JPPIPA 9(2) (2023)

Jurnal Penelitian Pendidikan IPA

Journal of Research in Science Education

http://jppipa.unram.ac.id/index.php/jppipa/index

Activity Test of Antibacterial Compounds of Aloe Vera Extract Against *Edwardsiella Tarda* Bacterial Infection in Vitro (technique)

Nuranti Anarkhis[1*], Arief Prajitno[2], Maftuch[3], Karimah[4]

[1] Master's Degree Program in Faculty of Fisheries and Marine Science, University of Brawijaya, Malang, East Java, Indonesia.
[2] Department of Aquculture, Faculty of Fisheries and Marine Science, University of Brawijaya, Malang, East Java, Indonesia.
[3] Department of Aquaculture, Faculty of Fisheries and Marine Science, University of Brawijaya, Malang, East Java, Indonesia.
[4] Master's of Physics Study Program, University of Brawijaya, Malang, East Java, Indonesia.

Received: December 12, 2022
Revised: February 13, 2023
Accepted: February 25, 2023
Published: February 28, 2023

Corresponding Author:
Nuranti Anarkhis
nuranti_ar@student.ub.ac.id

DOI: 10.29303/jppipa.v9i2.2610

Abstract: *Edwardsiella tarda* is a type of gram-negative intracellular pathogen that can cause mass demise in fish farming. The development of edwarsiellosis due to *E.tarda* infection causes significant economic losses for cultivators. Aloe vera is a perennialsucculent plant like a cactus, drought withstand, and belongs to the Liliaceae family,of which there are more than 360 known species (Wijaya and Masfufatun, 2022). *Aloe vera* is one of the plants known to contain active ingredients that can inhibit the growth of bacteria. The results of phytochemical analysis of a crude extract of *Aloe vera* showed the presence of active compounds, specifically flavonoids, tannins, triterpenoids, and saponins. MIC analysis showed that the lowest absorbance value of *Aloe vera* was obtained at a concentration of 50 mg/L with a value of 0.316, indicating a decrease at each concentration. The concentration of 50 mg/L is close to the positive control which indicates that at a concentration of 50 mg/L, it can bethe reference for the lowest concentration in determining the dose of *Aloe vera* extract which is known that the presence of antibacterial compounds in *Aloe vera* extract can inhibit the growth of *E.tarda* bacteria. The inhibition zone test was carried out with graded concentrations of 50 mg/L, 100 mg/L, 150 mg/L, 200 mg/Land 250 mg/L with an observation time of 24 hours post-incubation and 48 hours post-incubation. Inhibition zone analysis with the highest diameter was at 200 mg/L. SEM analysis by distributing a crude extract of *Aloe vera* affected *E.tarda* bacteria specifically, lysis occurring in bacterial cells.

Keywords: Antibacterial; *Aloe vera;* Phytochemicals; SEM; Zona of inhibition

Introduction

One of the factors causing a decrease in fish production is a bacterial infection. *Edwardsiella tarda* is a bacterium that causes edwardsiellosis.Edwardsiellosis can inflict mass fatality and is reported as a severe disease attack for freshwaterfish species reported as a severe disease attack for freshwater fish species (Abraham *et al*., 2015). *Edwardsiella tarda* is a common fish pathogen, causing septicemic diseases and formidable economic losses in freshwater fish farming (Algammal *et al*., 2022).

Fish infected with edwardsiellosis will show clinical symptoms such as pale skin color, produce excess mucus, and wounds, whenscratched, it will emit stink, and inflammation occurs from the anus to the base of the tail (Ali *et al*., 2014).

In general, the treatment of *E.tarda* bacterial infection uses antibiotics. However, the continuous use of antibiotics can cause resistance to microorganisms. Thus, the use of antibiotics for treatment is currently not recommended. Several chemicals and antibiotics widely used are tetracycline, chloramphenicol, formalin, methylene blue and gentian violet (Atma, 2019).

The efforts to reduce the use of antibiotics regularly, it is necessary to use natural ingredients at this time. Natural ingredients are known to have active compounds that can be used as antibacterials. One of the

How to Cite:
Anarkhis, N., Prajitno, A., Maftuch, M., & Karimah, K. (2023). Activity Test of Antibacterial Compounds of Aloe Vera Extract Against Edwardsiella Tarda Bacterial Infection in Vitro (technique). *Jurnal Penelitian Pendidikan IPA*, *9*(2), 563–567. https://doi.org/10.29303/jppipa.v9i2.2610

plants that have anactive ingredient is *Aloe vera*. *Aloe vera* is a succulent perennial plant like cactus, drought-resistant, and belongs to the *Liliaceae* family that there are more than 360 known species (Wijaya & Masfufatun, 2022).

The Aloe vera plant has long been usedas a natural medicinal plant packaged as herbal powder. Aloe vera contains active compounds such as flavonoids, saponins, tannins, and flavonoids, which inhibit bacterial growth. Aloe vera has various therapeutic characteristics such as antivirus, antioxidant, anti-allergic, anti-inflammatory, anti-cancer, antibacterial, andimmunostimulating (Bendjedid *et al*., 2021).

Based on this description, this study aimed to examine and study the antibacterialcompounds of aloe vera extract against bacterial infections *Edwardsiella tarda*.

Method

Location and Time of Research

This research was conducted in November 2021-Mei 2022 in the laboratory of fish parasites and diseases (Faculty of Fisheries and Marine Sciences Brawijaya University), fish reproduction laboratory (Faculty of Fisheries and Marine Sciences Brawijaya University), Materia Medica Batu, Batu City, East Java.

Production of Aloe vera Extract

Aloe vera samples were extracted by the maceration method. 100 g of Aloe vera powder soaked in 400 ml ethanol for 48 hours. The samples that have been macerated and then filtered using Whatman paper no. 42. Then, the filtered sample is putinto the rotary vacuum evaporator to form a paste.

E.tarda bacterial preparation

The bacteria used were obtained from the parasite and fish disease laboratory, Faculty of Fisheries and Marine Sciences, Brawijaya University. The bacteria were stored in Trypticase Soy Broth (TSB) mediaat -30°C.

MIC test (Minimum Inhibitory Concentrantion)

On the MIC Test, the first thing to do is prepare a Sterile test tube. The test tube was filled with 4.5 ml of sterile TSB media. Then 0.5 ml of A.vera extract was added to a test tube containing TSB at a specific concentration. Test tubes 1 to 5 were filled with aloe vera extract A.vera with concentrations of 50, 100, 150, 200 and 250 ppm. The 6th and 7th tubes were filledwith two controls, the negative control and the positive control. The positive control was given 50 ppm of synthetic antibacterial (Chloramphenicol) with a volume of 0.5 ml while the negative control was without treatment. Then, each tube was given 0.1 ml of bacterial isolate (107 CFU/ml) and incubated at 320C for 24 hours. Next, the turbidity level of the test medium was examined and its absorbance was measured with a spectrophotometer (wavelength of 600 nm). The MIC test can also be seen for turbidity in the TSB media,which has been added to the extract, planted with bacteria, incubated for 24 hours, and then compared with the control.

Zone of Inhibition Test

The procedure for this inhibition zonetest refers to the research by Maftuch et al.(2018), the procedure for the disc test is asfollows: (1) Trypticase Soy Agar (TSA) media is prepared and then poured into a petri dish and then waited for it to solidify. (2) Sterile paper discs were soaked with *A.vera* extract with various treatment doses. (3) Treatment dose is determined based on the MIC test results that were done beforehand. (4) Bacteria with a density of 107 were taken using a micropipette as much as 0.1 ml and spreadover the entire surface of the TSA media using a triangle. (5) The disc paper was soaked for 10-15 minutes, then the disc paper was carefully taken and placed into the TSA media. (6) Next, the media was incubated at 32oC for 18-24 hours. The media was observed by measuring the diameter of the clear zone formed around the disc paper using a vernier caliper.

SEM Test(Scanning Electron Microscope)

E.tarda preparations were carried out with two treatments. The first treatment of *E.tarda* was normal, and the second treatment of *E.tarda* bacteria was given *A.vera* extract at a dose of 250 ppm. Then, the two preparations are ready to be observed using SEM *(Scanning Electron Microscope*).

Data Analysis

The data obtained were analyzed statistically with data using a completely randomized design (CRD). Data were processed and analyzed using the (SPSS) 25.0for Windows software application. Theanalysis method for damage to *E. tarda* bacteria can be done by comparing photos of SEM observations between normal preparation conditions and bacteria treated with Aloe vera extract and then looking at the damage to the bacterial cell wall.

Result and Discussion

Content of Active Compounds in Aloe vera Extract

Based on the phytochemical screening, the results of Aloe vera extraction using 96% ethanol solvent contained several compounds, including flavonoids, alkaloids, tannins/phenols, triterpenoids and saponins can be seen in Table 1.

Table 1. Content of active compound in *Aloe vera*

Identification of compound	Characteristics	Result
Flavonoid	Orange, Brick red, Pink, Crimson	(+) Positive
Alkanoid	White precipitate	(-) Negative
Meyer	Orange precipitate	(-) Negative
Dragendrof	Brown precipitate	(+) Positive
Bouchardat		
Tanin/Fenol	Blackish brown, blue-black	(+) Positive
Terpenoid	Bluish-green	(-) Negative
Steroid	Green Orange	(+) Positive
Triterpenoid	Orange brown	
Saponin	Permanent foam	(+) Positive

Note: (+) There are chemical ingredients, (-) No chemical ingredients.

Saponins, tannins, and flavonoids are active compounds that can be used as antibacterials and these compounds are present in the aloe vera plant. Based on theresults of the phytochemical test, the antibacterial compounds are flavonoids, saponins, and tannins. positive (+)contained in the crude extract of Aloe vera. Kristianingsih *et al.* (2021) with the result of a phytochemical screening test showing that Aloe vera extract contains saponins, tannins, and flavonoids. Another study by Royani *et al.* (2022), that as an antibacterial plant, *A.vera* contains active substances such as saponins, tannins, and flavonoids. Phytochemical screening of Aloe vera extract using 96% ethanol solvent also showed that the sample contained active compounds, namely phenolic compounds, flavonoids, tannins, and saponins, and was negative for alkaloid compounds (Yasir *et al.*, 2021).

Aloe vera ethanol extract contains active substances that have been identified as antibacterial compounds which are a class of phenolic compounds, so the mechanism of action of Aloe vera extract as an antibacterial is by inhibiting bacterial growth. Active compounds enter thebacterial cell through the cell wall and cytoplasmic membrane. These active compounds cause denaturation of the proteins making up the bacterial protoplasm, so metabolism becomes inactive and bacterial growth is inhibited (Sari *et al.*, 2018).

One active compound that plays a rolebased on the results of phytochemicals is saponin. Saponin by their mechanism ofaction can reduce the surface tension of thecell wall and then damage the permeability of the membrane, this is because the surface of the active substance of saponins is similar to that of detergents so that saponins bind to the cytoplasmic membrane by diffusing through the outer membrane and vulnerable cell walls which can then reduce and disrupt the stability ofthe cell membrane (Wijaya and Masfufatun, 2022).

The Ability of Aloe vera Extract to Inhibit the Growth of E.tarda Bacteria

1). Minimun Inhibitory Concentration

The result of MIC test (Minimum Inhibitory Concentration) measured by the absorbance value indicates Aloe vera extract's ability to inhibit the growth of *E.tarda* bacteria. The results of measuring the absorbance value can be seen in Table 2.

Table 2. The result of MIC test

Concentration	Absorbance Value
Kontrol (-)	0.819
50 mg/L	0.316
100 mg/L	0.402
150 mg/L	0.369
200 mg/L	0.477
250 mg/L	0.412
Kontrol (+)	0.121

Note: Positive control (K+) using clorampenicol 5 ppm, Negative control (K-) without giving extracts.

The lowest absorbance value of Aloe vera was obtained at a concentration of 50 mg/L with a value of 0.316, indicating a decrease at each concentration. The concentration of 50 mg/L is close to the positive control, indicating that the concentration of 50 mg/L can be the reference for the lowest concentration in determining the dosage of Aloe vera extract. This can show that the presence of antibacterial compounds in aloe vera extract can inhibit the growth of *E.tarda* bacteria. The results of research by Royani *et al*. (2022) MIC testing to inhibit *P.aeruginosa* bacteria at the lowest concentration of 250 mg/L Aloe vera extract could inhibit the growth of *P.aeruginosa* bacteria. The MIC test is affected by the turbidity level of the extract used. After 24 hours of incubation, the clarity level in the medium was challenging to distinguish. This is because the concentration of the extract used results in the medium looking cloudy (Wardani *et al.*, 2019).

Aloe vera extract at a dose of 50 mg/L isthe minimum dose reference for disc dosing. The Minimum Inhibitory Concentration (MIC) test is used as a reference to determine the minimum concentration of antimicrobials that can inhibit microorganisms after 18 to 24 hoursafter the incubation period (Azaldin *et al.*, 2020).

2). *Inhibition Zone Test*

The result of precise zone measurements are presented in Table 3. The inhibition zone test (cakram) was carried out to see the value of the clear zone formed around the disc paper. The clear zone formed shows the ability of Aloe vera extract to inhibit the growth of *E.tarda* bacteria. The disc test was carried out with graded concentrations of 50 mg/L, 100 mg/L, 150 mg/L, 200 mg/L and 250 mg/L with an observation

time of 24 hours post- incubation and 48 hours post-incubation.

Table 3. The result of the inhibition zone test

Concentration Mg/L	Average Cleare Zone Diameter (mm)		Clear Zone Classification
	24 Jam	48 Jam	
50	4.91± 0,28[a]	5.31± 0,17[a]	Medium
100	5.40± 0,11[b]	5.62± 0,18[b]	Strong
150	5.98± 0,25[c]	5.99± 0,29[c]	Strong
200	6.01± 0,18[d]	4.89± 0,44[d]	Strong
250	5.84± 0,47[e]	5.04± 0,60[e]	Strong
K (-)	0.00± 0,00[f]	0.00± 0,00[f]	Weak
K (+)	12.49± 0,97[g]	12.49± 0,97[g]	Very Strong

Note: Inhibition zone classification, weak 1-3 mm, moderate 3-5 mm, strong 5-7 mm, and very strong>7

Measurement of the diameter of the inhibition zone at each dose treatment with24-hour intervals showed that the administration of extract doses could have an effect, namely increasing the diameter ofthe inhibition zone. The result of 50 mg/L isincluded in the moderate category, which is 4.91 mm. Extract concentrations of 100, 150, 200, and 250 mg/L are included in the 5.84 mm. Several factors affect the size of the inhibition zone formed, namely growth sensitivity, environmental pH, media components, incubation temperature, reactions between active ingredients,inoculum size, and metabolic activity of microorganisms (Mulqi *et al.*, 2022).

In *E.tarda* bacterial infection, aloe vera extract can also inhibit the growth of fungi. Administration of aloe vera leaf extracts significantly affected the diameter of the inhibition zone on the growth of C. Albicansin vitro (Huslina, 2017).

It can be seen from the results of this study that the higher the concentration value used, the larger the diameter of the inhibition zone formed. Huslina (2017) alsoreported that the greater the aloe vera leaf extract concentration, the greater thegrowth inhibition zone. C. albicans formed. Another study was also reported by Dewi and Marniza (2019) that the higher the concentration of aloe vera gel, the larger thediameter of the inhibition zone formed in *S.aureus*. The difference in the diameter of the inhibition zone at each concentration is due to the difference in the active substance contained therein, so the inhibition zone formed will differ at each concentration.

3). SEM (Scanning Electron Microscope)

The results of the SEM (Scanning Electron Microscope) test showed an antibacterial effect ofaloe vera extract on Etarda bacteria, as seen from changes in the structure of the bacteria. Changes in the structure of Etarda bacteria can be seen in Figure1.

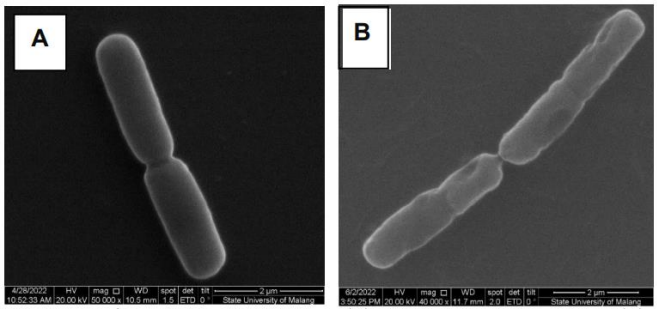

Figure 1. Edwardsiella tarda morphology; (A) Without Giving Extract (B) Giving Aloe Vera Extract

The content of active compounds in the form of flavonoids, tannins, alkaloids, and saponins in aloe vera extract affects changesin the structure of *E.tarda* bacteria as seen from *E.tarda* bacteria which experience lysisand shrinkage and the morphology of bacteria which becomes uneven after being given Aloe vera extract. The content present in aloe vera has the potential to inhibit bacterial growth by reducing the AB stress from the surface of the cell wall and damaging membrane permeability resulting in leakage of enzymes and proteins within the cell. This is in line with the research of Wijaya dan Masfufatun (2022) that the ingredients contained in Aloe vera extract have significant potential to inhibit the growth of microorganisms. The mechanism of Aloe vera in inhibiting bacterial growth is by reducing the cell wall's surface tension and damaging the membrane's permeability, resulting in leakage of enzymes and proteinswithin the cell.

Active compounds that successfullyplay a role in inhibiting bacterial growth show morphological characteristics. When abacterial cell undergoes lysis, it will appear to shrink, the bacterial cell wall is porous, and then the cell wall dies. The cell wallundergoing lysis will secrete fluid, which shrinks the bacterial cell wall (Hariati *et al.*, 2018).

Conclusion

Based on the phytochemical tests, it can be concluded that Aloe vera extract contains active compounds which areflavonoids, triterpenoids, tannins, and saponins. Based on the results of the MIC test, Disc test, and SEM test, it can be concluded that Aloe vera extract can inhibit the growth of *E.tarda* bacteria.

Acknowledgments

The authors would like to express their heartfelt appreciation and gratitude to all employeesof the Fish Cultivation Laboratory, Division of Fish Disease and Health, Faculty of Fisheries and Marine Sciences, Brawijaya University.

References

Abraham, T., Mallick, P., Adikesavalu, H. & Banerjee, S. (2015). Pathology of Edwardsiella tarda infection in African catfish, Clarias gariepinus (Burchell 1822), fingerlings. Fisheries & Aquatic Life,23(3) 141-148. https://doi.org/10.1515/aopf-2015-0016

Algammal, A. M., Mabrok, M., Ezzat, M., Alfifi, K. J., Esawy, A. M., Elmasry, N., & El-Tarabili, R. M. (2022). Prevalence, antimicrobial resistance (AMR) pattern, virulence determinant and AMR genes of emerging multi-drug resistant Edwardsiella tarda in Nile tilapia and African catfish. Aquaculture, 548, 737643. https://doi.org/10.1016/j.aquaculture.2021.737643

Azaldin, M., Syawal, H., & Lukistyowati, I. (2020). Sensitivity of pineapple peel (Ananas comosus) extract against Edwardsiella tarda bacteria. Jurnal Ruaya, 8(1) Hal.53-59. http://download.garuda.kemdikbud.go.id/

Bendjedid, S., Lekmine, S., Tadjine, A., Djelloul, R., & Bensouici, C. (2021). Analysis of phytochemical constituents, antibacterial, antioxidant, photoprotective activities and cytotoxic effect of leaves extracts and fractions of Aloe vera. *Biocatalysis and Agricultural Biotechnology*, *33*, 101991. https://doi.org/https://doi.org/10.1016/j.bcab.2021.101991

Dewi, R., & Marniza, E. (2019). Aktivitas antibakteri gel lidah buaya terhadap Staphylococcus aureus. *Jurnal Saintek Lahan Kering*, *2*(2), 61-62. https://doi.org/10.32938/slk.v2i2.888

Hariati, S., Wahjuningrum, D., Yuhana, M., Tarman, K., Effendi, I., & Saputra, F. (2018). Aktivitas Antibakteri Ekstrak Kapang Laut Nodulisporium sp. KT29 terhadap Vibrio harveyi. *Jurnal Pengolahan Hasil Perikanan Indonesia*, *21*(2), 250-257. https://doi.org/10.17844/jphpi.v21i2.22855

Huslina, F. (2017). Pengaruh ekstrak daun lidah buaya (Aloe vera L.) terhadap pertumbuhan jamur Candida albicans secara in vitro. *BIOTIK: Jurnal Ilmiah Biologi Teknologi dan Kependidikan*, *5*(1), 72-77. http://dx.doi.org/10.22373/biotik.v5i1.2977

Kristianingsih, I., Shoviantari, F. and Novriyanti, F.A. (2021). Uji Aktivitas Sediaan Hydrogel Kombinasi Lidah Buaya (Aloe vera l.) Dan Buah Belimbing Wuluh (averrhoa bilimbi l.) Hal. 28-38. https://prosidingonline.iik.ac.id/index.php/PSHP/article/view/143/135

Maftuch, F.H. Setyawan dan H. Suprastyani. (2018). Uji daya hambat ekstrak Chaetoceros calcitrans terhadap Aeromonas salmonicida. JFMR. 2(1) Hal. 39-46. https://doi.org/10.21776/ub.jfmr.2018.002.01.6

Mulqi, K. R., Silvia, E., Husna, I., & Hamzah, M. S. (2022). Efektivitas Antibiotik Benzoil Peroksida Terhadap Bakteri Propionibacterium Acnes Dengan Metode Difusi Pada Pasien Acne Vulgaris. Medical Profession Journal of Lampung, 12(2), Hal.206-211. https://doi.org/10.53089/medula.v12i2.350

Ogbonne, F. C., Ukazu, E. R., & Egbe, F. C. (2018). Antibiotics resistance pattern and plasmid profiling of Edwardsiella tarda isolated from Heterobranchus longifilis. *Journal of Biosciences and Medicines*, *6*(4), 95-105. 10.4236/jbm.2018.64008

Royani, A., Hanafi, M., Julistiono, H., & Manaf, A. (2022). The total phenolic and flavonoid contents of Aloe vera and Morinda citrifolia extracts as antibacterial material against Pseudomonas aeruginosa. Materials Today: Proceedings. Hal. 2214-7853. https://doi.org/10.1016/j.matpr.2022.06.466

Sari, N., Apridamayanti, P., & Sari, R. (2018). Penentuan Nilai MIC Ekstrak Etanol Kulit Lidah Buaya (Aloe vera Linn) Terhadap Isolat Bakteri Pseudomonas aeruginosa Resisten Antibiotik. *Jurnal Pendidikan Informatika dan Sains*, *7*(2), 219-232. https://doi.org/10.31571/saintek.v7i2.1062

Wardani, R., Jekti, D. S. D., & Sedijani, P. (2019). Uji aktivitas antibakteri ekstrak kulit buah jeruk nipis (citrus aurantifolia swingle) terhadap pertumbuhan bakteri isolat klinis. *Jurnal Penelitian Pendidikan IPA*, *5*(1). https://doi.org/10.29303/jppipa.v5i1.101

Wijaya, I. K. W. A., & Masfufatun, M. (2022). Potensi Lidah Buaya (Aloe vera) sebagai Antimikroba dalam Menghambat Pertumbuhan Beberapa Fungi: Literature Review. Jurnal Kedokteran dan Kesehatan, 18(2), Hal. 202- 211. https://doi.org/10.24853/jkk.18.2.202-211

Yasir, A.S., Marcellia, S., Wijaya, L.B., & Putri, T.R. (2021). formulasi dan uji aktivitas gel kombinasi ekstrak etanol daun lidah buaya (*Aloe Vera*) dan daun kemangi *(Ocinum sanctum L.)* Sebagai Anti Jerawat Terhadap Bakteri Staphylococcus epidermidis. Pharmacoscript. Vol.4(1),70-86. https://doi.org/10.36423/pharmacoscript.v4i1.610