ISSN:xxxx-xxxx

Proceedings of the 1st ACEH INTERNATIONAL CONFERENCE ON HEALTH Poltekkes Kemenkes Aceh November 10-11, 2024, Banda Aceh, Indonesia

ANTIBACTERIAL INHIBITORY OF *Acetobacter aceti* BACTERIA FROM ISOLATE PALM VINEGAR (*Arenga pinnata*) AGAINST *Salmonella* sp. BACTERIA

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ABSTRACT

Salmonella sp. is a bacterium that causes a disease commonly known as salmonellosis. This disease is treated with antibiotics. However, long-term administration of antibiotics can cause side effects or negative effects on the body and trigger resistance to these antibiotics. Therefore, another alternative is needed by utilizing acetic acid bacteria as a probiotic that can suppress the growth of Salmonella sp. One of the acetic acid bacteria that can be a probiotic is *Acetobacter aceti*. This research aims to determine the potential of *Acetobacter aceti* bacteria from aren palm vinegar (*Arenga pinnata*) isolates in inhibiting the growth of Salmonella sp. This reasrch uses the kirby-bauer method by differentiating the concentration of *Acetobacter aceti* bacteria that have been isolated from aren palm vinegar (*Arenga pinnata*) with concentrations of 25%, 50%, 75%, and 100% against *Salmonella* sp. Negative control (distilled water), and positive control (*chloramphenicol*) with 3 repetitions. The test results were analyzed by Annalisis of Varian (ANOVA) test using SPSS. Based on the test results, the four concentrations of *Acetobacter aceti* obtained produced inhibition zones with the highest inhibition zone at 100% concentration of 11.48 ± 1.11 mm. The positive control *chloramphenicol* also produced a high inhibition zone of 13.58 ± 2.64mm, so it was more effective than the concentration of *Acetobacter aceti* bacteria given. However, the concentration of *Acetobacter aceti* bacteria obtained from aren palm vinegar (*Arenga pinnata*) isolates has the potential to inhibit the growth of *Salmonella* sp. bacteria and can be used as probiotics.

Keywords: *Acetobacter aceti, Probiotic, Arenga pinnata, Salmonella sp.*

INTRODUCTION

Salmonella is one of a group of pathogenic bacteria that can cause disease in humans. Until now Salmonella is still a complicated problem, because of the transmission of Salmonellosis which can be transmitted through unhygienic food media. Salmonella bacteria are the cause of zoonosis which is very much transmitted through food, namely as much as 80.1%, transmission between humans 6.3% and through animals 4.3% (Darmawan & Muslimin, 2020). Treatment of pathogenesis originating from Salmonella mainly uses antibiotics (Handayani *et al.*, 2017). The use of antibiotics that can harmful human and animal health makes it important to apply the use of herbal ingredients that can be utilized as a substitute for the use of antibiotics that can be used in inhibiting the growth of unwanted bacteria (Sudarman *et al*., 2017).

Palm vinegar (*Arenga pinnata*) is a medicinal plant that contains a type of acetic acid formed from the fermentation process of nira water (Zulkifli *et al*., 2014). *Acetobacter aceti* is one of the probiotics that produce bacteriocins. Bacteriocins are antimicrobial components that can inhibit the growth of pathogenic bacteria. Bacteriocins can be bacteriocidal or bacteriostatic (Poernomo *et al*., 2015). Based on the information obtained above, aren palm vinegar is known to have many benefits and advantages, because one of its contents is *Acetobacter aceti* bacteria which can be used as an antimicrobial. In addition, according to research conducted previously by Ester (2020), *Acetobacter aceti* bacteria were found to be one of the probiotic bacteria isolated in banana mas (*Musa acuminata* L.) fruit vinegar. For this reason, researchers want to conduct research related to the inhibition of *Acetobacter aceti* bacteria contained in palm vinegar (*Arenga pinnata*) against *Salmonella* sp.

METHODS

This type of research is laboratory experimental research. This study uses the Kirby-bauer method by differentiating the concentration of Acetobacter aceti bacteria that have been isolated from palm vinegar (*Arenga pinnata*) with concentrations of 25%, 50%, 75%, and 100% against Salmonella. Then the blank papper disk is soaked in each concentration of *Acetobacter aceti* that has been provided. Next, the paper disk is placed on the surface of the PYG media which has been applied to *Salmonella* sp. bacteria culture and then the inhibition is calculated using a vernier callipers.

The procedure for identifying *Acetobacter aceti* bacteria isolated from palm vinegar (*Arenga pinnata*) was carried out as follows: 1 ml of vinegar liquid was taken and put in a test tube containing PYG broth, which was then

incubated. Once obtained, it was planted on PYG agar and subjected to gram staining. The bacteria were then subjected to further tests, namely biochemical tests using the indole test, methyl red, Voges-Proskauer, citrate, and fermentation of sugars (maltose, sucrose, lactose, glucose, and mannitol). Once the results were in, we replanted the *Acetobacter aceti* bacteria in PYG broth, which we then diluted by adding PYG broth to the bacterial preparation so that it was available in concentrations of 25%, 50%, 75%, and 100%.

After a 24-hour incubation period, the antibacterial activity was determined by measuring the diameter of the inhibition zone formed around the disc paper. A caliper was used to determine the size of the inhibition zone formed. The test results were analyzed with the Analysis of Variance (ANOVA) test using SPSS to see the inhibition of *Acetobacter aceti* bacteria isolated from palm vinegar (*Arenga pinnata*) against *Salmonella* sp. bacteria.

RESULTS AND DISCUSSION

The results of the inhibition test of *Acetobacter aceti* bacteria against Salmonella sp. bacteria can be known from the observation of whether or not there is an inhibition zone formed around the well. The results obtained showed that of the four concentrations of *Acetobacter aceti* bacteria from *Arenga pinnata* vinegar isolates tested, all produced inhibition zones indicated by white and clear zones around the wells. The test results can be seen in table 1.

Table 1. Mean zone of inhibition of various concentrations of *Acetobacter aceti* bacteria against *Salmonella* sp.

Concentration (%) *Acetobakter aceti* Bacteria	N	Average zone of inhibition
K+	3	13,58mm ±2,64^{d}
0	3	0,00mm ±0,00^{a}
25	3	7,11mm ±1,58^{b}
50	3	7,93mm ±1,36^{b}
75	3	9,81mm ±2,19^{bc}
100	3	11,48mm ±1,11^{cd}
Total	18	8,32mm ±4,65

Note: $^{a,b,c,\text{ dan }d}$ superscripts indicate significant differences $(P < 0.05)$.

The table above shows the average zone of inhibition formed and the magnitude of the standard deviation on the concentration of *Acetobacter aceti* bacteria with a concentration of 0%, 25%, 50%, 75%, and 100%, respectively 0.00mm ±0.00, 7.11mm ±1.58, 7.93mm ±1.36, 9.81mm ±2.19, 11.48mm ±1.11, and 13.58mm ±2.64. The inhibition zone formed is a white zone around the treatment that is overgrown with *Acetobacter aceti* bacteria and a clear zone around the zone where no *Salmonella* sp. bacterial colonies can be grown. From the results of the table above also shows that there is a significant difference (P <0.05) between the inhibition of *Acetobacter aceti* concentration treatment against *Salmonella* sp. bacteria. The test results showed a difference in the size

of the inhibition zone produced by *Acetobacter aceti* bacteria. This can be influenced by several factors such as pH, carbon source, temperature, and bacterial growth phase affecting the activity of bacteriocins produced by *Acetobacter aceti* bacteria. In addition to this, nutritional sources such as carbon and nitrogen contained in the growth medium also play an important role in the growth rate of *Acetobacter aceti* bacterial cells, which ultimately has an impact on the rate of metabolism and bacteriocin production (Fauziah *et al*., 2015).

The inhibition zone is formed due to various antimicrobial compounds produced by *Acetobacter aceti* bacteria such as acetic acid, bacteriocins, hydrogen peroxide, reutrin, diacetyl, and other organic acids. These organic acids include short chain fatty acids (SCFA) (Lacob *et al*., 2019). Acetic acid plays an important role in inhibiting the growth of *Salmonella* sp. bacteria by regulating pH. This can interfere with the survival of Salmonella sp. and prevent excessive colonization of the intestinal epithelium when the bacteria are accidentally consumed (Prayoga *et al*., 2021). The results of statistical tests on the treatment of *Acetobacter aceti* bacteria from palm vinegar (*Arenga pinnata*) isolates against *Salmonella* sp. bacteria showed that the four concentrations of *Acetobacter aceti* bacteria had the potential to inhibit the growth of *Salmonella* sp. bacteria with a concentration of 100% significantly different from the other three concentrations, namely 25%, 50%, 75%, and 100%. Based on the inhibition zone criteria of Davis & Stout (1971), the 100% concentration is categorized as "strong" with an inhibition zone formed of 11.48mm.

Concentrations of 25%, 50%, and 75% were categorized as medium with inhibition zones formed of 7.11mm, 7.93mm, and 9.81mm. The diameter of the inhibition zone of *Acetobacter aceti* is categorized as weak if the inhibition zone formed is less than 5mm. While the inhibition zone of *Acetobacter aceti* is said to be moderate if the inhibition zone formed is 5-10mm. If the inhibition zone formed is 10-20mm, the inhibition zone is said to be strong, while the inhibition zone formed is greater than 20mm, this is categorized as a very strong inhibition zone. Meanwhile, the control treatment is divided into two parts, namely negative control and positive control. In the negative control treatment using distilled water, showed no inhibition zone response. The positive control used chloramphenicol antibiotic which showed an inhibition zone of 13.58mm. This shows that the positive control of chlomphenicol is more effective than the treatment of *Acetobacter aceti* bacteria concentration. Based on the criteria according to CLSI (Clinical and Laboratory Standards Institute) (2016), the standard inhibition of chloramphenicol against Salmonella sp. bacteria is categorized as intermediate because it is in the range of 13-17mm.

CONCLUSION

Based on the research that has been done, the results of 4 concentrations of *Acetobacter aceti* bacteria isolated from palm vinegar (*Arenga pinnata*) are 25%, 50%, 75 &, and 100% concentrations. The four concentrations affect the inhibition of *Salmonella* sp. bacteria so that the four concentrations of *Acetobacter aceti* have the potential to inhibit the growth of Salmonella sp. bacteria with the highest inhibition produced by 100% concentration of 11.48 ± 1.11 mm.

ACKNOWLEDGEMENT

Praise and gratitude I express to Allah Subhanahu Wa Ta'ala who has given His grace, guidance and guidance so that the author can complete the thesis entitled "Antibacterial inhibitory of *Acetobacter aceti* bacteria from isolate palm vinegar (*Arenga pinnata*) against *Salmonella* sp. bacteria". Blessings and greetings the author conveys to the Great Prophet Muhammad Shallallahu 'Alaihi Wasallam who has brought us from the realm of darkness to this bright realm and may we receive his intercession on the day of resurrection.

The author would like to express his infinite respect and gratitude to Father Joni Naswar Refli and Mother Anggraini who have poured out prayers, given invaluable love and encouragement. Thank you to my entire extended family for all the prayers, love, sincere affection that is always given for the success and happiness of the author, as well as the motivation and support to complete this writing.

The author would like to express his sincere respect and gratitude to drh Rastina, M.Si, the supervisor who has provided knowledge, advice, advice, energy and free time to discuss from the beginning to the end of the research and writing of this journal.The author would like to thank the Dean of the Faculty of Veterinary Medicine, Syiah Kuala University, drh. Teuku Reza Ferasyi, M.Sc., Ph.D., coordinator of the Veterinary Education study program, Faculty of Veterinary Medicine, Syiah Kuala University Dr. drh. Darniati, M.Si. and to all the lecturers involved along with the administrative staff who have helped the author a lot during his studies at the Faculty of Veterinary Medicine, Syiah Kuala University.

The author would like to express his gratitude to Muhammad Hafizh as his beloved brother and Abyan as his beloved brother and fellow comrades who the author cannot mention one by one who are always ready to provide assistance to the author.
The author realizes that this journal is still far from perfect, therefore constructive criticism and suggestions are always expected. Hopefully this research will be useful in the development of science. Aamiin Yaa Rabbal ‘Alamin.

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