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Antibacterial Activity Test of *Citrus jambhiri* L to inhibit the Growth of *Aeromonas hydrophila*

Uji Aktivitas Antibakteri dari Perasan Asam Jungga (Citrus jambhiri L.) untuk Menghambat Pertumbuhan Aeromonas hydrophila

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Abstract

Jungga acid (*Citrus jambhiri* L.) contains compounds such as limonene, flavonoids, and essential oils that have potential as natural antibacterial agents. This study was conducted from September 2024 to June 2025 at the Parasite and Fish Disease Laboratory, Faculty of Fisheries and Marine, Universitas Riau. The objective of this study was to determine the antibacterial activity of jungga lime against *Aeromonas hydrophila* and to determine the MIC (Minimum Inhibitory Concentration). The methods used were experimental, employing the disk diffusion technique (Kirby-Bauer) to measure the clear zone and dilution methods (turbidity test and total plate count) to determine the MIC. The treatments consisted of jungga lime concentrations ranging from 100% to 1%, with a positive control using the antibiotic Oxytetracycline. Each treatment was repeated three times. The results showed that Jungga lime could inhibit the growth of *A. hydrophila* at concentrations ranging from 100% to 1%, with clear zone diameters of 13.93 mm to 6.56 mm. The best MIC value for inhibiting the growth of *A. hydrophila* was 0.6% (285 CFU/mL). It can be concluded that Jungga lime has potential as a natural antibacterial agent for controlling diseases in farmed fish.

Keywords: *Citrus jambhiri* L., Antibacterial, MIC

Abstrak

Asam jungga mengandung senyawa seperti limonene, flavonoid, dan minyak esensial yang berpotensi sebagai agen antibakteri alami. Penelitian ini dilakukan dari September 2024 hingga Juni 2025 di Laboratorium Parasit dan Penyakit Ikan, Fakultas Perikanan dan Kelautan, Universitas Riau. Tujuan penelitian ini adalah untuk menentukan aktivitas antibakteri jeruk jungga dalam menghambat pertumbuhan bakteri *Aeromonas hydrophila* serta menentukan nilai MIC (*Minimum Inhibitory Concentration*). Metode yang digunakan adalah metode eksperimental dengan teknik difusi cakram (Kirby-Bauer) untuk mengamati zona jernih, serta metode pengenceran (uji kekeruhan dan penghitungan total koloni) untuk menentukan nilai MIC. Perlakuan terdiri dari konsentrasi asam jungga berkisar antara 100% hingga 1%, dengan kontrol positif menggunakan antibiotik Oxytetracycline. Setiap perlakuan diulang tiga kali. Hasil menunjukkan bahwa asam jungga dapat menghambat pertumbuhan *A. hydrophila* pada konsentrasi berkisar antara 100% hingga 1%, dengan diameter zona jernih 13,93 mm hingga 6,56 mm. Nilai MIC terbaik untuk menghambat pertumbuhan *A. hydrophila* diperoleh pada konsentrasi 0,6% (285 CFU/mL). Dapat disimpulkan bahwa asam jungga memiliki potensi sebagai agen antibakteri alami dalam mengendalikan penyakit pada ikan budidaya.

Kata kunci: *Citrus jambhiri* L., Antibakteri, MIC

1. Introduction

Fish farming has strong market prospects, as domestic and non-domestic demand continues to increase. To meet these needs, fish farmers must implement intensive farming systems to increase productivity on limited land. Intensive farming systems are characterized by high stocking densities that can significantly increase production. This was stated by Fahmi et al. (2023), who explained that intensive systems allow the use of narrow land to produce multiple yields. However, intensification also increases stress on fish and reduces the quality of the media, thereby increasing the likelihood of disease emergence. Fish disease results from complex interactions among host conditions, pathogen presence, and suboptimal water quality. One of the diseases that often attacks aquaculture commodities is MAS (*Motile Aeromonas Septicemia*) caused by *Aeromonas hydrophila*. This bacterium can cause bleeding symptoms and lead to mortality rates of 50–100% within 1–2 weeks (Sinubu et al., 2022).

Efforts to control fish disease have thus far relied on antibiotics such as oxytetracycline, erythromycin, streptomycin, and kanamycin. However, the continuous use of antibiotics has given rise to new problems, namely pathogen resistance, environmental pollution, and the risk of residues that threaten consumer health. According to Azhar et al. (2022), the effectiveness of antibiotics is declining due to these impacts. Therefore, safer, more environmentally friendly treatment alternatives are needed, including the use of herbal ingredients as natural antibacterials.

One plant that has antibacterial potential is the jungga fruit (*Citrus jambhiri* L.). Baymolo (2002) states that the jungga fruit is similar to kaffir lime but has a different aroma. According to Hamdan et al. (2013), jambhiri essential oil has anti-inflammatory, antitumor, antibacterial, and antifungal activities. Limonene is the major component of fresh fruit peel essential oil, accounting for 84.5%. Given these biological activities, zone inhibition and MIC tests are important steps in assessing jambhiri's ability to inhibit *A. hydrophila*. However, research on the use of *C. jambhiri* L. against these two bacteria remains limited, so this study is needed to evaluate its potential as an antibacterial herbal ingredient in fish farming. The objectives of this study are to obtain the inhibition zone of jungga acid (*C. jambhiri* L.) against *A. hydrophila* and to determine the MIC dose that can inhibit the growth of *A. hydrophila*

2. Material and Method

2.1. Time and Place

This research was conducted from September 2024 to January 2025 at the Parasite and Fish Disease Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Riau.

2.2. Methods

The research method used was an experimental method with the Kirby-Bauer disc diffusion technique using 6 mm diameter disc blanks. Each treatment was repeated three times. In this method, disc blanks that had been treated with jungga acid solution were placed on the surface of agar media that had been colonised by *A. hydrophila*. The clear zone that appeared around the disc indicated the antibacterial activity of citric acid against the two test bacteria:

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

2.3. Procedures

2.3.1. Preparation of Jungga Acid Extract

Sour jungga fruit is produced by preparing ripe, greenish-yellow fruit, weighing it, washing it with running water, and drying it for 15 minutes. The clean fruit is cut lengthwise, squeezed through gauze, and rinsed with distilled water. The juice is then filtered again using Whatman No. 42 filter paper. From 100 g of fruit, approximately 36.8 mL of initial juice is obtained, and after filtering, 20 mL of solution is obtained. The filtered juice is collected in an Erlenmeyer flask, covered with aluminium foil, and then diluted as needed for sensitivity testing and MIC testing.

2.3.2 Preparation of A.hydrophila Bacteria Isolate

Inoculum preparation was carried out by taking one colony of *A. hydrophila* from the selective media and inoculating it into a test tube containing 10 mL of TSB. The tube was vortexed for two minutes to ensure homogeneity, then incubated for 24 hours. After incubation, the bacterial culture is ready for use in antibacterial activity tests and MIC tests of jungga acid.

2.3.3. Activity Test of Citric Acid and MIC Test

The inhibition zone test of *C.jambhiri* L. extract against *A.hydrophila* was conducted using the Kirby-Bauer disc method with 6 mm diameter blank discs. Fifty microliters of bacterial suspension from TSB medium was taken, then dropped onto the surface of TSA medium and spread using a glass spreader. Blank discs that had been dipped in a citric acid solution for 1 minute according to the treatment dose were placed on TSA media inoculated with bacteria. The procedure was carried out separately for both bacteria, and all dishes were incubated for 18–24 hours. After incubation, the inhibition zones formed around the blank discs were observed, and their diameters were measured using calipers.

The MIC test was conducted to determine the minimum concentration of tamarind juice required to inhibit the growth of *A. hydrophila*. The test dose was determined based on the results of previous activity tests, namely, from the dose that produced the smallest inhibition zone to the dose that did not produce an inhibition zone. The doses were diluted, and 50 µL of each was added to 9 mL of TSB medium. 1 mL of *A. hydrophila* suspension was added, vortexed, and incubated for 18–24 hours. After incubation, observations were made based on the level of media turbidity.

In addition, a mixture of *C.jambhiri* L and 100 µL of bacterial inoculum was also planted on the surface of TSA medium using a glass spreader and incubated for 18–24 hours. The colonies that grew were counted using a colony counter, with the stipulation that the number of colonies should not exceed 300 to maintain data accuracy. The concentration that produced a colony count of 250–300 was determined to be the Minimum Inhibitory Concentration.

2.4. Data Analysis

The data obtained during the study, namely the inhibition zone and MIC (Minimum Inhibitory Concentration) values of Jungga acid against *A. hydrophila*, were tabulated and analysed descriptively.

3. Result and Discussion

3.1. Biochemical Test of A.hydrophila

The bacteria used in the study were tested through a series of biochemical tests to confirm their identity and distinguish between species. Biochemical testing is important because it can reveal the physiological and metabolic characteristics of bacteria through specific reactions to various chemical substrates, thereby improving identification accuracy. The results of the biochemical testing for *A. hydrophila* are shown in Table 1.

Table 1. *Aeromonas hydrophila* Biochemical Test Results

	Biochemical Tests					
Morphology	Motility	Oxidase	Catalase	O/F	Gram	Species
Short rods	+	+	+	F	-	*A.hydrophila*

Table 1 shows that the bacteria are *A. hydrophila*, characterised by positive motility, positive oxidase, positive catalase, O/F (fermentative OF), and short rod morphology. These results are consistent with the identification made by Sri (2016), who stated that the genus *Aeromonas* is Gram-negative, catalase-positive, oxidase-positive, and fermentative.

3.2. Activity of Jungga Acid

The presence of an inhibition zone, a clear area around the disc, indicates antibacterial activity. Diameter measurements can be obtained by forming a clear zone around the disc paper. The positive control, oxytetracycline, showed the largest average inhibition zone diameter of 19.53 mm (Yanti & Mitika, 2017). This value is higher than all concentrations of tamarind juice, confirming that oxytetracycline is more effective in inhibiting the growth of *A. hydrophila*. Jungga acid extract at 100% produced an inhibition zone of 13.93 mm (strong category), while 1% produced 6.56 mm (moderate category), indicating that higher concentrations of the solution correlate with greater antibacterial activity (Figure 1). The diameter of the inhibition zone increases with increasing jungga acid concentration. The inhibition zone formed around the disc indicates the diffusion of active compounds from the tamarind juice into the medium, thereby inhibiting the growth of *A. hydrophila*. The size of the inhibition zone is a direct indicator of a compound's antibacterial activity (A'yunin et al., 2020).

The antibacterial activity of tamarind juice is associated with its secondary metabolite content, including essential oils and bioactive compounds such as limonene, eugenol, thymol, carvacrol, and citral. These lipophilic compounds can damage the structure of Gram-negative bacterial cell membranes, including the lipopolysaccharide (LPS) layer in *A. hydrophila*, leading to leakage of cell contents and disruption of membrane function (Muslikha et al., 2016). This damage is also described by Haque et al. (2021), who state that phenolic compounds disrupt membrane integrity, inhibit nutrient transport, and inactivate enzymes, thereby inhibiting bacterial growth. This shows the relationship between tamarind juice concentration and the increase in the diameter of the inhibition zone.

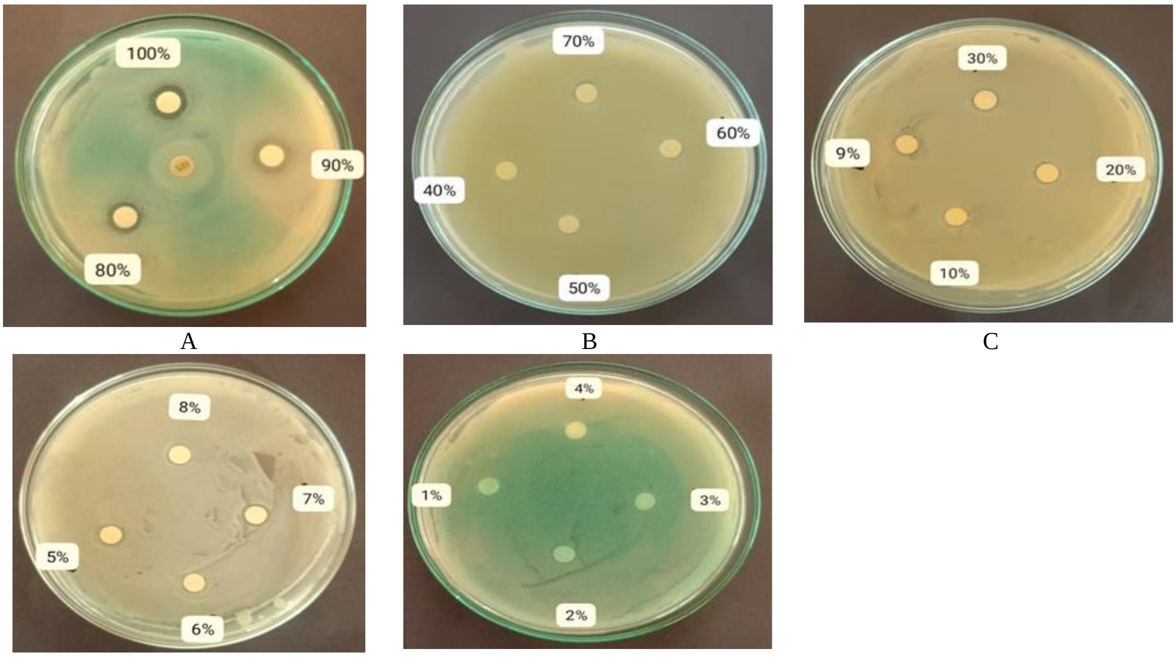

Figure 1. Results of Antibacterial Testing of Jungga (*C. jambhiri* L.) against *Aeromonas hydrophila*

3.3. MIC Test of Jungga Acid (C. jambhiri L.) against A. hydrophila

The MIC (Minimum Inhibitory Concentration) test was conducted to determine the minimum concentration of tamarind juice required to inhibit the growth of *A. hydrophila*. The test was conducted using concentrations derived from previous sensitivity tests, namely 0.9%–0.5%, the lowest concentration with an inhibition zone. The methods used in the MIC test included the turbidity test (observation of turbidity) and the Total Plate Count (TPC) test to confirm the presence or absence of bacterial growth. The results of the Turbidity method MIC test for *A. hydrophila* are shown in Figure 3.

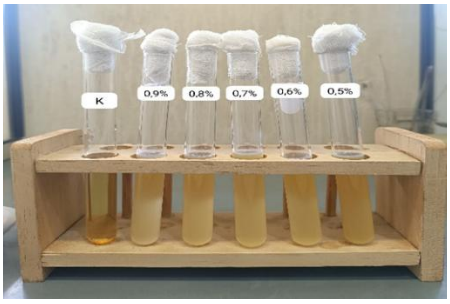

Figure 3. MIC Test Results using the Turbidity Method of Jungga Acid Extract (*C. jambhiri* L.) against *A. hydrophila*

The turbidity method in the MIC test is performed by visually assessing the solution's turbidity; a clearer solution indicates stronger inhibitory activity against bacteria. Based on Figure 3, the 0.9% concentration appears to be the clearest and closest to the control medium (K) without bacteria, while the 0.8%, 0.7%, 0.6%, and 0.5% concentrations appear to be more turbid. The turbidity at these concentrations indicates that the antibacterial activity is insufficient to inhibit *A. hydrophila* growth. This is in line with Azaldin et al. (2020), which states that the lower the concentration of an antibacterial agent, the closer the medium turbidity is to that of the positive control containing bacteria. The results of the MIC test for the number of colonies of *Citrus jambhiri* L. juice against *A. hydrophila* are shown in Table 2.

Based on Table 2, a 0.5% concentration of tamarind juice did not inhibit the growth of *A. hydrophila*. This concentration was still too low, so that the number of bacterial colonies that grew exceeded the ideal limit for calculation. Conversely, at a concentration of 0.6% and an average of 285 CFU/mL, jungga acid has shown a minimum inhibitory concentration (MIC), as the number of colonies falls within the range of 30-300 CFU/mL. This inhibitory effect is related to citric acid's natural antibacterial properties, especially its essential oils and limonene, which can suppress bacterial activity. The standard deviation in the MIC results also indicates how consistent the bacterial response is across test repetitions; a low standard deviation indicates that the antimicrobial inhibitory power is stable across repetitions.

Table 2. MIC Test Results for the Number of Colonies of *C. jambhiri* L. juice against *A. hydrophila*

Concentration (%)	Turbidity	Number of Colonies			Average Colonies (CFU/mL)
		I	II	III	
0.9	+	107	112	109	109,33±2.516[a]
0.8	+ +	198	202	192	197,33±5.033[b]
0.7	+ + +	221	225	223	245,33±2.000[c]
0.6	+ + + +	287	288	282	285,33±3.214[d]
0.5	+ + + + +	336	328	331	331,67±4.041[e]
K	∞	∞	∞	∞	∞

Description: ∞ = infinite number of colonies growing, + = Clear, ++ = Slightly cloudy, +++ = Slightly cloudy, ++++ = Cloudy, +++++ = Very cloudy

4. Conclusions

The results of this study indicate that Jungga acid (*C. jambhiri* L.) can inhibit the growth of *A. hydrophila* at concentrations ranging from 100% to 1%, with average inhibition zones of 13.93-6.56 mm. The MIC value of citric acid juice against *A. hydrophila* was obtained at a concentration of 0.6% with a colony count of 285 CFU/mL. These results indicate that Jungga acid has potential as a natural antibacterial agent against *A. hydrophila*.

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