Prisma Sains: Jurnal Pengkajian Ilmu dan Pembelajaran Matematika dan IPA IKIP Mataram
http://ojs.ikipmataram.ac.id/index.php/prismasains/index
e-mail: prismasains.pkpsm@gmail.com

April 2026. Vol. 14, No. 2
p-ISSN: 2338-4530
e-ISSN: 2540-7899
pp. 876-890

Study Biopotency of Rambusa (*Passiflora foetida* L.) Fruit Extract As An Antibacterial for *Aeromonas hydrophila*

[1]*Fatmawati, [2]Agussyarif Hanafie, [3]Siti Aisiah, [4]Ririen Kartika Rini, [5]Olga, [6]Rizka Agustina, [7]Aminah, [8]Elvina Kartiani

[1,2,3,4,5,6] Program Study Aquaculture, Faculty of Fisheries and Marine Sciences, Lambung Mangkurat University, Banjarmasin in Banjarbaru, Indonesia

[7] Program Study Aquaculture, Faculty of Agriculture, Achmad Yani University Banjarmasin, Indonesia

[8] Fish Quarantine and Quality Control Center (BKIPM) Banjarmasin. South Kalimantan

*Corresponding Author e-mail: fatmawati01@ulm.ac.id

Received: May 2025; Revised: December 2025; Published: April 2026

Abstract

Motile Aeromonad Septicemia (MAS) is a disease affecting freshwater fish and is caused by *Aeromonas hydrophila*. The control of MAS commonly relies on synthetic drugs and antibiotics. This study aimed to evaluate the biopotential of rambusa (*Passiflora foetida* L.) fruit extract as an antibacterial agent against *A. hydrophila*. The antibacterial activity was assessed using the disc diffusion and Minimum Inhibitory Concentration (MIC) methods, supported by qualitative and quantitative phytochemical analyses. The results showed that rambusa fruit extract produced inhibition zones against *A. hydrophila*. The inhibition zone of the aqueous extract after 24 hours of incubation was 3.25 mm, while the methanolic extract produced an inhibition zone of 11.42 mm. These findings indicate that rambusa fruit extract prepared using both distilled water and methanol as solvents can inhibit the growth of *A. hydrophila*. Qualitative phytochemical screening of the aqueous extract revealed the presence of flavonoids, alkaloids, tannins, saponins, and hydroquinones based on the 10% KOH test. Quantitative analysis also detected flavonoids, alkaloids, tannins, saponins, steroids, phenolics, anthocyanins, and triterpenoids. The MIC test showed that concentrations of 100–600 mg/L were effective in inhibiting the growth of *A. hydrophila* within the weak inhibition category, while concentrations of 700–900 ppm showed higher inhibitory activity. These results suggest that rambusa fruit extract has potential as a natural antibacterial agent against *A. hydrophila*.

Keywords: Rambusa; Antibacterial; Inhibition zone; Phytochemicals

How to Cite: Fatmawati, F., Hanafie, A., Aisiah, S., Rini, R. K., Olga, O., Agustina, R., … Kartiani, E. (2026). Study Biopotency of Rambusa (Passiflora foetida L.) Fruit Extract As An Antibacterial for Aeromonas hydrophila. *Prisma Sains: Jurnal Pengkajian Ilmu Dan Pembelajaran Matematika Dan IPA IKIP Mataram*, *14*(2), 876–890. https://doi.org/10.33394/j-ps.v14i2.15708

https://doi.org/10.33394/j-ps.v14i2.15708

INTRODUCTION

Aeromonas hydrophila is an opportunistic pathogenic bacterium that is widely distributed in aquatic environments, including freshwater, brackish water, sediments, and fish culture systems. This bacterium is recognized as one of the important causative agents of bacterial diseases in freshwater aquaculture because it can infect fish under unfavorable environmental and physiological conditions. Infection by *A. hydrophila* commonly occurs when fish experience stress due to poor water quality, high stocking density, sudden temperature changes, physical injury, or reduced immune response. Under these conditions, the bacterium can invade host tissues and cause systemic infection, leading to severe pathological changes and high mortality in cultured fish (Chopra et al., 2000). Several aquaculture species have been reported to be susceptible to *A. hydrophila* infection, including snakehead fish (*Channa striata*) (Olga & Fatmawati, 2018), climbing perch (*Anabas *testudineus*) (Hayati et al., 2012), catfish, tilapia, koi, and African catfish (Mulia et al., 2015). These reports indicate that *A. hydrophila* has a broad host range and remains a serious threat to the sustainability of freshwater fish farming.

Infections caused by *A. hydrophila* frequently develop into Motile Aeromonad Septicemia (MAS), a bacterial disease characterized by hemorrhagic lesions, ulceration, fin erosion, abdominal swelling, exophthalmia, and internal organ damage. MAS can spread rapidly in fish populations and may cause mortality rates ranging from 80% to 100% within one to two weeks, particularly when disease control is delayed or when fish are maintained under stressful culture conditions (Kamiso, 2004; Olga & Aisiah, 2007). These outbreaks cause not only significant biological losses but also serious economic damage to aquaculture industries, where fish survival directly affects farmers' productivity and income (Pridgeon & Klesius, 2011; Austin & Austin, 2016). In Indonesia, freshwater aquaculture plays an important role in supporting food security, income generation, and rural livelihoods. Therefore, effective control of MAS is essential to maintain fish health, improve production stability, and support the development of sustainable aquaculture systems.

The control of MAS in aquaculture has commonly relied on synthetic drugs and antibiotics because these treatments are considered fast and effective in suppressing bacterial infections. Antibiotics such as tetracycline and other broad-spectrum antibacterial agents have been widely used to reduce bacterial growth and prevent disease outbreaks. However, the repeated and excessive use of antibiotics in aquaculture has raised serious concerns, including antimicrobial resistance, disruption of aquatic microbiota, accumulation of antibiotic residues in edible fish tissues, and contamination of the surrounding environment (Ajane & Patil, 2019; Cabello et al., 2013). These problems are increasingly important because antimicrobial resistance is now recognized as a global public health, food safety, and environmental issue. The World Health Organization and the Food and Agriculture Organization have warned against the indiscriminate use of antibiotics in aquaculture and have encouraged the development of alternative approaches to disease management (FAO, 2020; WHO, 2021). In Indonesia, similar concerns have emerged as several studies have reported resistant *Aeromonas* strains, while regulatory discussions have increasingly addressed the need to limit antibiotic application in fish farming (Sari et al., 2021). These issues highlight the urgency of developing safe, effective, and sustainable antibacterial agents derived from natural products.

The limitations of synthetic antibiotics have encouraged researchers to explore natural antibacterial agents derived from medicinal plants. Plants are considered promising sources of antibacterial compounds because they contain various secondary metabolites with antimicrobial, antioxidant, anti-inflammatory, and immunostimulatory activities. Plant-derived antibacterial agents may offer several advantages, including lower environmental risk, wider availability, biodegradability, and potential compatibility with sustainable aquaculture practices. However, natural extracts cannot automatically be assumed to replace antibiotics without sufficient evidence. Their antibacterial activity, safety, effective dosage, toxicity, and application methods must be scientifically validated before being recommended for practical use. Therefore, studies on local medicinal plants are important, particularly in regions with rich biodiversity and long traditions of using plants for health-related purposes.

Passiflora foetida L., commonly known in Indonesia as rambusa or wild passion fruit, is one of the medicinal plants that has potential as a source of natural bioactive compounds. This plant grows widely in tropical regions and is commonly found in open fields, plantations, roadsides, and rural areas. Traditionally, rambusa has been used to treat several ailments, including urinary tract infections, sepsis, and diabetes mellitus (Dewi & Afsari, 2017; Khairati et al., 2015). Its traditional use suggests that the plant may contain compounds with biological activity. Previous phytochemical studies have shown that *P. foetida* contains alkaloids, flavonoids, phenols, glycosides, and passifloricins, which are associated with antibacterial, antioxidant, antiviral, and immunostimulatory properties (Jufri et al., 2022; Badolova et al., 2022). These compounds may contribute to the inhibition of bacterial growth through several mechanisms, such as damaging bacterial cell walls, disrupting membrane permeability, inhibiting essential enzymes, interfering with nucleic acid synthesis, and reducing bacterial metabolic activity.

Several studies have reported the antibacterial potential of rambusa and other *Passiflora* species. Rambusa leaves have been shown to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* (Noviyanti et al., 2014), while ethanolic extract of rambusa fruit has demonstrated activity against diarrhea-inducing *E. coli* (Budianto & Budiono, 2023). These findings indicate that rambusa contains bioactive compounds that may act against pathogenic bacteria. Nevertheless, most previous studies have focused on human-related pathogens or on specific plant parts such as leaves and stems. The antibacterial activity of rambusa fruit against fish pathogens, particularly *A. hydrophila*, remains largely unexplored. This gap is important because the fruit may contain a metabolite profile that differs from other plant parts and may serve as a potential source of antibacterial compounds for aquaculture applications.

The selection of rambusa fruit as the main material in this study is therefore relevant for both scientific and practical reasons. Scientifically, this study contributes to the limited information on the antibacterial potential of rambusa fruit against *A. hydrophila*. Practically, rambusa is a locally available plant that may be developed as an alternative antibacterial source if its effectiveness and safety are supported by further evidence. In addition, examining different solvents, such as distilled water and methanol, is important because solvent polarity can influence the extraction of bioactive compounds. Water tends to extract more polar compounds, while methanol may be more effective in extracting semi-polar compounds such as certain flavonoids and phenolics. These differences can influence the antibacterial activity of the resulting extract.

Therefore, this study aimed to evaluate the antibacterial activity of rambusa (*Passiflora foetida* L.) fruit extract against *Aeromonas hydrophila*. The antibacterial activity was assessed using the disc diffusion method to measure inhibition zones and the Minimum Inhibitory Concentration (MIC) test to determine the lowest concentration capable of inhibiting bacterial growth, both of which are widely used in antimicrobial evaluation of natural products (Balouiri et al., 2016; CLSI, 2021). Qualitative and quantitative phytochemical analyses were also conducted to identify the bioactive compounds present in the extract. The findings of this study are expected to provide scientific information on the potential use of rambusa fruit extract as a natural antibacterial agent for controlling *A. hydrophila* infection in freshwater aquaculture. More specifically, this study may serve as an initial basis for further research on toxicity, in vivo efficacy, dosage optimization, and formulation before rambusa extract can be recommended for practical application in fish disease management.

METHOD

Research Design

This study used a laboratory experimental design to evaluate the antibacterial activity of rambusa (*Passiflora foetida* L.) fruit extract against *Aeromonas hydrophila*. The experiment was arranged using a Completely Randomized Design (CRD), in which each treatment was designed to be tested with repeated measurements to ensure the reliability of the observed antibacterial response.

The treatments consisted of rambusa fruit extracts prepared using different solvents and concentrations, while the controls consisted of tetracycline 30 µg as the positive control and distilled water as the negative control. The positive control was used to compare the antibacterial activity of the plant extract with a standard antibiotic, whereas the negative control was used to ensure that the solvent did not independently inhibit bacterial growth.

Extraction Procedure

Rambusa fruits were collected from Bangkal Village, Cempaka District, Banjarbaru City, South Kalimantan. The fruits used in this study consisted of green and yellow fruits with their membranes. The collected samples were cleaned using tissue to remove adhering dirt and impurities, and then weighed to obtain 250 g of fresh sample material.

For the aqueous extract, the rambusa fruit samples were blended with distilled water at a ratio of 1:5 (w/v). The mixture was then filtered using a vacuum filter to obtain the filtrate. The filtrate was evaporated using a dehydrator at 50 °C until a paste-like extract was formed. The same sample preparation procedure was also applied for the methanolic extract, with methanol used as the extracting solvent. The resulting extracts were placed in dark bottles, wrapped with aluminum foil to minimize light exposure, and stored at 1–4 °C until further use.

Bacterial Culture and Rejuvenation

The bacterial isolate used in this study was *Aeromonas hydrophila*. The stock culture was maintained on Tryptic Soy Agar (TSA) slants to preserve bacterial viability before testing. Prior to antibacterial activity testing, the bacterial isolate was rejuvenated to obtain an active and fresh bacterial culture.

For rejuvenation, the *A. hydrophila* isolate was subcultured on Glutamate Starch Phenol Red (GSP) agar and incubated at 28–30 °C for 18–24 hours. Characteristic yellow colonies were then selected and transferred into Tryptic Soy Broth (TSB) for inoculum preparation. Some colonies were also transferred onto TSA slants for culture maintenance during the study.

Antibacterial Activity Testing Using the Disc Diffusion Method

The antibacterial activity of rambusa fruit extract against *A. hydrophila* was evaluated using the Kirby-Bauer disc diffusion method following CLSI (2020). Sterile paper discs with a diameter of 5 mm were loaded with 25 µL of rambusa fruit extract. The extract-loaded discs were prepared according to the treatment groups and placed on agar media that had been inoculated with the bacterial suspension.

The bacterial suspension was standardized to 0.5 McFarland, equivalent to approximately 1.5×10^8 CFU/mL. The standardized suspension was inoculated onto Mueller-Hinton Agar (MHA) using the double-layer technique to obtain uniform bacterial growth. The test discs, positive control discs containing tetracycline 30 µg, and negative control discs containing distilled water were then placed on the surface of the agar. The plates were incubated at 28–30 °C for 18–24 hours. After incubation, the inhibition zones formed around the discs were measured using a calibrated caliper with an accuracy of ±0.1 mm. The results were recorded as inhibition zone diameters and used to determine the antibacterial activity of the extract.

Phytochemical Testing

Phytochemical testing was conducted to identify the secondary metabolite compounds present in rambusa fruit extract. Qualitative phytochemical screening included tests for alkaloids, flavonoids, tannins, saponins, steroids, phenolics, anthocyanins, triterpenoids, and hydroquinones. The qualitative tests were carried out using standard phytochemical procedures based on Harborne (1998) and validated protocols described by Upadhyay and Dixit (2015).

Quantitative phytochemical analysis was also conducted to determine the concentration of major bioactive compounds contained in the extract. The compounds analyzed quantitatively included flavonoids, alkaloids, tannins, saponins, steroids, phenolics, anthocyanins, and triterpenoids. Spectrophotometric analysis was used for selected compounds to estimate their concentration ranges. Each phytochemical test was repeated to improve reliability and to support the interpretation of the antibacterial activity observed in the disc diffusion and MIC tests.

Minimum Inhibitory Concentration (MIC) Test

The Minimum Inhibitory Concentration (MIC) test was conducted to determine the lowest concentration of rambusa fruit extract capable of inhibiting the growth of *A. hydrophila*. The test was performed using the aqueous extract of rambusa fruit at different concentration levels. The concentrations tested ranged from 100 to 900 ppm, and the antibacterial response was observed after 24 and 48 hours of incubation.

The standardized *A. hydrophila* suspension was exposed to each extract concentration and incubated at 28–30 °C. The inhibitory response was evaluated based on the inhibition zone formed at each concentration. The lowest concentration that showed inhibition of bacterial growth was considered the minimum effective concentration of the extract. The inhibition zones were then categorized according to their antibacterial activity level to determine whether the extract produced weak, moderate, or strong inhibition.

Data Analysis

The data obtained from the antibacterial activity test were expressed as inhibition zone diameters in millimeters. Replicate values were summarized using descriptive statistics, including mean values and, where applicable, standard deviation. The mean inhibition zone was used to compare the antibacterial activity of rambusa fruit extract prepared with different solvents and concentrations.

The antibacterial activity was categorized based on inhibition zone diameter following Surjowardojo et al. (2015). In general, a smaller inhibition zone indicates weaker antibacterial activity, while a wider inhibition zone indicates stronger antibacterial activity. When the data met the assumptions for statistical analysis, one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test at a significance level of $p < 0.05$ was used to determine significant differences among treatments.

Instrument Validation

Instrument validation was conducted before the antibacterial and phytochemical tests were performed. The caliper used to measure the inhibition zones was calibrated to ensure measurement accuracy of ±0.1 mm. This calibration was important because small differences in inhibition zone diameter could affect the interpretation of antibacterial activity.

For quantitative phytochemical analysis, the spectrophotometer was validated using standard curves to ensure the accuracy of compound concentration measurements. Recovery and reproducibility checks were also considered to support the reliability of the quantitative data. The qualitative phytochemical tests were performed using recognized reference methods, including Harborne (1998) and Upadhyay and Dixit (2015), while the antibacterial testing procedure referred to CLSI standards to improve methodological consistency and repeatability.

RESULTS AND DISCUSSION

Antibacterial Activity Test of Rambusa Fruit Extract using the Disc Diffusion Method

The results of the antibacterial activity test of rambusa fruit extract using distilled water, methanol and a control using antibiotics (tetracycline) using the disc diffusion method are presented in Table 1 and Figure 1.

Table 1. Results of *A. hydrophila* antibacterial activity test

No	Sample	Inhibition Zone (mm)	
		Test I	Test II
1	Rambusa Fruit-Aquades	2.6	4.0
		3.3	3.3
		2.5	3.8
		3.8	3.2
		3.6	-
		3.7	-
Average		3.25	3.575
2	Rambusa Fruit - Methanol	0	11.3
		0	12.1
		0	10.8
		0	11.5
Average		0	11.425
3	Tetracycline	4.8	12.3
		4.6	9.0
Average		4.7	10.65

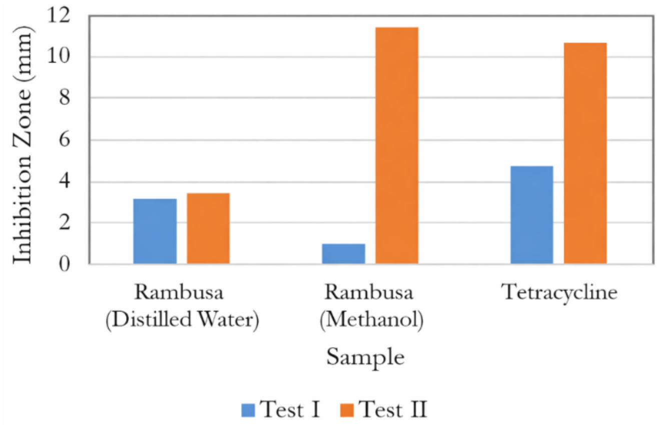
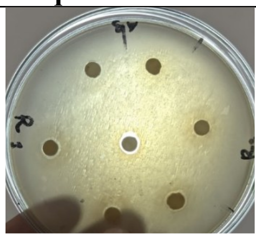
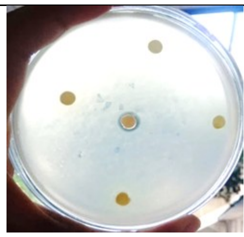
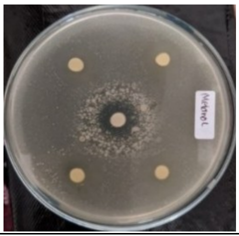


Figure 1. Antibacterial activity test graphic

Test	Aquades Solvent	Methanol Solvent
1		
2		

Description: :

- Test I: Aquades solvent in repetitions 1-6. Repeat 7 tetracycline antibiotics
- Methanol solvent in repetitions 1-4. Repeat 5 tetracycline antibiotics
- Test II: Aquades solvent in repetitions 1-4. Repeat 5 tetracycline antibiotics
- Methanol solvent in repetitions 1-4. Repeat 5 tetracycline antibiotics

Figure 2. Disc diffusion assay of rambusa fruit extract against *Aeromonas hydrophila*

Phytochemical Test of Rambusa Fruit Extract

The results of phytochemical tests on rambusa fruit extract qualitatively and quantitatively are presented in Table 2.

Table 2. Results of Qualitative and Quantitative Phytochemical Tests

Compounds	Qualitative*)	Quantitative**)	
	Aquades Solvent	Aquades solvent	Methanol Solvent
Flavonoids	Pb acetate (+), Mg+HCL (+), Dragendroff (-)	19.500 mg/ml QE	22.500 mg/ml QE
Alkaloids	Mayer (+), Wagner (-)	21.040%	25.640%
Tannins	FeCl3 (+)	1.467 mg/ml QE	1.370 mg/ml QE
Saponins	Aquades+, CL (+)	13.636%	15.480%
Hydroquinones	KOH 10% (+)	td	earlier
Steroids	td	20.193 mg/ml QE	20.703 mg/ml QE
Phenolics	td	46.733 mg/ml	59.400 mg/ml
Anthocyanins	td	0.741 mg/gr	0.768 mg/gr
Triterpenoids	td	243.8 mg/ml	256.8 mg/ml

Description: *) Basic Laboratory test of FPIK ULM; **) Biochemistry and Biomolecular Lab Test of FK ULM; td=not tested; (-)=none; (+)=exists

Minimum Inhibitory Concentration (MIC) Test

The MIC test for rambusa fruit extract was determined with distilled water solvent, while the MIC test was not carried out with methanol solvent. The MIC test for rambusa fruit extract with distilled water solvent was to see the inhibitory ability within 24 hours and 48 hours, as in Table 3 as follows.

Table 3. Average values of the inhibition zone of distilled rambusa fruit extract at various concentrations

Concentration (ppm)	Inhibition Zone Diameter (mm)		Description
	24 Hours	48 Hours	
100	3.5	3.0	Bacteriostatic
200	3.9	3.1	Bacteriostatic
300	4.0	3.4	Bacteriostatic
400	4.2	3.5	Bacteriostatic
500	4.4	3.7	Bacteriostatic
600	4.7	3.9	Bacteriostatic
700	5.0	4.0	Bacteriostatic
800	5.5	3.9	Bacteriostatic
900	6.4	4.5	Bacteriostatic

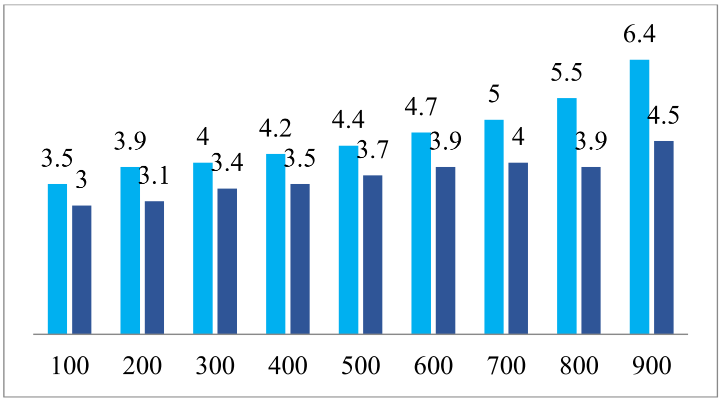
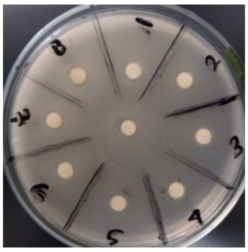


Figure 3. Graph of average MIC test results 24 and 48 hours

A



B

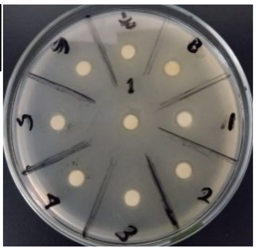


Figure 4. MIC Test Results (Personal Doc. 2024)
Description: (A) 24 hours (B) 48 hours

Discussion

Antibacterial Activity Test of Rambusa Fruit Extract using the Disc Diffusion Method

The results of the antibacterial activity test of rambusa fruit extract against *A. hydrophila* bacteria using distilled water as a solvent with six repetitions showed an average zone of inhibition of 3.25 mm. Meanwhile, rambusa fruit extract with methanol solvent in the first four replications did not show an inhibition zone (0 mm), but in the second repetition, the average inhibition zone increased to 11.425 mm. As a control, tetracycline antibiotics showed a larger inhibition zone with an average of 4.7 mm. The antibacterial activity of the control was stronger than rambusa fruit extract (Rahman et al., 2001). Tetracycline is a broad-spectrum antibiotic that inhibits protein synthesis by preventing the attachment of aminoacyl-tRNA to ribosome acceptors, effective against Gram-positive and Gram-negative bacteria (Chopra and Roberts, 2001). The results of measuring the inhibition zone using the disc diffusion method showed that rambusa fruit extract with aquadest solvent had bacteriostatic activity with an average inhibition zone of 3.25 mm. In contrast, the extract with methanol solvent in the initial test did not show inhibition, but in the second test an inhibition zone was found indicating antibacterial activity.

Antibacterials can be bactericidal (kill bacteria) or bacteriostatic (inhibit bacterial growth). Bacteriostatics can be bactericidal at high concentrations (Purmaningsih et al., 2017). The absence of antibacterial activity in the methanol extract in the initial test was probably caused by the evaporation time being too short or excessive heating which destroyed the active compound. In addition, too long extraction can increase the oxidation of secondary metabolite compounds due to higher oxygen exposure (Asworo and Widwiastuti, 2023).

Repeated antibacterial tests on methanol extracts showed antibacterial activity with an inhibition zone of >5 mm, although it did not appear clear. The criteria for the inhibition zone according to Surjowardojo et al. (2015), extracts with aquades solvent are categorized as weak (<5 mm), while extracts with methanol solvent (10.8-12.1 mm) are categorized as moderate to strong. This indicates that rambusa fruit extract, particularly with methanol as a solvent, has promising antibacterial potential, as the inhibition zone values achieved fall within the range that is often reported in plant-based antibacterial studies, suggesting that the bioactive compounds are effectively extracted and able to suppress the growth of *A. hydrophila*. These findings not only highlight the role of solvent selection in maximizing antibacterial efficacy but also provide a scientific basis for further exploration of rambusa fruit as a natural antibacterial agent.

The antibacterial activity of rambusa fruit extract with distilled water and methanol as solvents against *A. hydrophila* is in accordance with the function of the metabolite compounds contained in the extract. This compound acts as an antibacterial that inhibits bacterial growth (Saputra et al., 2020). The test results showed that the extract with distilled water as a solvent produced an inhibition zone of 3.25 mm after 24 hours of incubation, while the extract with methanol as a solvent had an average inhibition zone of 11.42 mm. These results prove that rambusa fruit extract with both solvents has the ability to inhibit the growth of *A. hydrophila* based on its inhibitory power category. The test was continued with a phytochemical test to identify the compounds that play a role in antibacterial activity against *A. hydrophila*.

Phytochemical Test of Rambusa Fruit Extract

The results of qualitative phytochemical tests showed that the extract of rambusa fruit with aquades solvent positively contained flavonoids (Pb acetate and Mg + HCl), alkaloids (Mayer), tannins (FeCl3), saponins, and hydroquinone (10% KOH). Quantitatively, this extract also contains flavonoids, alkaloids, tannins, saponins, steroids, phenolics, anthocyanins, and triterpenoids. Wardhani and Pardede (2022), extracts of rambusa stems and leaves contain secondary metabolites from the alkaloid, flavonoid, tannin/polyphenol, steroid, and saponin groups, while extracts of the skin and fruit contain alkaloids, flavonoids, tannins/polyphenols, and quinones. Wardhani and Pardede's (2022) research using the maceration method and methanol solvent found that rambusa fruit contains alkaloids, flavonoids, quinones, and tannins. The results of the study using the direct extraction method using aquades solvent showed similar results, namely flavonoids, alkaloids, tannins, and hydroquinones. The saponin test on the fruit showed negative results because no foam was formed, although quantitatively this extract contained eight secondary metabolite compounds.

The content of alkaloids, flavonoids, and hydroquinones in rambusa fruit extract has the potential to be antibacterial with bacteriostatic properties, which inhibit bacterial growth (Saputra et al., 2020). Phenolic compounds, flavonoids, tannins, and proanthocyanins in plants act as antioxidants that can be used in the treatment of degenerative diseases. The antibacterial effects of secondary metabolite compounds generally come from the content of saponins, flavonoids, tannins, quinones, phenols, and lectins (Gulci et al., 2012).

Flavonoids are natural bioactive compounds that are abundant and effective in damaging the envelope of Gram-negative and Gram-positive bacteria, as well as targeting molecules important for bacterial survival (Donadio et al., 2021). Antibacterial activity is influenced by the total flavonoid content, where the higher the content, the stronger the antibacterial activity (Manik et al., 2014).

Alkaloids have antibacterial activity through interaction with bacterial DNA, forming interchelates that inhibit topoisomerase enzymes, causing mutations or genetic damage (Rohama et al., 2023). Alkaloids are secondary metabolites with a broad antibacterial spectrum, low toxicity, and a lower tendency for drug resistance. The mechanism of action includes inhibition of bacterial cell wall synthesis, changes in cell membrane permeability, and disruption of metabolism and synthesis of nucleic acids and proteins (Yan et al., 2021).

Tannins interact with bacterial cell membranes, inactivate enzymes, and damage genetic material through the formation of protein complexes with hydrogen and hydrophobic bonds (Ningsih and Agustien, 2013). At low concentrations, tannins inhibit bacterial growth, while at high concentrations they function as antibacterials by coagulating bacterial protoplasm. Saponins act as antibacterials by damaging the cytoplasmic membrane, causing leakage of metabolites that inactivate bacterial enzymes (Dasopang, 2017).

Steroids inhibit bacterial growth by disrupting the integrity of the lipid membrane, causing liposome leakage due to membrane sensitivity to steroid components (Purba et al., 2023). Steroids are lipophilic and interact with the phospholipid membrane of cells, which reduces membrane stability and changes cell morphology, leading to cell lysis (Jeyaraj et al., 2023). Phenol is able to penetrate bacterial cell walls, disrupt membrane integrity, and precipitate bacterial cell proteins. At low concentrations, phenol inactivates essential enzymes in cells (Oliver et al., 2001).

Anthocyanins and catechins have the potential to replace antibiotics by inducing bacterial cell damage, destroying wall and membrane structures, and inhibiting microbial enzymes and biofilm formation (Ma et al., 2019; Jeyaraj, 2023). Triterpenoids work by lysing bacterial cell walls through interactions with porins (transmembrane proteins), forming polymer bonds that cause protein damage to bacterial cell walls (Jeyaraj et al., 2023).

The antibacterial activity of rambusa extract has been shown to inhibit the growth of *A. hydrophila* bacteria due to the content of secondary metabolite compounds, such as flavonoids, alkaloids, tannins, saponins, steroids, phenolics, anthocyanins, triterpenoids, and hydroquinones. Rambusa extract with distilled water contains active compounds which function as static antibacterials which are effective in inhibiting the growth of *A. hydrophila*.

Minimum Inhibitory Concentration (MIC) Test

The MIC test results show that a minimum inhibition zone is formed at a concentration of 100 ppm with bacteriostatic properties. Table 3 shows that at concentrations of 100–900 ppm, the inhibition zone is bacteriostatic after 24 hours of incubation, but shrinks after 48 hours. This indicates that rambusa fruit extract with a concentration of 100–900 ppm inhibits the growth of *A. hydrophila*. Table 3. The MIC value is set at a concentration of 100 ppm as the minimum concentration that effectively inhibits the growth of *A. hydrophila*. At concentrations of 100-600 ppm, the inhibition zone formed is categorized as weak, while at

700-900 ppm it is categorized as strong. This is in accordance with Surjowardojo et al. (2015), who classify the diameter of inhibition power as follows: <5 mm (weak), 6–10 mm (moderate), 11–20 mm (strong), and> 20 mm (very strong). MIC test results, the injection dose for the toxicity test was determined based on the lowest to highest inhibitory power, namely: A (100 ppm), B (500 ppm), C (900 ppm), and K (control) without treatment.

The results of this study demonstrate that rambusa fruit (*Passiflora foetida* L.) extract exhibits antibacterial activity against *Aeromonas hydrophila*, as evidenced by the formation of inhibition zones in the disc diffusion assay and the minimum inhibitory concentration (MIC) values observed at specific concentration ranges. This finding supports the growing body of evidence that natural plant-based compounds can serve as alternative strategies for controlling Motile Aeromonad Septicemia (MAS) in freshwater fish. The use of herbal extracts is particularly relevant in aquaculture, as they offer antibacterial effects while potentially reducing adverse environmental impacts and the risk of antimicrobial resistance. Similar outcomes were reported by Nya and Austin (2009), who demonstrated that dietary supplementation with garlic (Allium sativum) significantly reduced mortality and enhanced immune responses in rainbow trout challenged with *A. hydrophila*.

The antibacterial effectiveness of rambusa fruit extract can be attributed to the presence of multiple bioactive compounds identified through qualitative and quantitative phytochemical analyses. These compounds include flavonoids, alkaloids, tannins, saponins, phenolics, steroids, and triterpenoids, all of which are known to possess antimicrobial properties. Previous studies on *Passiflora* species have consistently highlighted flavonoids and saponins as major contributors to antibacterial activity. Simão et al. (2018) reported that extracts of P. foetida and related species exhibited selective inhibitory effects against both Gram-positive and Gram-negative bacteria, suggesting that the phytochemical composition plays a crucial role in determining antibacterial potency.

Flavonoids, which were detected as dominant constituents in the rambusa fruit extract, are known to exert antibacterial effects through multiple mechanisms. According to Cushnie and Lamb (2005), flavonoids can inhibit bacterial DNA gyrase, interfere with energy metabolism, disrupt cytoplasmic membrane function, and suppress nucleic acid synthesis. These mechanisms are consistent with the inhibition zones observed in this study and explain the concentration-dependent antibacterial activity reflected in the MIC values. The ability of flavonoids to target multiple cellular pathways also reduces the likelihood of rapid resistance development, making them particularly attractive as alternative antimicrobial agents.

In addition to flavonoids, phenolic compounds present in the rambusa fruit extract likely contributed significantly to the observed antibacterial activity. Phenolic compounds possess strong antioxidant and antimicrobial properties and are capable of damaging bacterial cell walls and membranes, particularly in Gram-negative bacteria such as *A. hydrophila*. Altemimi et al. (2017) emphasized that fruits are rich sources of phenolic compounds with broad-spectrum antimicrobial potential. The moderate antibacterial activity observed in this study aligns with previous reports indicating that phenolic-rich extracts often require relatively higher concentrations to achieve strong inhibition against Gram-negative pathogens.

The presence of tannins and saponins further enhances the antibacterial profile of rambusa fruit extract. Tannins are known to precipitate proteins and inhibit microbial enzymes, while saponins can increase membrane permeability, leading to leakage of intracellular contents. These combined effects can synergistically suppress bacterial growth. Similar findings were reported by Sibi et al. (2012) and Abastillas et al. (2023), who observed that plant extracts containing tannins and saponins exhibited notable antibacterial activity, particularly when extracted using organic solvents.

The difference in antibacterial effectiveness between aqueous and methanolic extracts observed in this study highlights the importance of solvent selection in phytochemical extraction. The larger inhibition zones produced by methanolic extracts indicate that organic solvents are more efficient in extracting lipophilic and semi-polar bioactive compounds, such as flavonoids and phenolics. This observation is consistent with the findings of Al Laham and Al Fadel (2014), who reported that ethanol extracts of medicinal plants exhibited stronger antibacterial activity against antibiotic-resistant *A. hydrophila* compared to aqueous extracts. The limited efficacy of aqueous extracts may be attributed to the lower solubility of key antibacterial compounds in water.

The MIC results obtained in this study, ranging from weak inhibition at 100–600 mg/L to strong inhibition at 700–900 mg/L, further confirm the dose-dependent antibacterial activity of rambusa fruit extract. Although the antibacterial potency can be classified as moderate, these findings are significant when considering the growing concern over antibiotic resistance in aquaculture pathogens. Michel *et al*. (2003) reported increasing resistance of fish-pathogenic bacteria to commonly used antibiotics such as chloramphenicol, emphasizing the urgent need for alternative disease control strategies.

The relevance of plant-based antibacterials is further strengthened by studies demonstrating their immunostimulatory effects in fish. Wu et al. (2010) showed that hot-water extracts of Toona sinensis enhanced respiratory burst activity, phagocytosis, and lysozyme activity in tilapia, leading to increased survival following *A. hydrophila* infection. While the present study focused on in vitro antibacterial activity, the phytochemical profile of rambusa fruit extract suggests potential immunomodulatory properties that warrant further investigation through in vivo studies.

From an applied perspective, the use of rambusa fruit extract as a natural antibacterial agent aligns with sustainable aquaculture practices. Plant-derived antimicrobials are generally biodegradable, environmentally friendly, and less likely to induce resistance compared to synthetic antibiotics. Janda and Abbott (2010) emphasized that Aeromonas species are opportunistic pathogens with complex resistance mechanisms, reinforcing the need for integrated disease management approaches that include natural products alongside improved husbandry practices.

Overall, the findings of this study support the potential of *Passiflora foetida* fruit extract as a promising natural antibacterial agent against *Aeromonas hydrophila*. The demonstrated antibacterial activity, combined with the presence of diverse bioactive compounds, positions rambusa as a valuable candidate for further development in aquaculture disease control. Future research should focus on in vivo efficacy, toxicity assessment, optimal dosage determination, and formulation strategies to fully realize its application as a safe and effective alternative to conventional antibiotics.

CONCLUSION

Rambusa fruit extract has potential as an antibacterial against *A. hydrophila*. Testing with aquades solvent produced an inhibition zone of 3.25 mm, while methanol solvent was 11.42 mm, indicating effectiveness in inhibiting bacterial growth. Qualitative phytochemical tests identify flavonoids, alkaloids, tannins, saponins, and hydroquinones, while quantitative tests also detect steroids, phenolics, anthocyanins, and triterpenoids. The MIC test showed the effectiveness of the extract at 100–600 mg/L with weak inhibition, and 700–900 ppm with strong inhibition. The higher the extract concentration, the wider the inhibition zone formed, so that rambusa extract has the potential to be a natural antibacterial against *A. hydrophila*.

RECOMMENDATION

It is recommended to use rambusa fruit extract as a natural antibacterial alternative to inhibit the growth of *Aeromonas hydrophila* which causes MAS in freshwater fish.

ACKNOWLEDGMENT

The authors would like to express their gratitude to Lambung Mangkurat University for financially supporting this research through the 2024 Research Grant, contract number 1090.86/UN8.2/PG/2024.

FUNDING INFORMATION

This research was financially supported by Lambung Mangkurat University Research Grant year 2024 with grant number 1090.86/UN8.2/PG/2024.

AUTHOR CONTRIBUTIONS STATEMENT

Name of Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
Fatmawati	✓	✓		✓		✓	✓	✓		✓		✓	✓	✓
Agussyarif Hanafie	✓		✓		✓	✓		✓	✓		✓			
Siti Aisiah		✓		✓		✓	✓		✓	✓		✓	✓	
Ririen Kartika Rini		✓		✓		✓	✓		✓	✓		✓		
Olga	✓		✓		✓	✓		✓	✓		✓		✓	
Rizka Agustina		✓		✓		✓	✓		✓	✓		✓		
Aminah	✓		✓		✓	✓		✓	✓		✓		✓	
Elvina Kartiani		✓		✓		✓	✓		✓	✓		✓		

CONFLICT OF INTEREST STATEMENT
Authors state no conflict of interest.

DATA AVAILABILITY
The data that support the findings of this study are available on request from the corresponding author.

REFERENCES

Abastillas, M. K. A., Bagolcol, T. J. R., Buro, P. B., & Gayoba, M. I. B. (2023). Phytochemical composition and antibacterial activity of selected medicinal plants in a local community in the Philippines. *International Journal of Biosciences, 22*(2), 171–182. https://doi.org/10.12692/ijb/22.2.171-182

Adamczak, A., Ożarowski, M., & Karpiński, T. M. (2019). Antibacterial activity of some flavonoids and organic acids widely distributed in plants. *Journal of Clinical Medicine, 9*(1), Article 109. https://doi.org/10.3390/jcm9010109

Ajane, G. A., & Patil, A. S. (2019). Evaluation of antioxidant potential of *Passiflora foetida* extract and quantitative evaluation of its phytochemical content: A possible natural antioxidant. *The Pharmaceutical and Chemical Journal, 6*(4), 14–24.

Al Laham, S. A., & Al Fadel, F. M. (2014). Antibacterial activity of various plants extracts against antibiotic-resistant *Aeromonas hydrophila*. *Jundishapur Journal of Microbiology, 7*(7), Article e11370. https://doi.org/10.5812/jjm.11370

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., & Lightfoot, D. A. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants, 6*(4), Article 42. https://doi.org/10.3390/plants6040042

Asworo, R. Y., & Widwiastuti, H. (2023). Effect of simplicia powder size and maceration time on the antioxidant activity of soursop skin extract. *Indonesian Journal of Pharmaceutical Education (e-Journal), 3*(2), 256–263.

Badolova, V., Suleymanov, T., Mammadov, R., & Atay, M. (2022). Determination of the antimicrobial activity of ethanolic extracts of some *Passiflora* L. species, first introduced in Azerbaijan. *Bulletin of Science and Practice, 8*(5), 92–101.

Chopra, A. K., Xiu, X. I., Ribardo, D., Gonzales, M., Khul, K., Peterson, J. W., & Husto, C. W. (2000). The cytotoxic enterotoxin of *Aeromonas hydrophila* induces proinflammatory cytokine production and activates arachidonic acid metabolism in macrophages. *Infection and Immunity, 68*(5), 2808–2818.

Cushnie, T. P. T., & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents, 26*(5), 343–356.

Dasopang, E. S. (2017). Phytochemical screening and antibacterial activity test of ethanol extract of sangitan leaves (*Sambucus javanica* Reinw) against the growth of *Escherichia coli* and *Salmonella typhi*. *BioLink Journal, 4*(1), 54–62.

Dewi, S. T. R., & Afsari, Y. (2017). Activity test of rambusa fruit extract (*Passiflora foetida* L.) against tooth decay caused by *Streptococcus mutans* bacteria. *Pharmaceutical Media, 13*(2), 92–96.

Donadio, G., Mensitieri, F., Santoro, V., Parisi, V., Bellone, M. L., De Tommasi, N., Izzo, V., & Dal Piaz, F. (2021). Interactions with microbial proteins driving the antibacterial activity of flavonoids. *Pharmaceutics, 13*(5), Article 660. https://doi.org/10.3390/pharmaceutics13050660

Hayati, J. W., Susanti, B. S., Sihananto, & Olga. (2012). *Aeromonas hydrophila* BGB01 vaccine types of H antigen and O antigen to control MAS (Motile Aeromonad Septicemia) in catfish (*Anabas testudineus* Bloch). In *Proceedings of the 9th Annual National Seminar: Results of Fisheries and Marine Research in 2012*. Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada.

Janda, J. M., & Abbott, S. L. (2010). The genus *Aeromonas*: Taxonomy, pathogenicity, and infection. *Clinical Microbiology Reviews, 23*(1), 35–73. https://doi.org/10.1128/CMR.00039-09

Jeyaraj, E. J., Gamage, G. C. V., Cintrat, J. C., & Choo, W. S. (2023). Acylated and non-acylated anthocyanins as antibacterial and antibiofilm agents. *Discover Food, 3*(1). https://doi.org/10.1007/s44187-023-00062-8

Jufri, N., Laga, A., & Faidah, A. N. (2022). Effectiveness of rambusa extract (*Passiflora foetida* L.) in inhibiting bacteria, yeast, and its effect on total tofu microbes during storage. In *Seminar on the results of the Faculty of Agriculture, Hasanuddin University*. Faculty of Agriculture, Hasanuddin University.

Kamiso, H. N. (2004). Status of fish diseases and their control in Indonesia. In *Proceedings of the IV National Seminar on Fish and Shrimp Diseases*.

Khairati, K., Ihwan, I., & Maya, M. S. (2015). Antidiabetic effectiveness of rambusa leaf extract (*Passiflora foetida* L.) on mice (*Mus musculus*) with glucose induction. *Galenika Journal of Pharmacy (e-Journal), 1*(2), 99–104.

Lingga, A. R., Pato, U., & Rossi, E. (2016). Antibacterial test of torch ginger stem extract (*Nicolaia speciosa* Horan) against *Staphylococcus aureus* and *Escherichia coli*. *JOM Faperta, 3*(1), 1–15.

Ma, Y., Ding, S., Fei, Y., Liu, G., Jang, H., & Fang, J. (2019). Antimicrobial activity of anthocyanins and catechins against foodborne pathogens *Escherichia coli* and *Salmonella*. *Food Control, 106*.

Mangunwardoyo, W., Ismayasari, R., & Riani, E. (2010). Pathogenicity and virulence test of *Aeromonas hydrophila* Stanier on tilapia (*Oreochromis niloticus* Lin.) through Koch's postulate. *Jurnal Riset Akuakultur, 5*, 245–255.

Manik, D. F., Hertiani, T., & Anshory, H. (2014). Correlation analysis between flavonoid levels and antibacterial activity of ethanol extract and fractions of kersen leaves (*Muntingia calabura* L.) against *Staphylococcus aureus*. *Khazanah, 6*(2), 1–11.

Maulidiani, M. (2023). NMR analysis of *Passiflora foetida* extracts. *Universiti Malaysia Terengganu Journal of Undergraduate Research, 5*(2). https://doi.org/10.46754/umtjur.v5i2.404

Michel, C., Kerouault, B., & Martin, C. (2003). Chloramphenicol and florfenicol susceptibility of fish-pathogenic bacteria isolated in France: Comparison of minimum inhibitory concentration, using recommended provisory standards for fish bacteria. *Journal of Applied Microbiology, 95*(5), 1008–1015. https://doi.org/10.1046/j.1365-2672.2003.02093.x

Mulia, D. S., Khusniah, A., & Maryanto, H. (2015). Potential immunogenicity of *Aeromonas hydrophila* bacteria strain GPI-05 and GL-02 as vaccine candidates. *Aquasains, 3*(3), 320–329.

Nendissa, D. M. (2012). Analysis of the ability of silpau green algae (*Dictyiosphaeria versluysii*) as antibacterial. *Jurnal Ekosain, 1*(1), 47–51.

Ningsih, A. P., & Agustien, A. (2013). Antibacterial activity test of thick extract of yellow kepok banana plant (*Musa paradisiaca* Linn.) against *Staphylococcus aureus* and *Escherichia coli*. *Jurnal Biologi UNAND, 2*(3), 207–213.

Noviyanti, Y., Pasaribu, S. P., & Tarigan, D. (2014). Phytochemical, toxicity and antibacterial activity test of ethanol extract of rambusa leaves (*Passiflora foetida* L.) against *Staphylococcus aureus* and *Escherichia coli* bacteria. *Mulawarman Chemistry Journal, 12*(1), 31–35.

Nya, E. J., & Austin, B. (2009). Use of garlic (*Allium sativum*) to control *Aeromonas hydrophila* infection in rainbow trout (*Oncorhynchus mykiss* Walbaum). *Journal of Fish Diseases, 32*(11), 963–970. https://doi.org/10.1111/j.1365-2761.2009.01100.x

Olga, & Aisiah, S. (2007). *Aeromonas hydrophila* extracellular product protein vaccine to increase patin (*Pangasius hypophthalmus*) immune response to Motile Aeromonad Septicemia (MAS). *Aquatic Science, 10*(2), 105–110.

Olga, & Fatmawati. (2018). Efficacy of *Aeromonas hydrophila* ASB-01 vaccine route in snakehead fish (*Ophiocephalus striatus*). In *Proceedings of the 4th National Maritime and Fisheries Seminar: Development of marine and fisheries science and technology to support food security in facing the MEA* (p. 51). ISBN: 878-602-6906-39-7

Oliver, S. P., Gillespie, B. E., Lewis, M. J., Ivey, S. J., Almeida, R. A., Luther, D. A., Johnson, D. L., Lamar, K. C., Moorehead, H. H., & Dowlen, H. H. (2001). Efficacy of a new premilking teat disinfectant containing a phenolic combination for the prevention of mastitis. *Journal of Dairy Science, 84*, 1545–1549.

Purba, A. U. C., Naliani, S., & Sugiaman, V. K. (2023). Antibacterial effectiveness of red fruit fraction (*Pandanus conoideus* Lam) as a removable partial denture cleaner against *Staphylococcus aureus*. *e-GiGi, 11*(2), 143–151.

Purmaningsih, N. A., Kalor, H., & Atun, S. (2017). Antibacterial activity test of temulawak (*Curcuma xanthorrhiza*) extract against *Escherichia coli* ATCC 11229 and *Staphylococcus aureus* ATCC 25923 bacteria. *Journal of Science and Technology Research, 22*(2), 140–147.

Rahman, M. H., Suzuki, S., & Kawai, K. (2001). The effect of temperature on *Aeromonas hydrophila* infection in goldfish, *Carasius auratus*. *Journal of Applied Ichthyology, 17*, 282–285.

Rohama, R., Melviani, M., & Rahmadani, R. (2023). Antibacterial activity and determination of flavonoid content of kalangkala leaf fraction (*Litsea angulata*) and thin layer chromatography profile. *Jurnal Surya Medika (JSM), 9*(1), 267–276.

Saputra, A., Arfi, F., & Yulian, M. (2020). Literature review: Phytochemical analysis and benefits of moringa leaf extract (*Moringa oleifera*). *Amina, 2*(3), 114–119.

Sarmira, M., Purwanti, S., & Yuliati, F. N. (2021). Antibacterial activity of oregano leaf extract against *Escherichia coli* and *Staphylococcus aureus* bacteria as alternative poultry feed additives. *Animal Science Journal of Padjadjaran University, 21*(1), 40–49.

Sibi, G., Naveen, R., Dhananjaya, K., & Ravikumar, K. R. (2012). Potential use of *Muntingia calabura* L. extracts against human and plant pathogens. *Pharmacognosy Journal, 4*(34), 44–47. https://doi.org/10.5530/pj.2012.34.8

Simão, M. J., Barboza, T. J. S., Vianna, M. G., Garcia, R., Mansur, E., Ignacio, A. C. P. R., & Pacheco, G. (2018). A comparative study of phytoconstituents and antibacterial activity of in vitro derived materials of four *Passiflora* species. *Anais da Academia Brasileira de Ciências, 90*(3), 2805–2813. https://doi.org/10.1590/0001-3765201820170809

Surjowardojo, P., Tri, E. S., Gabriel, R., & Sirait. (2015). Inhibitory power of Manalagi apple peel decoction (*Malus sylvestris* Mill.) on the growth of *Staphylococcus aureus* and *Pseudomonas* sp. causing mastitis in dairy cows. *Tropical Animal Science Journal, 16*(2), 40–48.

Upadhyay, S., & Dixit, M. (2015). Role of polyphenols and other phytochemicals on molecular signaling. *Oxidative Medicine and Cellular Longevity, 2015*, Article 504253. https://doi.org/10.1155/2015/504253

Wardhani, R. R. A. A. K., & Pardede, A. (2022). Phytochemical analysis and antioxidant activity of methanol extract of stems, leaves, fruit skin and fruit of kelubut plant (*Passiflora foetida*). *Dalton: Journal of Chemical Education and Chemical Science, 5*(2), 62–74.

Wu, C.-C., Liu, C.-H., Chang, Y.-P., & Hsieh, S.-L. (2010). Effects of hot-water extract of *Toona sinensis* on immune response and resistance to *Aeromonas hydrophila* in

Oreochromis mosambicus. *Fish & Shellfish Immunology, 29*(2), 258–263. https://doi.org/10.1016/j.fsi.2010.04.021