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EFFECTIVENESS TEST OF ONION EXTRACT (*Allium cepa* L.) ON BACTERIA GROWTH *Aeromonas hydrophila IN* VITRO

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ABSTRACT

Aeromonas hydrophila is a bacterium that causes MAS disease (*Motile Aeromonas Septicemia*), often found by cultivators. This study aimed to determine the effectiveness of onion extract (*Allium cepa* L.) in replacing chemical antibiotics and the correct dosage to inhibit the growth of *Aeromonas hydrophila bacteria* in *vitro*. The research method used was a Completely Randomized Design (CRD) with 7 treatments and 3 replications, P1 (Control-), P2 (Control+), P3 (10% onion extract), P4 (25% onion extract), P5 (50% onion extract), P6 (75% onion extract), P7 (100% onion extract). The results showed that 30 ml of onion extract obtained from 101.3 grams of onion powder had a yield value of 29.812%. Some secondary metabolite compounds in onion extract are flavonoids, alkaloids, saponins, tannins, steroids, and phenols, which function as antibacterial compounds. The diameter of the clear zone of 10% onion extract has a value of 26 mm and has a value that is not significantly different from the use of 50% *chloramphenicol antibiotics* with an inhibition zone diameter of 26.67 mm, treatment with onion extract with a concentration of 100% gives the same inhibition zone diameter. The highest is 34.17 mm.

INTRODUCTION

Earth the equator crosses Indonesia's position as an archipelagic country in Southeast Asia. It is between 2 oceans (the Indian Ocean and the Pacific Ocean) and 2 continents (the Asian and Australian continents), with an area of around 9 million km2 and 1.3% of the total area. Indonesia is included in the tropical region, which has a very high level of variation in life (Whitemore, 1985 *in* Kusmana, 2015). Indonesia is estimated to have potential medicinal plants of 30,000 out of a total of 40,000 plant species worldwide. Of these, 940 species are known to have medicinal properties, or around 90% of the total medicinal plants on the Asian continent (Nugroho, 2010 *in* Maryani, 2020). Presently, the use of herbal plants in Indonesia for medicinal purposes is still far from the potential in nature. One of the natural and inexpensive ingredients that can be used is onions. Onions are natural foods that contain active ingredients such as allin, flavonoids, saponins, pectin, and allicin, and these active ingredients can inhibit the growth of bacteria (Pakekong, 2016).

In the fisheries sector, one of the bacteria often found in a hatchery and grow-out cultivation is *Aeromonas hydrophila* which can cause *Motile Aeromonas Septicemia* (MAS). These bacteria include gram-negative bacteria and can cause fish death in a short time, ranging from 80-100% (Lukistyowati and Kurniasih, 2012 *in* Muslikha, 2016). In Indonesia, *Aeromonas hydrophila bacteria* became known in 1980. This bakery spread disease outbreaks and caused the death of 125 tons of carp (Triyanto, 1990 *in* Arwin, 2016).

Treatment of diseases caused by bacteria can use antibiotics. However, using antibiotics with chemicals, if used continuously, will cause bacterial resistance because bacteria naturally develop tolerance to the surrounding environment and become resistant to drugs often used (Skou and Jensen, 2007 *in* Putri, 2012).

In a previous study by Wuryanti (2009), using onion extract to inhibit the growth rate of *Pseudomonas aeruginosa bacteria* gave good results. In contrast, onion extract has antibacterial properties against gram-negative bacteria. The results of this study can be used as a reference for developing knowledge about the effect of onion extract on the growth of Aeromonas hydrohila bacteria which are included in gram-negative bacteria.

METHODOLOGY

Time and place

The research location for the phytochemical screening test occurred at the Analytical Chemistry Laboratory at the University of Mataram. In contrast, the *in vitro test* occurred at the Aquaculture Fish Health Laboratory, Faculty of Agriculture, University of Mataram. This research was conducted for 3.5 months, starting November 23, 2021- February 24, 2022.

Tools and materials

The tools used in this study were petri dishes**,** wire loops **,** calipers **,** paper discs **,** autoclaves **,** digital scales **,** erlenmeyer **,** measuring pipettes **,** test tubes **,** sample bottles **,** analytical balances **,** test tube racks **,** hot plates **,** spectrophotometers **,** computers . **,** blender **,** plastic tray **,** spatula **,** oven **,** aluminum foil **,** bunsen **,** gloves **,** paper **,** matches **,** iron tongs **,** tube brush **,** plastic wrap **,** drigalski **,** 1 ml syringe and sponge . While the materials used were Aeromonas hydrophila, onion, 76% alcohol, 96% ethanol, *chloramfenicol* , tissue, label paper, sunlight.

Research design

The research design used was a Completely Randomized Design (CRD), which used 7 treatments (P1, P2, P3, P4, P5, P6, P7) and 3 replications, namely treatment P1 (Control-), P2 (Control+), P3 (10% onion extract), P4 (25% onion extract), P5 (50% onion extract), P6 (75% onion extract), P7 (100% onion extract). The negative control used 96% ethanol (P1), and the positive control used 50% *chloramphenicol.*

Research procedure

Preparation of onion extract

Fresh onions are peeled off the outer skin and washed, then sliced thin and then in the oven for ± 7 hours with a temperature of 60 ℃. After that, the dried onions were blended and sifted to obtain the onion powder used to make the extract. A total of 101.3 grams of onion powder was macerated with 0.5L 96% ethanol for 2 days and then evaporated using a rotary evaporator, and 30 ml of onion extract was obtained.

Phytochemical screening assay

The phytochemical screening test was carried out by direct observation to see color changes and specific reactions indicating the presence of secondary metabolism in onion extract. The compounds tested in this phytochemical screening test are flavonoids, alkaloids, saponins, phenols, steroids, and tannins.

Tool sterilization

Tool sterilization is carried out by washing clean the tool to be used then, followed by 3 ways. The first is using an autoclave for heat-resistant and extensive tools such as Erlenmeyer, Petri dishes, and reaction tubes. The second way is spraying alcohol on small tools such as spatulas, and tube clamps, using needles and drigalski. The third way is to use a Bunsen to loop the needle just before it is used to take bacterial cultures.

Preparation of *Nutrient Agar* (NA) media

Dry NA media was used as much as 4 grams, dissolved in 200 ml of distilled water, and then heated with a hotplate until boiling. Pouring into the petri dish is done after the Erlenmeyer is not too hot. After that, it was sterilized by autoclaving for ± 15 minutes at 121 ℃ and 1 atm pressure.

Regeneration of test bacteria

Pure Aeromonas hydrophila bacteria cultures from the slanted media were transferred to new solid agar media, then incubated for 18-24 hours at 20-37 ℃.

Antibacterial activity test i

Bacteria resulting from culture for 18-24 hours were taken as much as 1-2 ose and dissolved in 10 ml of distilled water, after which the bacterial suspension was calculated using Mc Farland 0.5 using a spectrophotometer. Measurements were made on a 625 mm wave with a susceptible absorbance value of 0.08-0.13. The bacterial density was based on the Mc Farland 0.5 standard, 1-2 x 10 8 CFU/ml (Clinical and Laboratory Standards Institute, 2009 *in* Aristyawan, 2017). The bacterial suspension was taken as much as 0.2 ml and spread over solid NA media, then left for ± 30 minutes.

The onion extract and *chloramphenicol were* diluted according to the required concentration, after which the sterile disc paper was immersed and placed on the NA media, which already contained bacteria marked using label paper, incubated for 24 hours, and measured the clear zone formed around the disc paper using a caliper.

Data collection technique

Data collection techniques in this study used qualitative and quantitative methods. The qualitative method was used because the research results were obtained by direct and descriptive observation of the phytochemical screening test. At the same time, the quantitative experimental method is carried out because its implementation is through experimental research conducted in the laboratory and statistical data processing to show the effect of a treatment on results under controlled conditions. Data is taken from the calculations measuring the area of the clear zone formed in each treatment in each replication. The results will be calculated statistically to test the established hypothesis.

Data analysis

Data analysis on this research plan used the Analysis of Variance (ANOVA) statistical test with a 95% confidence level in the Minitab program. The data were obtained by measuring the clear zone of the antibacterial activity test using a caliper. The data showed a significant effect, then a further test (Tukey) was carried out to compare the average results of all treatments.

RESULT

The yield of Onion Extract

The yield value of the onion extract was calculated by dividing the resulting extract weight divided by the weight of the dry raw material used and then multiplying it by 100%. According to Budiyanti (2015) *in* Senduk (2020), the higher the yield value of the extract, the higher the content of substances or compounds that are attracted to a material. The yield of onion extract was obtained at 29.812%. According to Mardina (2011) *in* Senduk (2020), the yield value is affected by the length of extraction time. The longer the extraction time, the higher the yield obtained. This is because the opportunity for the material to react with the solvent is extended so that more and more compounds will diffuse out of the cell. The yield calculation results can be used to estimate the number of raw materials needed when making a certain amount of onion extract.

Phytochemical Test of Onion Extract

No	Test/Test	Test Sample (Onion Extract
1	Flavonoids	+
2	Alkaloids	+
3	Saponins	+
4	tannins	+
5	Steroids	+
6	Phenol	+

Table 1. Phytochemical Test of Onion Extract

Based on direct observation of the phytochemical screening test, it is known that the prepared onion extract contains flavonoids, alkaloids, saponins, tannins, steroids and phenols.

Antibacterial Inhibitory Test

Antibacterial activity test on NA media showed a clear zone formed in all treatments except for the control- (96% ethanol). The smallest inhibition zone diameter was in P3 treatment (10% onion extract) with a diameter of 26 mm, while the largest inhibition zone diameter was in P7 (100% onion extract) with a value of 34.17 mm.

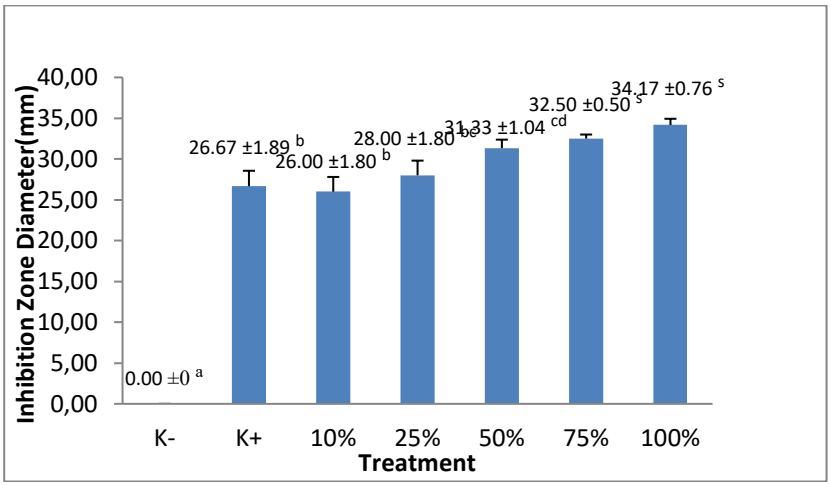

Figure 1. Inhibition zone diameter

After the ANOVA test was carried out, it was shown that the treatment using onion extract had a significant effect on the inhibition of the growth of *Aeromonas hydrophila bacteria*. Tukey's further test results showed that the treatment with the smallest inhibition zone diameter was P3 (10% onion extract) had significant results with P2 (*50% chloramfenicol antibiotic*). The treatment with the largest diameter of the inhibition zone P7 (100% onion extract) had a significant value with P6 (75% onion extract).

DISCUSSION

A phytochemical screening test was conducted to determine the secondary metabolites in the prepared onion extract. *Secondary metabolites* are compounds that can help inhibit the growth of bacteria, such as flavonoids, alkaloids, saponins, steroids, tannins, and phenols. The presence of the compounds mentioned can be determined by direct observation, such as the flavonoid compound group marked by the change in color of the solution to yellow, alkaloid compounds characterized by the formation of a white precipitate, saponin compounds characterized by the appearance and presence of foam for a few minutes after the solution was shaken vigorously, the formation of precipitates can identify the presence of Tannin compounds, steroid compounds are characterized by a change in color to bluish green and phenol compounds are indicated by a change in color to green.

Secondary metabolite compounds contained in onion extract have a role in inhibiting bacterial growth. This is based on several statements, the first from Robinson (1995) *in* Hasibuan (2018), which states that alkaloids and flavonoids have antibacterial properties, alkaloids with a mechanism that disrupts the part that makes up the peptidoglycan of bacterial cells so that a complete cell wall is formed and can kill the cell. . Then the statement from Sirait (2007) *in* Hasibuan (2018) terpenoids are divided into several categories namely monoterpenes, sesquiterpenes, diterpenes, triterpenes and tetraterpenes, where according to Robinson (1995) *in* Hasibuan (2018) triterpenoids which are a class of terpenoids can be antimicrobial and have antifungal, antiviral, antibacterial and insecticidal properties. Then there is a statement from Harborne (1987) *in* Hasibuan (2018) that saponins can be used as poisons and antimicrobials, including viruses, fungi, and bacteria, where saponins can cause damage to erythrocytes at low concentrations so that they can be used as antibacterials.

The antibacterial activity test on NA media was carried out using the *paper disc diffusion method* (Kirby-Bauer) and measuring the diameter of the clear zone vertically and horizontally

around the paper disc. The treatment used was P1 as a negative control, P2 as a positive control, P3 with 10% onion extract concentration, P4 with 25% onion extract concentration, P5 with 50% onion extract concentration, P6 with 75% onion extract concentration and P7 as an extract concentration, onions 100%.

Based on *One Way* test data *Analysis of Variance* (ANOVA) with *Tukey's advanced test*, it can be seen that the inhibition of bacteria by 50% *chloramphenicol antibiotics* and 10% concentration of onion extract has values that are not significantly different. This shows that 10% onion extract can match and replace 50% *chloramphenicol antibiotics* to inhibit the growth of *Aeromonas hydrophila* bacteria, which are gram-negative. It is also known that the average diameter of the inhibition zone for *Aeromonas hydrophila bacteria* using onion extract for all treatments is > 20 mm, based on the antibacterial activity category table by Davis and Stout (1971) *in* Tampongangoy (2019), the diameter of the inhibition zone ≥20 mm is included in the category extreme antibacterial activity.

The graph shows that the 100% concentration has the largest diameter of the clear zone among the other treatments. However, after being tested using the *Way test Analysis of Variance* (ANOVA) with *Tukey's advanced test*, the bacterial inhibition test between 100% and 75% concentrations appears to have a significant or not significantly different value. So the selection of using onion extract with a concentration of 75% can be made with the consideration that the use of onion extract is less but has antibacterial results on a laboratory scale which are not significantly different from the use of a higher onion concentration of 100%.

CONCLUSION

Based on the research that has been done, it is concluded that:
a. Onions have antibacterial compounds that can inhibit the growth of *Aeromonas hydrophila bacteria*.
b. inhibition zone of *Aeromonas hydrophilla* was found in the treatment with 100% onion extract concentration with a diameter of 34.7 mm**.**
c. A 10% concentration of onion extract can replace 50% *chloramphenicol antibiotics.*

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