

Antibacterial Activity Test of Papaya Seed Extract (*Carica papaya* L.) on the Growth of *Aeromonas hydrophila*

Uji Aktivitas Antibakteri Ekstrak Biji Pepaya (*Carica papaya* L.) terhadap Pertumbuhan *Aeromonas hydrophila*

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Abstract

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Aeromonas hydrophila is a pathogenic bacterium that can cause systemic disease and mass death. The disease caused by *A. hydrophila* bacteria is MAS (Motile Aeromonas Septicemia). One alternative treatment for *A. hydrophila* infection in fish is papaya seed extract (*Carica papaya* L.). This research was conducted from June 2024 to June 2025 at the Laboratory of Parasites and Fish Diseases, Aquaculture, Faculty of Fisheries and Marine, Universitas Riau. The purpose of this study was to determine the inhibition zone of papaya seed extract, as seen from the inhibition zone formed in inhibiting the growth of *A. hydrophila*, and also to determine the best MIC dose that can inhibit the growth of *A. hydrophila*. As well as to get a safe dose by conducting LD₅₀ tests of papaya seed extract within 24 hours against common carp (*Cyprinus carpio*) with immersion techniques. The research method used was the experimental method, using the Kirby-Bauer disc method and a 6 mm diameter blank disk, with 3 repetitions per treatment. The results of the research indicate that papaya seed extract can inhibit the growth of *A. hydrophila* bacteria at concentrations of 100% - 1%, with an average inhibition zone diameter of 9, 86-6.2 mm. The MIC (Minimum Inhibition Concentration) of papaya seed extract inhibits the growth of *A. hydrophila* at 3.5%, with an average bacterial colony count of 280 CFU/mL. The LD₅₀ value of papaya seed extract against common carp with the immersion technique for 24 hours is at a concentration of 3.73%.

Keywords: Common carp, LD₅₀, MIC

Abstrak

Aeromonas hydrophila adalah jenis bakteri yang bersifat patogen dan dapat menyebabkan penyakit sistemik serta kematian massal. Penyakit yang disebabkan oleh bakteri *A. hydrophila* adalah MAS (*Motile Aeromonas Septicemia*). Salah satu alternatif pengobatan yang dapat mengatasi infeksi *A. hydrophila* yang menyerang ikan adalah ekstrak biji pepaya (*Carica papaya* L.). Penelitian ini dilakukan dari Juni 2024 hingga Juni 2025 di Laboratorium Parasit dan Penyakit Ikan, Budidaya Perikanan, Fakultas Perikanan dan Kelautan, Universitas Riau. Tujuan penelitian ini adalah untuk menentukan zona inhibisi ekstrak biji pepaya yang terlihat dari zona inhibisi yang terbentuk dalam menghambat pertumbuhan *A. hydrophila*, serta untuk menentukan dosis MIC terbaik yang dapat menghambat pertumbuhan *A. hydrophila*. Selain itu, untuk mendapatkan dosis aman dengan melakukan uji LD₅₀ ekstrak biji pepaya dalam 24 jam terhadap ikan mas (*Cyprinus carpio*) dengan teknik perendaman. Metode penelitian yang digunakan adalah metode eksperimental dengan menggunakan metode Kirby-Bauer disc dan disk kosong berdiameter 6 mm dengan 3 ulangan untuk setiap perlakuan. Hasil penelitian yang telah dilakukan dapat disimpulkan bahwa,

ekstrak biji pepaya dapat menghambat pertumbuhan bakteri *A. hydrophila* pada konsentrasi 100-1% dengan diameter rata-rata zona hambat yang terbentuk sebesar 9,86-6,2 mm. Dosis MIC (*Minimum Inhibition Concentration*) ekstrak biji pepaya dalam menghambat pertumbuhan bakteri *A. hydrophila* adalah pada konsentrasi 3,5% dengan rata-rata koloni bakteri 280 CFU/mL. Nilai LD₅₀ ekstrak biji pepaya terhadap ikan mas dengan teknik perendaman selama 24 jam adalah pada konsentrasi 3,73%.

Kata kunci: Ikan mas, LD₅₀, MIC

1. Introduction

Aquaculture businesses often face serious obstacles, including disease outbreaks that can cause production failures. One of the diseases commonly found in freshwater fish is *Aeromonas hydrophila* bacterial infection, known as *Motile Aeromonas Septicemia* (MAS). This infection can affect fish of various sizes, with a high mortality rate of 80-100% (Endang et al., 2018). Clinical symptoms of MAS include peeling skin, red spots on the body, pale gills, bleeding at the anus, and fin damage. This disease can cause common carp mortality in just 1-2 weeks (Kurniaji et al., 2022). The spread of bacteria generally occurs through direct contact between healthy fish and infected fish, and is triggered by high stocking density and poor water quality.

So far, farmers' efforts to control *A. hydrophila*-related diseases have relied on antibiotics. However, long-term use of antibiotics at inappropriate doses can lead to bacterial resistance and negatively impact the aquatic environment (Syawal, 2019). Therefore, safer, environmentally friendly alternatives are needed, one of which is the use of natural materials as antibacterial sources.

Papaya (*Carica papaya* L.) is widely found in Indonesia, and its seeds contain various bioactive compounds, including alkaloids, saponins, flavonoids, terpenoids, and tannins, with potential antibacterial activity. These compounds can damage bacterial cell membranes and inhibit pathogen growth (Sukadana & Santi, 2020). Previous research also showed that papaya seed extract can inhibit the growth of several pathogenic bacteria, such as *Escherichia coli* and *Staphylococcus aureus* (Ristianti et al., 2015). The purpose of this study was to determine the inhibition zone produced by papaya seed extract (*C. papaya* L.) against the growth of *A. hydrophila*; to obtain the best MIC dose that inhibits the growth of *A. hydrophila*; and to obtain a safe dose for fish by conducting the LD₅₀ test of papaya seed extract within 24 hours against common carp (*Cyprinus carpio*) with immersion technique.

2. Material and Method

2.1. Time and Place

This research was conducted from October 2024 to June 2025 at the Laboratory of Fish Parasites and Diseases, Faculty of Fisheries and Marine, Universitas Riau.

2.2. Methods

The research method is the experimental method, using the KIRBY-BAUER disc method with a blank disk of 6 mm diameter, and each treatment is repeated 3 times. The Kirby-Bauer method is an antimicrobial test included in the diffusion method. This method is to determine microbial activity. Disc blanks containing antibacterial substances are placed on the surface of agar media that has grown *A. hydrophila*. The clear zone formed indicates the inhibition by antimicrobials contained in papaya seeds, as for the treatment as follows:

| | | |
|--------------------------------|-------------------------------|------------------------------|
| P0 : Control (Oxytetracycline) | P6 : Papaya Seed Extract 50% | P13 : Papaya Seed Extract 7% |
| P1 : Papaya Seed Extract 100% | P7 : Papaya Seed Extract 40% | P14 : Papaya Seed Extract 6% |
| P2 : Papaya Seed Extract 90% | P8 : Papaya Seed Extract 30% | P15 : Papaya Seed Extract 5% |
| P3 : Papaya Seed Extract 80% | P9 : Papaya Seed Extract 20% | P16 : Papaya Seed Extract 4% |
| P4 : Papaya Seed Extract 70% | P10 : Papaya Seed Extract 10% | P17 : Papaya Seed Extract 3% |
| P5 : Papaya Seed Extract 60% | P11 : Papaya Seed Extract 9% | P18 : Papaya Seed Extract 2% |
| | P12 : Papaya Seed Extract 8% | P19 : Papaya Seed Extract 1% |

2.3. Procedures

2.3.1. Preparation of Papaya Seed Extract

Ripe papaya seeds are dried by drying in the sun for 3-4 days until completely dry, then mashed into a powder. A total of 1.5 kg of simplisia from 10 kg of wet seeds was then extracted by the maceration method using 96% ethanol at a 1:3 ratio. The maceration was carried out in a dark glass bottle for 3 days at room temperature with

periodic stirring. Filtering was carried out every 24 hours until a clear filtrate was obtained. The collected filtrate was then evaporated using a rotary evaporator at 40°C to yield a thick, brown extract (Sukadana & Santi, 2020).

2.3.2 Preparation of *A. hydrophila* Bacteria Isolate

Aeromonas hydrophila used is an isolate of the collection of Parasites and Fish Diseases Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Riau. Isolates were cultured on TSA media and incubated 18-24 hours at 28-30°C, then transferred to GSP media and incubated 18-20 hours. Changes in the colour of GSP media from red to yellow indicate *Aeromonas* growth. Furthermore, the bacteria were re-cultured on TSA media and tested physically and biochemically, including catalase, O/F, indole, motility, H₂S, and gas tests. After the test results showed suitability, the bacteria were used to make an inoculum.

Inoculum is prepared by taking 1 loopful of *A. hydrophila* culture and growing it in a test tube containing 10 ml TSB media. The test tube is then homogenized using a vortex for 2 minutes and then incubated in an incubator for 24 hours. After 24 hours, the bacteria were ready for activity and MIC tests using papaya seed extract solution.

2.3.3. Activity Test of Papaya Seed Extract and MIC Test

Observation of the zone of inhibition of papaya seed extract against *A. hydrophila* was performed by the Kirby-Bauer disc method using a 6 mm diameter blank disk. 100 µL of bacterial suspension from TSB media was spread evenly on TSA media, and a blank disk soaked in 50 µL of papaya seed extract at the treatment concentration was placed on it. The samples were incubated for 18-24 hours, and the inhibition zone formed around the disk was observed, and its diameter was measured using a calliper (Kumara et al., 2021).

The Minimum Inhibitory Concentration (MIC) test was conducted to determine the minimum dose of papaya seed extract that could inhibit the growth of *A. hydrophila*. The test dose was determined from previous antibacterial activity results, then diluted and tested using the turbidity method on TSB media with 18-24 hours of incubation. Furthermore, a sensitivity test was performed by soaking a blank disk in the test solution at the specified dose, placing it on TSA medium, and incubating for 18-24 hours at 28-30 °C to observe the inhibition zone. The TPC test was also carried out by growing a mixture of extracts and a bacterial inoculum on TSA for 18-24 hours, then counting the colonies using a colony counter. The results of the calculation indicated that bacterial colonies ranging from 250 to 300 were used to determine the MIC value.

2.3.4. Toxicity Test of LD₅₀ to Common Carp

The LD₅₀ toxicity test was conducted using common carp measuring 8-12 cm in length, with 10 fish per aquarium (40 × 30 × 30 cm). The treatments consisted of doses of papaya seed extract at 5%, 4.5%, 4%, 3.5%, and 3%, as well as a no-dose control, each repeated three times, for a total of 18 containers. Each aquarium contained 10 L of water to which the appropriate dose of extract was added. The test fish were then introduced to observe their behaviour and mortality for 24 hours. This acute toxicity test aims to detect the toxic effects of a compound by measuring fish mortality (Sulastra et al., 2020). Mortality data were tabulated and calculated using the Reed & Muench (1938) method to determine the LD₅₀ value.

2.4. Data Analysis

Data obtained during the study, namely the inhibition zone and MIC tests, were tabulated and analysed descriptively. While the LD (50) (Lethal Dose 50) toxicity test was analysed using the method of Ramadhi (2019).

3. Result and Discussion

3.1. Activity Test of Papaya Seed Extract (*C. papaya* L.) against *A. hydrophila*

A clear zone of inhibition around the disk indicates antibacterial activity. The papaya seed extract activity test was conducted to determine its ability to inhibit the growth of pathogenic bacteria. The diameter of the inhibition zone for papaya seed extract is shown in Table 1. The antibacterial test results showed that the positive control, in the form of the Oxytetracycline antibiotic, produced an average inhibition zone diameter of 19.06 mm and was classified as very strong. This value was higher than that of papaya seed extract, indicating that the antibiotic inhibition was more effective against *A. hydrophila*. The mechanism of action of Oxytetracyclin is to inhibit protein synthesis by binding to the 30S ribosomal subunit of bacteria (Dewi et al., 2019), whereas papaya seed extract acts through secondary metabolites such as alkaloids, flavonoids, and saponins, whose activity is relatively weaker.

Papaya seed extract treatment at 100% concentration produced the largest inhibition zone (9.86 mm), while 5% concentration produced a 6.53 mm zone. This shows that the higher the extract concentration, the larger the inhibition zone, because more bioactive compounds increase bacterial sensitivity. Based on the classification of Yanti & Mitika (2017), papaya seed extract at 100% concentration with a diameter of 9.86 mm and at 1% concentration with a diameter of 6.2 mm is included in the medium potential category. This shows that, although less potent than antibiotics, papaya seed extract still exhibits antibacterial activity against *A. hydrophila*.

Table 1. Observation Results of the Inhibition Zone of Papaya Seed Extract (*C. papaya* L.) against *A. hydrophila*

| Concentration of papaya seed extract solution (%) | Zone of Inhibition (mm) in each replicate | | | Average (mm) |
|---|---|------|------|--------------|
| | I | II | III | |
| K (Oxytetracycline) | 19.2 | 19.1 | 18.9 | 19.06 |
| 100 | 10.0 | 9.8 | 9.8 | 9.86 |
| 90 | 9.5 | 9.2 | 9.3 | 9.33 |
| 80 | 9.0 | 8.7 | 8.9 | 8.86 |
| 70 | 8.8 | 8.5 | 8.3 | 8.53 |
| 60 | 8.5 | 8.2 | 7.9 | 8.20 |
| 50 | 8.3 | 7.9 | 7.4 | 7.86 |
| 40 | 7.8 | 7.7 | 7.2 | 7.56 |
| 30 | 7.7 | 7.3 | 7.1 | 7.36 |
| 20 | 7.4 | 7.1 | 7.0 | 7.16 |
| 10 | 7.0 | 7.0 | 6.9 | 6.96 |
| 9 | 6.9 | 6.9 | 6.8 | 6.86 |
| 8 | 6.9 | 6.8 | 6.8 | 6.83 |
| 7 | 6.8 | 6.7 | 6.6 | 6.70 |
| 6 | 6.7 | 6.7 | 6.5 | 6.63 |
| 5 | 6.6 | 6.6 | 6.4 | 6.53 |
| 4 | 6.5 | 6.5 | 6.4 | 6.46 |
| 3 | 6.4 | 6.3 | 6.3 | 6.33 |
| 2 | 6.3 | 6.3 | 6.3 | 6.30 |
| 1 | 6.2 | 6.2 | 6.2 | 6.20 |

The concentration of the extract influences variations in the diameter of the inhibition zone, the content of secondary metabolites, and the mechanism of action of the active substances. These factors synergistically determine the effectiveness of papaya seed extract in inhibiting *A. hydrophila* growth. The results of the antibacterial test of papaya seed extract against *A. hydrophila* are shown in Figure 1.

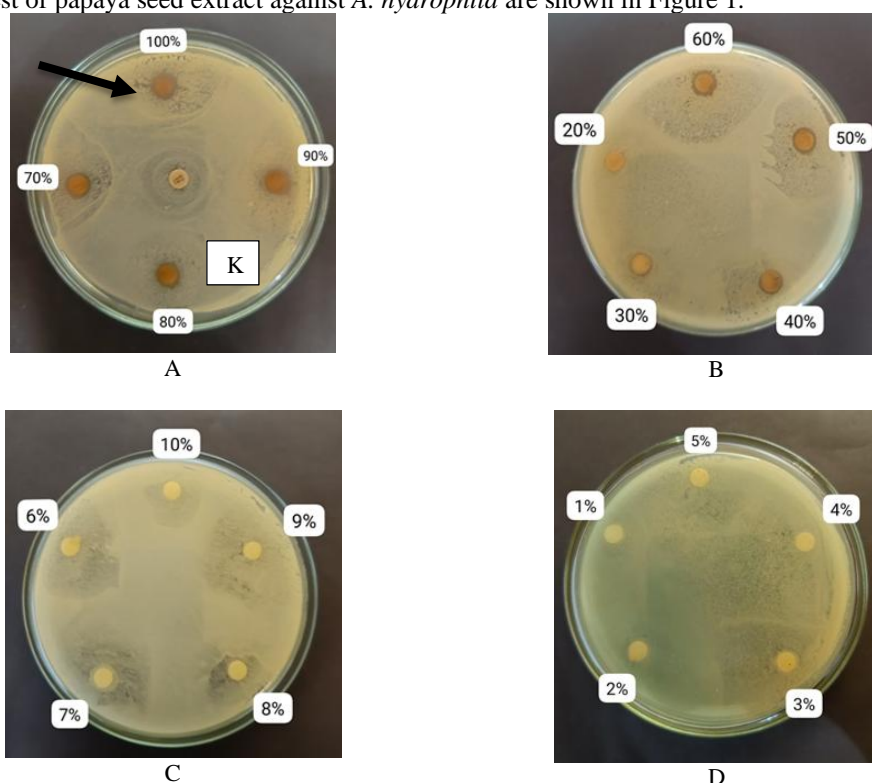
Figure 1. Antibacterial Test Results of Papaya Seed Extract against *A. hydrophila*

Figure 1 shows that the higher the dose of papaya seed extract used, the larger the inhibition zone formed. Conversely, the lower the treatment dose, the smaller the inhibition zone formed. This aligns with the opinion of (Hasanuddin & Salnus, 2020), which states that the higher the concentration of an antimicrobial substance, the stronger the inhibition, leading to more bacteria being killed or their growth being inhibited. The zone of inhibition around the disc paper on the media indicates that papaya seed extract has antibacterial activity against *A. hydrophila*. Treatment at high concentrations (100% - 50%) produces a clearer inhibition zone than at medium to low concentrations (40% - 6%), which tend to be only bacteriostatic, inhibiting growth but not killing bacteria.

The pattern of inhibition zone diameter in the 100% to 1% treatment showed a dose-response relationship, where high concentrations produced a larger inhibition zone diameter than low concentrations. This indicates that papaya seed extract exhibits antibacterial activity against *A. hydrophila*, although the level of effectiveness varies. Large zones of inhibition, such as at 100% concentration, indicate that the bacteria are sensitive to the treatment. In contrast, small zones of inhibition at low concentrations indicate low effectiveness or a tendency towards resistance. Differences can also influence variations in the size of the zone of inhibition, the ability of the extract to diffuse into the agar medium, and the content of active compounds at each concentration.

The antibacterial activity of papaya seed extract is influenced by dose-dependent levels of secondary metabolites, including alkaloids, flavonoids, tannins, saponins, steroids, and triterpenoids. Alkaloids inhibit the formation of peptidoglycan in the bacterial cell wall, so the cell wall is not formed intact, leading to cell death. Flavonoids can form complexes with extracellular proteins, damage the bacterial cell membrane, and trigger the release of intracellular compounds. Tannins can precipitate bacterial cell wall proteins and disrupt cell metabolism (Nurhalimah et al., 2015). In addition, saponins, steroids, and triterpenoids are known to denature proteins and damage cell membranes, thereby inhibiting and even killing bacteria (Ahmad et al., 2023). Thus, the bioactive compounds in papaya seed extract synergistically inhibit the growth of *A. hydrophila*.

3.2. MIC (Minimum Inhibitory Concentration) Test of Papaya Seed Extract against the Growth of *A. hydrophila*

The MIC (Minimum Inhibitory Concentration) test is conducted to determine the lowest dose that inhibits bacterial growth, as evidenced by absorbance and colour changes in the media (Hasanuddin & Salnus, 2020). The MIC test was performed using the Turbidity method, and the Total Plate Count (TPC) test was performed. The concentration tested was based on the results of the sensitivity test of papaya seed extract at 5%-3%. MIC test results for the Turbidity method, using papaya seed extract against *A. hydrophila* are shown in Figure 2.



Figure 2. MIC Test Results of Papaya Seed Extract against *A. hydrophila* with Various Doses

The turbidity method was used to assess media turbidity as an indicator of *A. hydrophila* growth, with the control (K) containing no extract. Based on Figure 2, the control media is more turbid than the 5%-4% treatment. This shows that papaya seed extract contains antibacterial compounds that inhibit the growth of *A. hydrophila*, making the media clearer. In contrast, in the 3.5% and 3% treatments, the media appeared almost identical to the control, indicating that these extract concentrations did not inhibit bacterial growth. Thus, the treatment that produces clear media is designated as MIC (Minimum Inhibitory Concentration). According to Najiya et al. (2022), MIC is the lowest concentration of an antimicrobial that can inhibit the growth of microorganisms after 24 hours of incubation.

Furthermore, Total Plate Count (TPC) testing was performed to determine the number of viable bacterial colonies after papaya seed extract treatment. The principle of this method is that living microbial cells will grow on agar medium and form colonies that can be counted directly by eye without a microscope (Angelia, 2020). The TPC test results for papaya seed extract against *A. hydrophila* are shown in Figure 3.

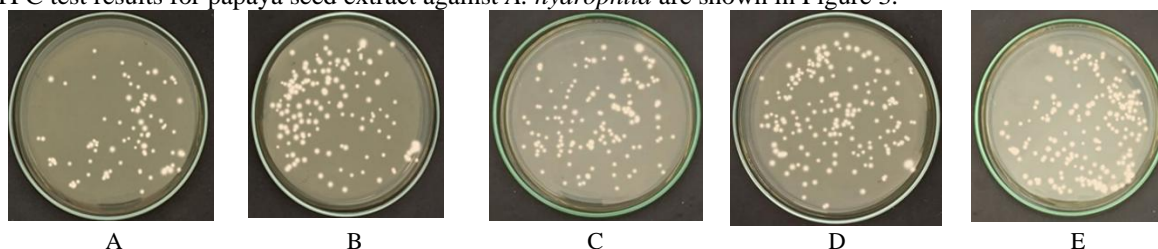


Figure 3. TPC (Total Plate Count) Test Results of Papaya Seed Extract against *A. hydrophila*

Based on Figure 3, the higher the concentration of papaya seed extract, the lower the density of bacterial colonies in the media. This can be seen in the comparison of the density of bacterial colonies at 5% with that at 3%, which is very dense, so that one colony merges with another. This is because papaya seed extract contains flavonoids, saponins, tannins, and alkaloids. Flavonoids and tannin compounds have antibacterial effects. For more details, the MIC test results of papaya seed extract against *A. hydrophila* bacteria are presented in Table 2.

Table 2. MIC Test of the Calculation of the Number of Colonies of Papaya Seed Extract against *A. hydrophila*

| Concentration (%) | Turbidity | Number of Colonies | | | Average Colonies (CFU/mL) |
|-------------------|-----------|--------------------|-----|-----|---------------------------|
| | | I | II | III | |
| 5,0 | + | 94 | 93 | 89 | 92,00 |
| 4,5 | ++ | 172 | 177 | 165 | 171,33 |
| 4,0 | +++ | 227 | 231 | 225 | 227,66 |
| 3,5 | ++++ | 280 | 284 | 278 | 280,66 |
| 3,0 | +++++ | 324 | 330 | 327 | 327,00 |
| 0 | | ∞ | ∞ | ∞ | ∞ |

Based on Table 2, the results of the *A. hydrophila* colony count calculation in papaya seed extract showed a difference in colony count between treatments. At a dose of 3.5%, the average number of colonies obtained was 280 CFU/mL, while at a dose of 3%, the number of colonies increased to 327 CFU/mL. The number of colonies is still within the range that can be calculated reliably, 30-300 CFU/mL (Azaldin et al., 2020). These results indicate that higher concentrations of papaya seed extracts are more effective at suppressing *A. hydrophila* growth.

The administration of papaya seed extract at various doses proved to affect the number of *A. hydrophila* colonies. This is because the higher the extract concentration, the higher the content of active antibacterial compounds. These active compounds can disrupt metabolism and damage bacterial cell structure, thereby inhibiting growth. The greater the concentration of an extract, the greater the amount of solute it contains, including bioactive compounds such as alkaloids, flavonoids, tannins, and saponins, which play an important role in antibacterial mechanisms (Weni et al., 2024).

3.3. LD₅₀ Value (Lethal Dose50) of Papaya Seed Extract against Common Carp (*C. carpio*)

The toxicity test of papaya seed extract was conducted to determine the dose that can cause 50% mortality (LD₅₀) within 24 hours in the test fish. The fish used were common carp fry with 30 fish per container. The treatment doses correspond to the MIC test results: 3%, 3.5%, 4%, 4.5%, and 5%, along with the control without extract. Common carp were chosen as test animals because they are sensitive to changes in water quality and dissolved compounds, enabling them to show clear responses to environmental influences. The results of the toxicity test observations are tabulated and presented in Table 3.

Table 3. Calculation results of LD₅₀ determination according to Reed & Muench (1938) for 24 hours.

| Concentration of papaya seed extract (%) | Dead (fish) | Live (fish) | Accumulation (Tails) | | | Ratio % | | LD ₅₀ |
|--|-------------|-------------|----------------------|-------------|--------------|-----------|-----------|------------------|
| | | | Dead (fish) | Live (fish) | Total (fish) | Mortality | Mortality | |
| Control | 0 | 30 | 0 | 89 | 89 | 0/89 | 0 | } 3,73 |
| 3,0 | 6 | 24 | 6 | 59 | 65 | 6/65 | 9 | |
| 3,5 | 13 | 17 | 19 | 35 | 54 | 19/54 | 35 | |
| 4,0 | 18 | 12 | 37 | 18 | 55 | 37/55 | 67 | |
| 4,5 | 24 | 6 | 61 | 6 | 67 | 61/67 | 91 | |
| 5,0 | 30 | 0 | 91 | 0 | 91 | 91/91 | 100 | |

Based on Table 3, the LD₅₀ calculated using the Reed & Muench (1938) method for 24 hours is 3.73%. This value indicates that papaya seed extract is not toxic to common carp at concentrations below 3.73%. The highest mortality was at 5% (91%), while the lowest was at 3%. This pattern indicates that the higher the extract concentration, the faster the active substance reaches the fish's body, leading to increased mortality. Conversely, at low concentrations, contact occurs more slowly, so fish mortality is lower and takes longer. The death of common carp in the papaya seed extract treatment is thought to be caused by toxic active compounds. According to Susilowati & Farfar (2019), one of the mechanisms of fish death due to exposure to bioactive compounds is the inhibition of cholinesterase activity in the respiratory muscles that move the operculum and gills. This condition causes disruption of respiration and osmoregulation, which leads to death.

In addition, observations showed changes in common carp behavior during the toxicity test. Symptoms include stress, abnormal swimming, frequent surfacing, and gasping movements before dying. The behavioral pattern of common carp during 24 hours of immersion in papaya seed extract is shown in Figure 4. The comparison of clinical symptoms of common carp before and after the LD₅₀ test is presented in Table 4.

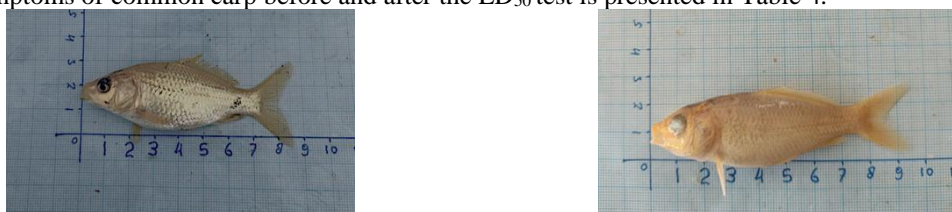
Figure 4. Clinical Symptoms of Common Carp Before and After the LD₅₀

Table 4. Observation of Fish Clinical Symptoms Before and After the LD50

| Fish before LD ₅₀ test | Common Carp after LD ₅₀ test |
|---|---|
| Bright eyes | Pale eyes |
| Bright body color | Pale body color and bloated abdomen |
| There is no wound on the body of the fish | There is excessive mucus |
| | Fish swim to the surface. |

Visual observations during the study showed changes in common carp behaviour, including swimming patterns toward the surface with abnormal movements, increased mucus production, and a change in body colour to pale. These clinical symptoms are thought to be caused by toxic secondary metabolites in papaya seed extract, such as alkaloids and saponins. According to Sari et al. (2021), high levels of alkaloids can be toxic to fish. Saponins are toxic to poikilotherms because they can cause red blood cell hemolysis. This hemolysis is thought to occur in the gills, thus disrupting respiratory function and leading to death. Alkaloid compounds themselves are known to have a bitter taste, are toxic, and in high levels can cause physiological disorders or death (Lumowa, 2011). In addition, alkaloids can reduce the number of leukocytes, especially granulocytes, which affects the immune system of fish (Wahyuni, 2013).

In addition to the direct influence of secondary metabolites, changes in fish behaviour can also be triggered by aquatic environmental conditions. The high concentration of extract dissolved in the test media is thought to inhibit the diffusion of oxygen from the air into the water, thereby reducing dissolved oxygen (DO) levels. According to Pujananto (2020), low DO levels force fish to increase respiratory flow by pumping water through the gills more frequently. This condition increases the likelihood that toxins from extracts are absorbed through the gill tissue. As a result, fish experience hypoxia, which disrupts metabolism, growth, and other physiological activities such as swimming and reproduction.

4. Conclusions

Based on the study's results, it can be concluded that papaya seed extract inhibits the growth of *A. hydrophila* at concentrations of 100%-1%, with an average inhibition zone diameter of 9.86-6.2 mm. The minimum inhibitory concentration (MIC) of papaya seed extract against *A. hydrophila* was 3.5%, with an average colony count of 280 CFU/mL. Meanwhile, the LD₅₀ value of papaya seed extract against common carp, determined by immersion for 24 hours, was 3.73%.

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