern sensitivity of Escherichia coli and Klebsiela Sp to antibiotic sefalosporin period of year 2008-2013. Lampung: *Medical Journal of Lampung Universit,* 2014, ISSN 2337-3776
13. Pelczar MJ, Chan ECS. Dasar - dasar mikrobiologi. Alih bahasa Hadioetomo RS, Imas T. Dkk. Jakarta: UI Press, 2005, p 26–28.
14. Matuschek, E., Brown, DFJ., and Kahlmeter, G. Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. *Clin Microbiol Infect*, 2013, 20(4); p O255-266
15. Mukhriani. Ekstraksi, pemisahan senyawa dan identifikasi senyawa aktif. Makasar: *Jurnal Kesehatan*, 2014, 7(2); p 361- 67.
16. Swami HS, Singh KSP, Gennaro L, Dutt RD. Extraction technologies for medicinal and aromatic plants. Trieste: United Nations Industrial Development Organization and the International Centre for Science and High Technology, 2008, p 200 – 66
17. Oktavianes, Fifendy M, Handayani D. Daya hambat sari belimbing wuluh (Averrhoa bilimbi L.) terhadap pertumbuhan bakteri Escherichia coli. *Jurnal Pendidikan Biologi*, 2013, 2(2); p 1 – 5.
18. Azis T, Febrizky S, Mario AD. Pengaruh jenis pelarut terhadap persen yieldalkaloid dari daun salam india (Murraya koenigii). Palembang: *Teknik kimia*, 2014, 20(2); p 1 – 6.
19. Marnoto T, Haryono G, Gustinah D, Putra FA. Ekstraksi tannin sebagai bahan pewarna alami dari tanaman putri malu (mimosa pudica) menggunakan pelarut organik. Yogyakarta: *Reaktor*, 2012, 14 (1); p 39 – 45
20. Sutton, S. Measurement of microbial cells by optical density. *Journal Of Validation Technlogy*, 2011, p 46–49
21. Wadhwani T, Desai K, Patel D, Lawani D, Bahaley P, Joshi P, Kothari V. Effect of various solvent on bacterial growth in context of determining MIC of various antimicrobials. *The Internet Journal of Microbiology*, 2008, 7(1); p 1 – 6.
22. Nugrahawati D. Pemanfaatan belimbing wuluh (Averrhoa bilimbi L) sebagai cairan akumulator secara alami dan ramah lingkungan. Surakarta: Universitas Sebelas Maret, 2009, p 1–15
23. Robinson T. Kandungan organik tumbuhan tinggi. Alih bahasa. Kosasih Padmawinata. Bandung: ITB, 1995, p 191–216
24. Taliningrum KK. Perbedaan berbagai konsentrasi ekstrak etanol 70% belimbing wuluh (Averrhoa bilimbi L) sebagai bahan obat kumur terhadap hambatan pertumbuhan bakteri Streptococcus sanguis in vitro. Solo, *Jurnal UMS*, 2015, 9(1); p 1–12
25. Dina R. Aktivitas antibakteri ekstrak etanol belimbing wuluh (Averrhoa bilimbi L) terhadap Bacillus sp. Solo: *Jurnal UMS*, 2012, 1(1); p 1–16
26. Yudistira B, Widodo E, Sjofjan O. The effect of Averrhoa bilimbi L juice as feed additive on layer hen gut microflora. Malang: Brawijaya university, 1(1); p 1–9
27. Hasdiana F, Kuswarini S, dan Koendhari EB. Antibacterial activity of belimbing wuluh (Averrhoa Bilimbi Linn) extract on Salmonella typhi growth. *Folia Medica Indonesiana*, 2012, 48(4); p 144-149
28. Chandra S, Sultana S, Roy S, Hasan SS. Antibacterial and cytotoxic activities of methabolic extracts of leaf and fruit parts of the plant Averrhoa bilimbi L (Oxalidaceae). Dhaka: *America Journal of Scientific and Industrial Research*, 2011, 2(4); p 531–536
29. Yuliandari R. Uji aktivitas antibiofilm sari buah belimbing wuluh (Averrhoa bilimbi L) terhadap biofilm Pseudomonas aeruginosa secara in vitro. Jakarta: UIN Syarif Hidayatullah, 2015, 1(1); p 1–74
30. Kristiani V, Halim FI. Pengaruh konsentrasi etanol dan waktu maserasi terhadap perolehan fenolik, flavonoid, dan aktivitas antioksidan ekstrak rambut jagung. Surabaya: UKWMS, 2014, 1(1); p 1 – 36
31. Abraham CM. Antibacterial effects of Averrhoa bilimbi L. fruit extracts. *Int. Res. J. Biological. Sci*, 2016, 5(8); p 72-74
32. Karon B, Ibrahim M, Mahmood A, Huq AKMM, Chowdury MMU, Hossain MA, dan Rashid MA. *Bangladesh Pharmaceutical Journal*, 2011, 14(2); p 127-131
33. Aziz NA. A review of the antimicrobial properties of three selected underutilizied fruits of Malaysia. *International journal of pharmaceutical and clinical research*, 2016, 8(9); p 1278-1283