

AN IN-VITRO STUDY OF ANTIBACTERIAL EFFECTS OF SOURSOP LEAVES EXTRACT (*Annona muricata*) ON *Staphylococcus aureus*

Hibban Rifai¹, Andri Pramono^{2*}, Retno Yulianti³

¹Program Studi Sarjana Kedokteran, FK UPN Veteran Jakarta

²Departemen Mikrobiologi, FK UPN Veteran Jakarta

³Departemen Patologi Anatomi, FK UPN Veteran Jakarta, Jl. RS Fatmawati, Pondok Labu, Jakarta Selatan 12450

*Corresponding email : andri.pramesti@upnvj.ac.id

ABSTRACT

Soursop (*Annona muricata*) is claimed to have an antimicrobial effect. This study was conducted to determine the antimicrobial effect of Soursop (*Annona muricata*) leaves extract and the correlation between its effect based on the inhibition zone measured in diameter and for every increases of the concentration of the extract while using wells diffusion method. A total of 700 gram of leaves was prepared and turned into ethanol extract using maceration technique and then the extract is diluted into various concentration, specifically 20%, 40%, 60%, 80%, and 100%. The process was repeated 3 times in order to obtain 24 samples. Ciprofloxacin was used as a positive control while the aquadest was used as the negative. All samples are incubated for 24 hours. Data obtained was analyzed using Pearson's r with Confidence interval of 95% and the data was statistically significant ($p=0,000$). The results shown that the Pearson's r was positive (variance = 0,731) with every increases of concentration of extract in 1%, the diameter of inhibition zone is also increased by amount of 0,15 mm. The Soursop extract shown its efficacy for every concentration which was tested and increases in concentration are correlated to the increases of the diameter of the inhibition zone.

Keywords : *Annona muricata*, *Staphylococcus aureus*, Wells Diffusion

1. INTRODUCTION

Staphylococcus aureus is the cause of most cases of microbial infections in the public and healthcare environments (healthcare-acquired). Epidemiology in the last few years began to appear *S. aureus* that is resistant to many antibiotics, namely penicillin and oxacillin and other beta-lactam groups. *S. aureus* infection starts from the entry of these microorganisms into the host body through the opening in the skin and mucous layers that will affect the structure of the local tissue or will spread to other organs resulting in life-threatening infectious diseases such as bacteremia, pneumonia, and osteomyelitis (Bergin et al., 2015; Idelevich et al., 2016; Olaniyi et al., 2016)

Staphylococcus aureus has evolved rapidly towards the introduction of antibiotics during the last seventy years. Although in general *S. aureus* is a species that is susceptible to antibiotics, over time, the bacteria become resistant to any antibiotic drug that has entered the clinical stage until 80% of *S. aureus* isolates are resistant to penicillin (Choo dan Chambers, 2016).

Antistafilococcal penicillin drug resistant to penicillinase, or methicillin, shows an adequate response to penicillin-resistant *S. aureus*. However, methicillin drug resistance appears evidenced by the identification of the first Methicillin-Resistant *Staphylococcus aureus* (MRSA)

in hospitals in the City of London (Chambers and Deleo, 2010). The use of glycopeptide drugs has a relationship with the emergence of *S. aureus* which has begun to be resistant to glycopeptide drugs, thus encouraging health institutions to develop insights and seek alternative therapies for *Staphylococcus aureus* isolates. In Indonesia, the public has the perception that plants can be used as a substitute for clinical medicine and to date development of science, insights, and research has been carried out to sort plants to be determined as medicines ranging from herbal medicines, herbal medicines, and phytopharmaca drugs. Soursop (*Annona muricata*) is a plant that can be used as an alternative treatment for cancer and as a substitute for chemotherapy. Things that cause soursop plants to be effective against microorganisms is the presence of several secondary metabolites in the form of phenolic compounds, flavonoids, tannins, alkaloids, saponins, and glycosides, and acetogenin (Ononiwu et al., 2017). Metabolite compounds such as flavonoids, alkaloids and terpenoids are known to have antimicrobial effects. In addition to metabolites, soursop plants also contain acetogenin compounds. Acetogenin is an active substance that can be found in the family Annonacea. This substance is known to have the effect of tumorisida, anti-malaria, anti-helminth, antiviral, and antimicrobial (Moghadamtousi et al., 2015).

Research on the antimicrobial effect of soursop leaf extract has been carried out with bacteria *Streptococcus mutans*, *Streptococcus mitis*, *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Candida albicans* mushrooms with concentrations of 1%, 5%, 10%, 15%, 20% by Pai et al. with the results of all effective concentration gradients on all the above microbes except *Prevotella intermedia* (Pai et al., 2016). Research on *Annona muricata* was also carried out by Vinothini and Growther to see the antimicrobial effects of leaf and fruit extracts on several types of bacteria including *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Escherichia coli* and *Proteus mirabilis*, and the results that there were antifungal effects. extract against the bacteria tested (Vinothini dan Growther, 2016).

Based on previous studies, researchers feel the results of the research from extraction need to be repeated with different concentration gradients and the variable *Staphylococcus aureus* bacteria are still not specific as no measurements were made with various concentrations in the form of percentages, knowing the research was also carried out in different regions. Based on this background, researchers are interested in continuing research on the effectiveness of soursop leaf testing with a welling method against *Staphylococcus aureus* bacteria. This study uses a variation of extract concentration of 20%, 40%, 60%, 80, and 100% because it refers to previous studies that use variations in the concentration.

2. METHODS

Research Design

This study uses an experimental test design in exploring the potential antimicrobial effects of *Annona muricata* extract on *Staphylococcus aureus* bacteria. The method used is the in vitro method of the wells method.

Material and Methods

The tools used in this research were: test tubes, test tube racks, autoclaves, bunsen, cloves, pumpkins, scales, micropipets, incubators, stoves, perforator vacuum pouring machines, sterile petri dishes.

The materials used in this study were MHA Media (Mueller-Hinton Agar), *Annona muricata* extract, bacterial culture of *Staphylococcus aureus* ATCC 25923, discs containing the antibiotic ciprofloxacin, empty test discs.

Obtaining Extract

Annona muricata extract was obtained from the Indonesian Spice and Medicinal Research Institute (Balitro). The extract was made using maceration method, soursop leaves obtained as much as 700 grams were washed. Then dried, kneaded and mashed until it becomes powder. The powder was then soaked in 70% ethanol for 3x24 hours, through filtration filtration. Then all the filtrates are combined, and evaporated or concentrated with a rotary

evaporator at a temperature of 39 degrees Celsius with a rotational speed of 50 rpm until an extract is obtained.

Sample Size

The sample size used in this study was divided into several groups namely 5 variations in the concentration of Soursop Leaf Extract (20%, 40%, 60% 80% and 100%), the positive antibiotic control group and the negative group using ethanol. The number of repetitions of each group was obtained through calculations with the formula $(t - 1) (r - 1) \geq 15$ where t is the number of treatments and r is the number of repetitions (Federer, 1983). The results obtained are based on calculations and the number of groups to be studied (5) obtained a total of 4 repetitions.

Statistical Analysis

The data processing method is carried out with an IBM SPSS 22.0 computer device. Based on the type of data processed in this study, the analysis used is a metric analysis of metrics, which will produce a scatterplot graph to indicate an increase or decrease in mathematical gradient at every 1% of the strength of soursop plant extracts against the S.aureus Disc Inhibiting Zone. In addition, the hypothesis test in this study involved the '95% Confidence Interval' ($p < 0.05$) and the significance test for Pearson's r values

Research Procedure

Researchers made some concentrations of Soursop leaf extract that will be carried out by the study. And prepare a comparative control solution consisting of antibiotics as a positive control antibiotic, and ethanol solution as a negative control. Culture on the media using the method of two-layer base layer for the main media and the seed layer containing *Staphylococcus aureus* bacteria.

In each inoculated test media a well was made with a 5mm diameter and a depth of 4 mm using a perforator and each hole was given extract concentration, negative control in the form of distilled water, and positive control of the ciprofloxacin antibiotic. Test media that have been given treatment are incubated in an incubator for 24 hours at 37 °. After incubating, the media was observed to see the shape of the inhibition zone around the disc paper and was calculated using a digital calipers from edge to edge.

3. RESULTS AND DISCUSSION

Phytochemistry test

Phytochemical testing is the initial stage to identify the content of a compound in a simplicia or plant that will be tested to determine the presence of active compounds that cause toxic effects or beneficial effects. Table 1 shows the results of the phytochemical test of soursop leaf extract (*Annona muricata*).

Table 1 Phytochemical Test Results of Soursop Leaf Extract

Testing Category	Result
Alkaloid	+
Saponin	+
Tanin	+
Fenotik	+
Flavonoid	+
Triterpenoid	+
Glikosida	+
Steroid	+

4. Discussion

Phytochemical test results in this study showed that all metabolites that were able to be tested by researchers were positive. These results are consistent with Agu and Okolie's (2017) research on the presence of flavonoids, saponins, tannins, phenolics, alkaloids, triterpenoids, glycosides, and saponins in soursop leaves (*Annona muricata*) (Agu and Okolie, 2017). Soursop leaves have the most lipid components compared to other plant parts and Acetogenin is a derivative of a long chain of fatty acids (C32 or C34). This statement reinforces research into the composition of lipids in soursop leaves (Kossouh et al., 2007; Smith, Tran dan Richards, 2014; Agu dan Okolie, 2017).

Inhibition Zone Measurement

Table 1. The results of various extract concentrations

Sample	20%	40%	60%	80%	100%
1	3,8	6,3	9,8	12,3	12,0
2	2,6	5,2	8,3	12,0	6,6
3	0,9	6,0	7,7	12,0	13,4
4	2,8	5,6	9,8	12,2	4,7
Median	2,7	5,8	9,05	12,1	9,3
Average	2,525	5,775	8,9	12,125	9,175

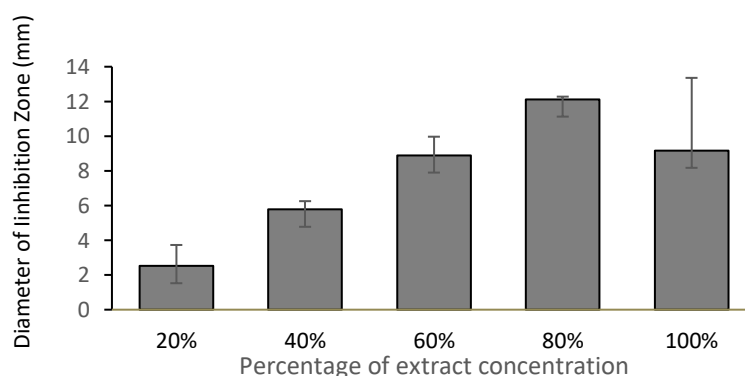


Figure 1. Inhibition zone measurement from various extract concentration

Table 1 shows the trend of increasing the average inhibition zone diameter in the variation of soursop extract concentration of 20% -80%, but the decrease in inhibition zone diameter was found in the concentration of soursop extract 100% (from 12,125 mm to 9,175 mm). In *S. aureus* culture given 100% soursop extract concentration obtained a small inhibitory zone diameter in the second and fourth experiments of 6.6 and 4.7 mm compared with other experiments. In this study, a significant correlation data was obtained with a coefficient value (r) of 0.855, exceeding the Pearson's (Pearson's r) critical correlation value of 0.388 (number of samples $n = 24$) and a variance value (r^2) of = 0.731 with a note of the significance value of used is at $\alpha = 0.05$.

Table 3. Pearson correlation analysis results

Konsentrasi Ekstrak Daun Sirsak

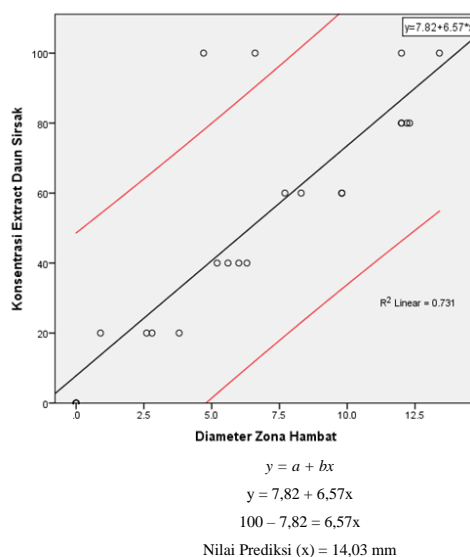
Diameter Zona Hambat	R = 0,855
	p < 0,00001
	n = 24
Uji korelasi Pearson	

5. Discussion

The results showed an antibacterial activity with a fluctuating pattern of the average wells inhibition zone per increase in the number of concentrations with variations of 20%, 40%, 60%, 80, and 100% in the descriptive table. Giving the impression that the most effective concentration is the extract concentration of 80%, with the data recorded in table 2 only giving an average picture without processing the test.

Statistical analysis. Correlation is performed to ascertain the average pattern of data in table 2. The reason the researcher considers this is because seen from the 100% concentration group in the second and fourth tests have very low diameter values (6.6 mm and 4.7 mm), which is outlier of statistical analysis. This causes the average value of the concentration group to be 100% lower than 80% which certainly gives the impression of a decrease. After doing a statistical test, the researcher can get a predictive value based on the results of interpretations from scatterplots that state positive values. This shows that the predicted inhibition zone diameter will continue to increase with increasing concentration because with a variance value of 0.731 approaching the value of one will indicate the interpretation of the Scatterplot graph that will continue to rise. Based on the statistical analysis, if repetition will be carried out in this study, then the variance value will remain positive and experience an increase. This is reinforced by the calculation of predictions with assumptions using the graph formula in chart 4.

Figure 4. Grafik Scatterplot dan nilai prediksi



It is likely that the predicted concentration of 100% (14.03mm) will give better results than the 80% concentration if the same research treatment is carried out in the future. Using the chart formula 4, researchers can determine the magnification of the minimum inhibition zone for each 1% increase in concentration with a value of 0.15 mm.

In a study conducted by Vinothini et.al., the researchers stated that soursop leaf extract on methanol solvents had a more effective antimicrobial effect compared to distilled water extracts. The research method was conducted with different dosage sizes namely methanol leaf extract and distilled water extracts obtained from soursop plants by 5 kg with variations in concentrations of 50 μ l, 100 μ l, and 200 μ l (Vinothini and Growther, 2016). The characteristics of the research results in the form of descriptive tables also indicate an upward trend. However, because there are only three concentration

variations in the data, the data cannot be analyzed metric by metric to see the predicted value of Vinothini's research. Vinothini's research also did not carry out statistical analysis tests. For future research, it is expected to be able to use more raw materials from this study and with reference to the scatterplot graph calculation can get a larger diameter of the inhibition zone.

Kemit et.al. conducted research on the extraction solvent fraction and proved by comparing 90% methanol solvent and 90% ethanol to the treatment of maceration duration of various avocado plants. The results of the study prove that ethanol solvents have a higher yield of flavonoid metabolites than methanol in maceration extraction methods. Flavonoids are polar compounds that require polar solvents such as methanol, ethanol, acetone and water. Ethanol possesses polar nature which is more polar than methanol so that it can get flavonoids (Kemit, Widarta and Nocianitri, 2017). The above is the reason why researchers use ethanol as a solvent rather than methanol in the extraction process.

This research has limitations. The number of samples of extract concentration variation is still small in the future. It is better to do a lot of samples in order to see the distribution of data more clearly. In addition, the effects of extract doses will vary if carried out on experiments on humans.\

6. CONCLUSION

Based on the results and discussion that has been presented, it can be concluded that the most effective concentration in experiments with the wells method is at a concentration of 80%. Significant correlation data with a coefficient (r) of 0.855, exceeding Pearson's critical correlation value. There is a correlation between the enlargement of the inhibition zone diameter for each 1% increase in concentration with an increase of 0.15 mm.

REFERENCES

- [1] Agu, K. C. dan Okolie, P. N. (2017) "Proximate composition , phytochemical analysis , and in vitro antioxidant potentials of extracts of *Annona muricata* (Soursop)," (April), hal. 1029–1036. doi: 10.1002/fsn3.498.
- [2] Bergin, S. P. et al. (2015) "Bacteremia, Sepsis, and Infective Endocarditis Associated with *Staphylococcus aureus*," *Current Topics in Microbiology and Immunology : Staphylococcus aureus*, 409, hal. 263–296. doi: 10.1007/82.
- [3] Chambers, H. F. dan Deleo, F. R. (2010) "Waves of Resistance: *Staphylococcus aureus* in the Antibiotic Era," 7(9), hal. 629–641. doi: 10.1038/nrmicro2200.Waves.
- [4] Choo, E. J. dan Chambers, H. F. (2016) "Treatment of methicillin-resistant *Staphylococcus aureus* bacteremia," *Infection and Chemotherapy*, 48(4), hal. 267–273. doi: 10.3947/ic.2016.48.4.267.
- [5] Idelevich, E. A. et al. (2016) "Staphylococcus aureus -Associated Musculoskeletal Infections," *Current Topics in Microbiology and Immunology : Staphylococcus aureus*, 409, hal. 229–262. doi: 10.1007/82.
- [6] Kemit, N., Widarta, I. W. R. dan Nocianitri, K. A. (2017) "Pengaruh Jenis Pelarut dan Waktu Maserasi Terhadap Kandungan Senyawa Flavonoid dan Aktivitas Antioksidan Ekstrak Daun Alpukat (*Persea Americana* Mill)," *Jurnal Ilmu dan Teknologi Pangan*, 5(2). Tersedia pada: <https://ojs.unud.ac.id/index.php/itepa/article/view/27509/17418>.
- [7] Kossouoh, C. et al. (2007) "Essential Oil Chemical Composition of *Annona muricata* L . Leaves from Benin," *Journal of Essential Oil Research*, 19(April 2006), hal. 307–309. Tersedia pada: <https://www.tandfonline.com/doi/abs/10.1080/10412905.2007.9699288>.
- [8] Moghadamtousi, S. Z. et al. (2015) "*Annona muricata* (*Annonaceae*): A Review of Its Traditional Uses , Isolated Acetogenins and Biological Activities," hal. 15625–15658. doi: 10.3390/ijms160715625.
- [9] Olaniyi, R. et al. (2016) "Staphylococcus aureus -Associated Skin and Soft Tissue Infections : Anatomical Localization , Epidemiology , Therapy and Potential Prophylaxis," *Current Topics in Microbiology and Immunology : Staphylococcus aureus*, 409(October), hal. 199–227. doi: 10.1007/82.

- [10] Ononiwu, F. O. et al. (2017) “Antimicrobial activities and phytochemical properties of *Annona muricata* leaf,” *Journal of Physical & Life Sciences*, 5(2), hal. 40–49.
- [11] Pai, B. H. M. et al. (2016) “Anti-microbial Efficacy of Soursop Leaf Extract (*Annona muricata*) on Oral Pathogens : An In-vitro Study,” *Journal of Clinical and Diagnostic Research*, 10(11), hal. 1–4. doi: 10.7860/JCDR/2016/18329.8762.
- [12] Smith, R. E., Tran, K. dan Richards, K. M. (2014) *Bioactive Annonaceous Acetogenins*. 1 ed, *Studies in Natural Products Chemistry*. 1 ed. Elsevier B.V. doi: 10.1016/B978-0-444-63294-4.00004-8.
- [13] Vinothini, R. dan Growther, L. (2016) “Antimicrobial and Phytochemical Analysis of Methanolic and Aqueous Extract of *Annona muricata* (Leaf and Fruit),” *International Journal of Current Microbiology and Applied Sciences*, 5(10), hal. 617–625. Tersedia pada: <http://dx.doi.org/10.20546/ijemas.2016.510.069>.