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Antibacterial Activity of Breadfruit Leaf (*Artocarpus altilis*) Ethanol Extract Against *Vibrio* sp.

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Abstract

Vibrio sp. infections remain a major challenge in aquaculture due to their negative impacts on aquatic animal health, productivity, and sustainability. This study aimed to evaluate the antibacterial potential of ethanol extract from breadfruit (Artocarpus altilis) leaves against Vibrio sp. and characterize its bioactive secondary metabolites. Breadfruit leaves collected from Tual, Maluku, Indonesia, were extracted through maceration using 70% ethanol, yielding a crude extract of 5.4%. The antibacterial activity was evaluated using the disc diffusion method with extract concentrations of 100%, 75%, 50%, 25%, 12.5%, 6.25%, and 3.125%, using tetracycline and distilled water as positive and negative controls, respectively. The highest antibacterial activity was obtained at 100% extract concentration with an inhibition zone of 10.275 ± 0.106 mm, classified as strong activity, although lower than tetracycline (21.550 ± 1.131 mm). Statistical analysis demonstrated significant differences among treatments (Kruskal–Wallis, $p = 0.048$) and a very strong positive correlation between extract concentration and inhibition zone diameter (Spearman $r = 0.950$, $p < 0.001$). Phytochemical screening revealed alkaloids, flavonoids, steroids, saponins, and tannins, while FTIR analysis confirmed hydroxyl, carbonyl, aromatic, and C–O functional groups. These findings indicate that breadfruit leaf extract has potential as a natural antibacterial agent against Vibrio sp.

Keywords: Artocarpus altilis, antibacterial activity, breadfruit leaf, ethanol extract, Vibrio sp.

INTRODUCTION

Diseases caused by *Vibrio* sp. remain one of the major constraints in fisheries and aquaculture sectors because they affect aquatic animal health, production stability, and the sustainability of seafood supply chains. These bacterial pathogens can infect various cultured organisms, including fish, shrimp, and other seafood commodities, leading to reduced growth performance, increased mortality, and economic losses for aquaculture industries (Ghosh et al., 2021). Several species, such as *Vibrio parahaemolyticus*, *Vibrio harveyi*, *Vibrio alginolyticus*, and *Vibrio anguillarum*, have been widely reported as important pathogens affecting aquatic organisms. In shrimp farming, vibriosis is considered a serious disease problem because it can cause mass mortality, reduced growth, and significant economic losses, particularly in intensive and semi-intensive aquaculture systems (Lian, Liu et al., 2024). Recent studies also indicate that *Vibrio parahaemolyticus* occurring in aquaculture environments may possess antibiotic resistance patterns and resistance genes, highlighting the need for more sustainable and environmentally responsible approaches to disease management in fisheries and aquaculture (Zhang et al., 2022)

The application of synthetic antibiotics has been widely used in aquaculture practices due to their rapid antibacterial effects in controlling bacterial infections. However, excessive and inappropriate antibiotic usage in aquatic farming systems may accelerate the development of antimicrobial resistance, increase the risk of antibiotic residues in fisheries products, and negatively affect microbial balance in aquatic ecosystems. These concerns have become increasingly important because fisheries and aquaculture industries require disease control strategies that can maintain productivity while ensuring food safety and environmental sustainability (Jubair, Rajagopal,

Chinnappan, Abdullah, & Fatima, 2021). Therefore, the exploration of natural antibacterial agents has become a promising approach for improving aquatic health management. Plant-based extracts are considered potential alternatives because they contain various bioactive secondary metabolites, including flavonoids, tannins, saponins, alkaloids, terpenoids, and phenolic compounds, which may inhibit bacterial growth through mechanisms such as cell membrane disruption, permeability alteration, enzyme inhibition, and interference with biofilm formation (Shohreh et al., 2025).

Several natural materials have been investigated as potential antibacterial agents for controlling *Vibrio* infections in aquatic organisms. *Shorea beccariana* stem extract showed antibacterial activity against *Vibrio parahaemolyticus* and contained bioactive compounds such as alkaloids, flavonoids, saponins, tannins, and steroids (Anisa, Mukti, Mahasri, Amin, & Siti, 2025). Similarly, leaf extracts of *Avicennia marina* and *Avicennia alba* demonstrated potential antibacterial effects against *Vibrio* sp. isolates obtained from intensive shrimp farming environments (Ong et al., 2023). Other studies have also reported the antibacterial potential of *Chromolaena odorata*, *Isotoma longiflora*, *Nothopanax scutellarium*, *Moringa oleifera*, *Cotylelobium lanceotatum*, *Averrhoa bilimbi*, and *Biancaea sappan* extracts against *Vibrio* sp. or *Vibrio parahaemolyticus*, indicating the increasing interest in natural products for sustainable disease management in aquaculture systems (Hitijahubessy et al., 2024).

Breadfruit leaves (*Artocarpus altilis*) represent a tropical plant resource with potential for future application in fisheries and aquaculture health management. This plant has been traditionally used for medicinal purposes and is known to contain various bioactive compounds, including flavonoids, phenolics, tannins, alkaloids, saponins, and terpenoids, which are associated with antimicrobial and biological activities (Yuswantina & Karminingtyas, 2025). Previous studies have demonstrated that breadfruit leaf extract exhibits antibacterial activity against several pathogenic bacteria, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Propionibacterium acnes*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella enterica* (Lenny & Zuhra, 2023). Furthermore, recent toxicity evaluation showed that ethanol extract of breadfruit leaves has a safety profile supporting its potential development as a herbal medicine candidate (Fitrya et al., 2025). However, despite its demonstrated antibacterial potential against various terrestrial bacterial pathogens, information regarding the antibacterial activity of breadfruit leaf ethanol extract against *Vibrio* sp. associated with fisheries and aquaculture remains limited. Previous studies have predominantly focused on the antibacterial activity of breadfruit leaves against human or terrestrial bacterial pathogens. In contrast, studies on natural antibacterial agents against *Vibrio* have mainly investigated other plant species. Consequently, there remains a research gap concerning the specific response of *Vibrio* sp. to breadfruit leaf ethanol extract, particularly regarding the relationship between extract concentration and bacterial growth inhibition. Therefore, the novelty of this study lies not only in evaluating breadfruit leaf ethanol extract against *Vibrio* sp. associated with aquatic production systems, but also in determining its concentration-dependent antibacterial activity and identifying an effective concentration capable of inhibiting bacterial growth. This study thus expands the research context of *Artocarpus altilis* by evaluating its previously reported antibacterial properties against an aquaculture-associated bacterial pathogen. At the same time, its practical use in aquaculture remains a potential area for future investigation. The findings are expected to provide preliminary scientific evidence supporting the future development of breadfruit leaf ethanol extract as a locally available, plant-based antibacterial candidate for more sustainable disease management in fisheries and aquaculture.

METHODOLOGY

Materials and Instrumentals

The materials used in this study were seawater samples from the coastal area of Watdek, Southeast Maluku Regency, breadfruit leaves (*Artocarpus altilis*), Thiosulfate Citrate Bile Salts Sucrose (TCBS) medium, distilled water, 70% ethanol, sterile cotton swabs, 6 mm diameter tetracycline antibiotic discs, 6 mm diameter blank paper discs, and red and blue litmus paper.

Methods

Sample Preparation

Fresh leaves of breadfruit (*Artocarpus altilis*) were collected from Tual, Maluku, Indonesia. The plant material was authenticated based on morphological characteristics before further processing. Healthy and mature leaves were selected based on their intact appearance and the absence of visible fungal infection, mechanical damage, or contamination. The collected leaves were washed thoroughly with running tap water to remove adhering soil and impurities, followed by rinsing with distilled water. The leaves were drained, cut into smaller pieces, and dried at room temperature in a clean, well-ventilated, and shaded area for five days to minimize

degradation of heat- and light-sensitive phytoconstituents. The dried leaves were subsequently ground into a fine powder using a laboratory blender and sieved to obtain a uniform particle size. The resulting powdered material (simplicia) was stored in an airtight container under dry conditions until extraction (Rahmawati et al.,2025).

Preparation of Breadfruit Leaf Extract

Dried breadfruit (*Artocarpus altilis*) leaf powder (500 g) was extracted using the maceration method with 70% ethanol as the extraction solvent. The plant material was soaked in 2 L of 70% ethanol (solid-to-solvent ratio 1:4, w/v) in a clean Erlenmeyer flask. The mixture was homogenized by stirring, covered with aluminum foil, and maintained at room temperature for five days while protected from direct light exposure. The extraction mixture was intermittently agitated to enhance solvent penetration and facilitate the release of bioactive compounds. After maceration, the extract mixture was filtered through clean muslin cloth followed by Whatman No. 1 filter paper to remove insoluble plant residues. The obtained filtrate was concentrated under reduced pressure using a rotary evaporator at a controlled temperature until a viscous crude extract was obtained. The concentrated extract was transferred into an amber sterile glass container, tightly sealed, and stored at 4 °C until further phytochemical and antibacterial analyses. The extraction yield was calculated as the percentage ratio between the weight of dried extract and the initial weight of dried plant powder.

Determination of Extract Yield

The extraction yield was calculated to determine the percentage of extract obtained from the dried breadfruit leaf powder. The yield was calculated using the following formula (Zhang et al., 2022):

$$\text{Extract yield (\%)} = \frac{Weight\ of\ concentrated\ extract}{Weight\ of\ dried\ breafriut\ leaf\ powder}\ x\ 100\%$$

Antibacterial Activity Assay and Measurement of Inhibition Zone Diameter

Sterilization of Instruments and Materials

All glassware and laboratory materials used for bacterial isolation and antibacterial assays were properly cleaned with detergent, rinsed thoroughly with distilled water, dried, and sterilized before use. Heat-resistant glassware, including Petri dishes, test tubes, pipettes, and Erlenmeyer flasks, was sterilized using a hot-air oven at 170 °C for 1–2 h. Culture media and other heat-sensitive microbiological materials were sterilized by autoclaving at 121 °C and 15 psi for 15 min, following standard microbiological procedures and manufacturer recommendations. All microbiological manipulations, including bacterial inoculation, culture preparation, and antibacterial testing, were conducted under aseptic conditions to minimize contamination and ensure the reliability of experimental.

Seawater Sample Collection

Seawater samples were collected from the coastal area of Watdek, Southeast Maluku Regency, Indonesia. Sampling was conducted using sterile sample bottles. The bottles were filled with seawater, tightly closed, and immediately transported to the laboratory for bacterial isolation. The samples were processed as soon as possible to maintain the viability of the bacteria.

Preparation of TCBS Medium

TCBS agar was used as a selective and differential medium for the isolation and presumptive identification of *Vibrio* spp. The medium was prepared according to the manufacturer's instructions. Briefly, 88 g of TCBS powder was suspended in 1 L of distilled water (equivalent to 8.8 g in 100 mL). The suspension was heated with continuous stirring until completely dissolved and was not autoclaved because excessive heating may reduce the selectivity and performance of TCBS medium. The prepared medium was cooled to approximately 37 °C and aseptically poured into sterile Petri dishes to obtain an agar depth of approximately 4 mm. The medium was then allowed to solidify at room temperature before inoculation. TCBS agar plates were subsequently used for the isolation of presumptive *Vibrio* colonies (Akbar et al.,2024).

Isolation and Preparation of *Vibrio* sp.

Seawater samples were processed for the isolation of presumptive *Vibrio* spp. using Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar as a selective and differential medium. Briefly, seawater samples were directly inoculated onto TCBS agar plates and incubated at 37 °C for 24 h. Colonies showing typical *Vibrio*-like characteristics on TCBS agar were selected based on colony morphology and pigmentation. The presumptive *Vibrio* colonies were purified by repeated streaking on fresh TCBS agar plates to obtain pure bacterial isolates. The purified isolates were cultured in appropriate broth medium to prepare bacterial suspensions for antibacterial activity testing. The bacterial suspension was adjusted to the turbidity equivalent of a 0.5 McFarland standard to obtain a standardized inoculum density before inoculation into agar plates. The standardized bacterial suspension

was subsequently used for the antibacterial assay (Haque et al., 2023).

Preparation of Extract Concentrations and Controls

The concentrated breadfruit leaf extract was used as the stock extract. The extract was prepared into seven concentrations: 100%, 75%, 50%, 25%, 12.5%, 6.25%, and 3.125%. The extract concentrations were expressed as % (w/v). A 100% extract concentration was defined as 500 mg/mL, prepared by dissolving 500 mg of crude extract in 1 mL of distilled water. Subsequent concentrations were prepared by serial dilution, resulting in concentrations of 75% (375 mg/mL), 50% (250 mg/mL), 25% (125 mg/mL), 12.5% (62.5 mg/mL), 6.25% (31.25 mg/mL), and 3.125% (15.625 mg/mL). Tetracycline antibiotic discs were used as the positive control, while sterile distilled water was used as the negative control. Sterile blank paper discs were used as carriers for the breadfruit leaf extract and as the negative control. In order to improve methodological clarity, the basis of the extract concentration should be clearly stated, for example, as a percentage of stock extract, weight per volume, or volume per volume. This is important because international journals require a clear and reproducible description of extract preparation.

Antibacterial Activity Assay

The antibacterial activity of breadfruit (*Artocarpus altilis*) leaf extract against *Vibrio* sp. was evaluated using the disc diffusion method. Briefly, a standardized bacterial suspension was prepared from fresh *Vibrio* sp. cultures and adjusted to a turbidity equivalent to a 0.5 McFarland standard to obtain a uniform inoculum density. The bacterial suspension was evenly spread onto the surface of Mueller–Hinton agar (MHA) plates using a sterile cotton swab. Sterile paper discs were impregnated with 2 µL of breadfruit leaf extract at each tested concentration and allowed to stand aseptically at room temperature for approximately 10–15 min to facilitate complete absorption of the extract. Before application, the discs were visually checked to ensure that the liquid had been fully absorbed and that the disc surface was no longer visibly wet. The impregnated discs were then placed onto the inoculated MHA plates using sterile forceps. Tetracycline discs were used as the positive control, while sterile paper discs treated with 2 µL of distilled water were used as the negative control and handled under the same conditions. The discs were positioned at an appropriate distance to avoid overlapping inhibition zones. The plates were incubated at 37 °C for 24 h (Bijang et al.,2024). After incubation, the diameter of the inhibition zones surrounding each disc was measured using a digital caliper. The antibacterial activity was expressed as the mean inhibition zone diameter ± standard deviation (SD) in millimeters (mm). All experiments were performed in duplicate, with each replicate conducted independently on a separate MHA plate to ensure independent measurements and minimize potential interference between replicates (Bontzolis et al., 2024).

Measurement of Inhibition Zone Diameter

The antibacterial activity was evaluated using the disc diffusion method. Sterile blank paper discs used for the breadfruit leaf extract and negative control, as well as the antibiotic discs used as the positive control, had a diameter of 6 mm. After incubation, the diameter of the inhibition zone formed around each paper disc was measured using a digital caliper. The measurement was performed in two perpendicular directions (horizontal and vertical), and the average diameter was calculated. Since the measured inhibition zone included the 6-mm disc diameter, the diameter of the paper disc was subtracted from the average measured value to obtain the net inhibition zone diameter. All experiments were performed in triplicate, and the antibacterial activity was expressed as the mean net inhibition zone diameter ± standard deviation (SD) in millimeters (mm). Similar approaches have been applied for evaluating the antibacterial activity of plant extracts using disc diffusion assays (Atta et al., 2023)

Classification of Antibacterial Activity

The antibacterial activity was classified based on the mean diameter of the inhibition zone (Hutapea et al., 2024). The classification criteria were as follows in Table 1.

Table 1. Classification of Antibacterial Activity

Inhibition Zone Diameter	Inhibitory Activity
D < 5 mm	Weak
5 mm < D < 10 mm	Moderate
10 mm < D < 20 mm	Strong
D > 20 mm	Very strong

The mean inhibition zone diameter of each treatment was used to determine the inhibitory activity category.

Phytochemical Screening

Phytochemical screening was performed to detect the presence of secondary metabolites in breadfruit leaf samples, including alkaloids, flavonoids, saponins, tannins, steroids, and triterpenoids.

1. Alkaloid Test

A total of 4 g of breadfruit leaf powder was mixed with chloroform and ground thoroughly. Then, 10 mL of

ammonia and 10 mL of chloroform were added to the mixture. The mixture was filtered into a test tube, and 10 drops of 2N sulfuric acid were added to the filtrate. The solution was shaken and allowed to stand until two layers were formed.

The upper layer was transferred into three separate test tubes and tested using Mayer, Dragendorff, and Wagner reagents. The formation of a white precipitate with Mayer reagent, an orange-red precipitate with Dragendorff reagent, or a brown precipitate with Wagner reagent indicated the presence of alkaloids.

2. Triterpenoid and Steroid Test

A total of 50–100 mg of breadfruit leaf powder was placed on a spot plate and mixed with acetic anhydride until the sample was completely submerged. The mixture was allowed to stand for approximately 15 minutes. Several drops of the solution were transferred into a test tube and mixed with concentrated sulfuric acid.

The formation of an orange-red or purple color indicated the presence of triterpenoids, while the formation of a blue or greenish-blue color indicated the presence of steroids.

3. Flavonoid Test

A total of 200 mg of breadfruit leaf sample was extracted with 5 mL of ethanol and heated for five minutes. Several drops of concentrated hydrochloric acid were added, followed by 0.2 g of magnesium powder. The formation of a dark red, orange, or magenta color within three minutes indicated the presence of flavonoids.

4. Saponin Test

A total of 2 g of breadfruit leaf powder was placed into a test tube and mixed with distilled water until the sample was fully submerged. The mixture was boiled for 2–3 minutes, cooled, and shaken vigorously. The formation of stable foam indicated the presence of saponins.

5. Tannin Test

A total of 20 mg of breadfruit leaf powder was mixed with ethanol. Then, 1 mL of the solution was transferred into a test tube and mixed with 2–3 drops of 1% ferric chloride solution. The formation of a bluish-black or greenish color indicated the presence of tannins.

Statistical Analysis

The inhibition zone diameter data were analyzed descriptively and statistically. Descriptive analysis was performed by calculating the mean and standard deviation for each treatment group. The results were presented as mean ± standard deviation. Because the number of replications was limited and the normality assumption could not be reliably tested, a non-parametric Kruskal-Wallis test was used to determine whether there were significant differences in inhibition zone diameter among treatment groups. Spearman correlation analysis was also performed to evaluate the relationship between extract concentration and inhibition zone diameter. All statistical analyses were interpreted at a 5% significance level. A p-value of less than 0.05 was considered statistically significant.

FTIR Analysis

The extract was analyzed using an Agilent Cary 630 FTIR spectrophotometer at the Organic Chemistry Laboratory of Pattimura University, equipped with Microlab PC software and an ATR sampling unit, with a resolution of 5 cm^{-1} and a scanning range of 4000 cm^{-1} to 500 cm^{-1}.

RESULTS AND DISCUSSION

Extract Yield

The extraction of breadfruit leaves (*Artocarpus altilis*) using 70% ethanol produced 27 g of thick extract from 500 g of dried leaf powder. The extract yield was calculated by dividing the weight of the obtained extract by the weight of the initial powdered sample and multiplying it by 100%. Based on this calculation, the extract yield was 5.4%. This result indicates that ethanol was able to dissolve and extract bioactive compounds from breadfruit leaves, although the yield was relatively moderate.

The use of ethanol as an extraction solvent is relevant because ethanol can dissolve polar and semi-polar secondary metabolites, including flavonoids, phenolics, tannins, alkaloids, and saponins. Previous studies on *Artocarpus altilis* leaves reported that ethanolic extracts contain flavonoids, phenolic compounds, tannins, and other bioactive constituents that are associated with antibacterial and antioxidant activities (Effendy et al., 2023)

Antibacterial Activity Based on Inhibition Zone

The antibacterial activity of ethanol extract of breadfruit leaves against *Vibrio* sp. was evaluated using the disc diffusion method. The inhibition zone was measured after incubation at 37°C for 24 hours (Bijang et al., 2025). The extract was tested at seven concentrations, namely 100%, 75%, 50%, 25%, 12.5%, 6.25%, and 3.125%. A 100% extract concentration was defined as 500 mg/mL, prepared by dissolving 500 mg of crude extract in 1 mL of distilled water. Subsequent concentrations were prepared by serial dilution, resulting in concentrations of 75% (375 mg/mL), 50% (250 mg/mL), 25% (125

mg/mL), 12.5% (62.5 mg/mL), 6.25% (31.25 mg/mL), and 3.125% (15.625 mg/mL). Tetracycline was used as the positive control, while distilled water was used as the negative control. The results obtained can be seen in Table 2.

Table 2. Mean inhibition zone and inhibitory activity classification

No.	Extract concentration	Mean Inhibition Zone (mm)	Inhibitory Activity Classification
1	100%	10.275 ± 0.106	Strong
2	75%	5.900 ± 3.111	Moderate
3	50%	3.600 ± 1.485	Weak
4	25%	0.600 ± 0.212	Weak
5	12.5%	0.000 ± 0.000	Weak
6	6.25%	0.000 ± 0.000	Weak
7	3.125%	0.000 ± 0.000	Weak
8	Positive Control (Tetracycline)	21.550 ± 1.131	Very Strong
9	Negative Control (Aquades)	0.000	Weak

Based on the results presented in Table 2, the ethanol extract of breadfruit (*Artocarpus altilis*) leaves showed concentration-dependent inhibitory activity against *Vibrio* sp., with greater inhibition observed at higher extract concentrations. The 100% concentration produced the highest mean inhibition zone among the extract treatments, measuring 10.275 ± 0.106 mm, and was classified as strong according to the classification criteria applied in this study. The small standard deviation indicates relatively consistent measurements between the two independent replicates, which produced inhibition zones of 10.35 and 10.20 mm, respectively.

At the 75% concentration, the mean inhibition zone decreased to 5.900 ± 3.111 mm and was classified as moderate. The relatively large standard deviation indicates substantial variation between the two replicates, which produced inhibition zones of 8.10 and 3.70 mm. Such variability could reflect differences in extract diffusion through the agar, distribution of extract within the paper disc, or other experimental variation between the independently prepared plates. At the 50% concentration, the inhibition zone further decreased to 3.600 ± 1.485 mm, while the 25% concentration produced only 0.600 ± 0.212 mm; both were classified as weak. No measurable inhibition was observed at concentrations of 12.5%, 6.25%, and 3.125%, indicating that under the conditions of this assay, these lower concentrations were insufficient to produce a detectable inhibition zone.

Compared with previous studies investigating plant-derived antibacterial agents against *Vibrio parahaemolyticus*, the maximum inhibition observed for breadfruit leaf extract in the present study was relatively modest. Kongchum et al. (2016) reported inhibition zones of approximately 14.4–16.4 mm for aqueous green tea (*Camellia sinensis*) extracts against *V. parahaemolyticus*. Their study also demonstrated an MIC of 10% (v/v) and reported improved survival of challenged Pacific white shrimp following green tea treatment. More recently, Linh et al. (2026) reported inhibition zones of 25.67 ± 0.58 mm for *Lagerstroemia speciosa* extract and 18.33 ± 0.58 mm for *Limnophila aromatica* extract against AHPND-associated *V. parahaemolyticus*. These reported values were greater than the 10.275 ± 0.106 mm observed at the highest breadfruit leaf extract concentration in the present study.

However, direct numerical comparison among these studies should be interpreted cautiously. Differences in plant species, extraction solvent, extract concentration, amount of extract applied, bacterial strain and inoculum density, agar composition, and diffusion assay procedure can substantially influence inhibition-zone diameter. For example, Kongchum et al. (2016) used an agar-well diffusion assay, whereas the present study employed 6-mm paper discs loaded with 2 µL of extract. In addition, the inhibition values in the present study were calculated as net inhibition zones after subtraction of the 6-mm paper-disc diameter; therefore, they may not be directly equivalent to values reported as total inhibition-zone diameters in other studies. Consequently, the smaller inhibition zone observed for breadfruit leaf extract should not by itself be interpreted as evidence of inferior antibacterial efficacy without standardized comparative testing.

The positive control, tetracycline, produced a substantially larger inhibition zone of 21.550 ± 1.131 mm and was classified as very strong, whereas the negative control (distilled water) produced no inhibition zone. This indicates that the observed inhibitory effect in the extract-treated groups was associated with the extract treatment rather than distilled water. Nevertheless, the considerably smaller inhibition zone of the breadfruit leaf extract compared with tetracycline indicates that, under the experimental conditions used, the crude extract exhibited lower in vitro inhibitory activity than the antibiotic control. Overall, the results demonstrate measurable antibacterial activity of breadfruit leaf ethanol extract against *Vibrio* sp., particularly at 100% concentration, while also indicating that further determination of MIC and MBC would be necessary to characterize its antibacterial potency more comprehensively.

At concentrations of 12.5%, 6.25%, and 3.125%, no inhibition zone was formed, as all inhibition zone

diameter values were 0 mm. This indicates that at these concentrations, breadfruit leaf extract was not able to produce a visible inhibitory effect macroscopically. Therefore, it can be stated that the effectiveness of the extract was strongly influenced by its concentration level. The Tetracycline group had the largest inhibition zone diameter, measuring 22.35 mm. If Tetracycline was used as the positive control, this result indicates that the positive control had a much stronger inhibitory effect than all concentrations of breadfruit leaf extract. Meanwhile, Aquades produced an inhibition zone of 0 mm, indicating that it did not have an inhibitory effect on the tested microorganism.

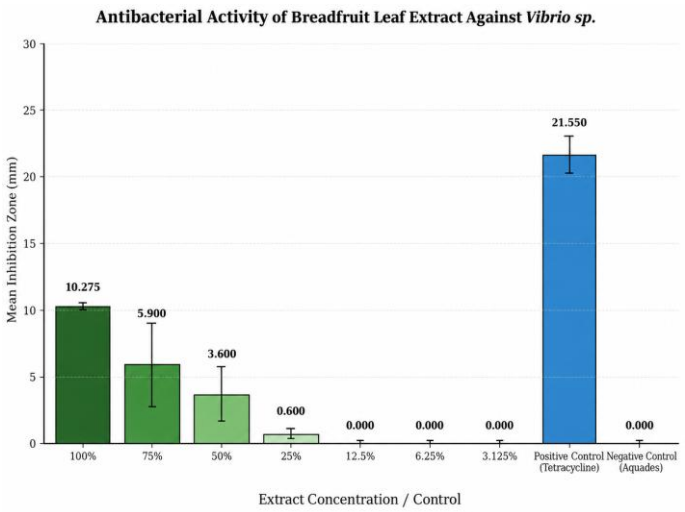

Figure 1. Mean inhibition zone diameter

The Kruskal-Wallis test showed a p-value of 0.048, indicating a significant difference among the concentrations of breadfruit leaf extract. This supports the descriptive findings that the higher the extract concentration, the larger the inhibition zone formed. The Spearman correlation test also supported this pattern, with a correlation coefficient of 0.950 and a p-value of < 0.001, indicating a very strong positive relationship between extract concentration and inhibition zone diameter.

Overall, the data indicate that breadfruit leaf extract has potential as an antibacterial or antimicrobial agent, particularly at higher concentrations. The most effective concentration in this study was the 100% extract, as it produced the largest inhibition zone among the extract groups and showed the most stable replicate results. However, to strengthen the conclusion, future studies should increase the number of replicates to at least 3–5 per group, so that the statistical results become more robust and further tests, such as ANOVA or post-hoc analysis, can be conducted more validly.

Phytochemical analysis

Phytochemical analysis was conducted on the ethanol extract of breadfruit leaves (*Artocarpus altilis*). The results of the phytochemical screening in this study are presented in Table 3.

Table 3. Phytochemical screening results of ethanol extract of breadfruit leaves (*Artocarpus altilis*)

No.	Phytochemical Test	Reagent	Test Result	Reaction Result
1	Alkaloids	Wagner	+	Brown precipitate was formed
		Mayer	+	White precipitate was formed
		Dragendorff	+	Orange-red precipitate was formed
2	Triterpenoids	—	-	No purple color was formed
3	Steroids	—	+	Dark green precipitate was formed
4	Flavonoids	—	+	Red coloration was formed
5	Saponins	—	+	Foam was formed
6	Tannins	—	+	Greenish-black precipitate was formed

Note:
(+) indicates the presence of secondry metabolits
(-) indicates the absence of secondary metabolites.

The phytochemical screening results presented in Table 3 showed positive qualitative reactions for alkaloids, steroids, flavonoids, saponins, and tannins in the ethanol extract of breadfruit leaves (*Artocarpus altilis*). In contrast, the triterpenoid test showed no characteristic positive reaction under the experimental conditions. These results should be interpreted as preliminary qualitative indications of the possible presence of these secondary metabolite groups based on characteristic color changes, precipitate formation, or foam formation. Therefore, the screening provides only qualitative indications of the presence of particular phytochemical groups and does not allow definitive identification of the individual phytochemical constituents present in the extract. Previous studies have also reported phenolic and flavonoid-related constituents in ethanol extracts of *A. altilis* leaves and described their potential biological activities (Effendy et al., 2023). However, the present qualitative screening alone cannot establish which specific compounds are responsible for the observed antibacterial activity or determine their individual contribution to the inhibition of *Vibrio* sp.

The alkaloid test showed positive qualitative reactions with Wagner, Mayer, and Dragendorff reagents. The formation of a brown precipitate with Wagner reagent, a white precipitate with Mayer reagent, and an orange-red precipitate with Dragendorff reagent suggested the possible presence of alkaloid-type compounds in the extract. These reactions should be interpreted as preliminary qualitative indications rather than definitive identification of individual alkaloid compounds. (Hasanela & Souhoka, 2022). Alkaloids have been reported to exhibit antibacterial activity through several mechanisms, including disruption of bacterial cell membrane permeability, inhibition of bacterial metabolism, and interference with nucleic acid and protein synthesis (Ngamsurach & Praipipat, 2022). However, the present qualitative screening did not determine the specific identity of alkaloid compounds or their individual contribution to the antibacterial activity observed in this study.

The triterpenoid test showed a negative qualitative result, as no purple color was formed under the experimental conditions used. This result indicates that no characteristic reaction associated with triterpenoids was observed; however, it does not necessarily confirm the complete absence of triterpenoid compounds because the response of qualitative phytochemical tests may vary depending on the extraction conditions and the characteristics of the compounds present. In contrast, the steroid test produced a positive qualitative reaction, marked by the formation of a dark green precipitate, suggesting the possible presence of steroid-related constituents. Steroid compounds have been reported to possess antimicrobial activity by affecting microbial cell membrane integrity, which may lead to leakage of intracellular components and subsequent cell damage (Effendy et al., 2023). Nevertheless, the present qualitative test does not establish whether steroid-related constituents were directly responsible for the antibacterial effect observed against *Vibrio* sp.

The flavonoid test also showed a positive qualitative reaction, indicated by the formation of a red coloration. This reaction suggests the possible presence of flavonoid-related constituents in the ethanol extract, but it does not provide definitive identification of individual flavonoid compounds. Flavonoids have been widely reported to possess antibacterial properties through mechanisms that may include disruption of bacterial cell membranes, inhibition of nucleic acid synthesis, inhibition of energy metabolism, and interference with bacterial biofilm formation (Farhadi et al., 2019). Therefore, the qualitative flavonoid reaction observed in this study may indicate the presence of constituents with chemical characteristics associated with flavonoids; however, their specific role in the inhibition of *Vibrio* sp. cannot be established from the present phytochemical screening alone.

The saponin test produced foam formation, which represents a positive qualitative reaction suggestive of saponin-like constituents in the extract. This observation provides preliminary qualitative evidence of compounds with surfactant-like characteristics but does not confirm the identity of individual saponins. Saponins have been reported to exhibit antimicrobial activity through interactions with lipid components of microbial membranes, which may increase membrane permeability and disturb membrane stability (Li & Monje-Galvan, 2023). Similarly, the tannin test produced a greenish-black precipitate, suggesting the possible presence of tannin-related polyphenolic constituents. Tannins have been reported to inhibit bacterial growth through several mechanisms, including iron chelation, inhibition of cell wall synthesis, disruption of the cell membrane, and inhibition of bacterial virulence factors (Farha et al., 2020). However, based on the qualitative nature of the present screening, the individual contribution of saponin- and tannin-related constituents to the antibacterial activity cannot be determined.

Overall, the qualitative phytochemical reactions observed in this study suggest the possible presence of several classes of secondary metabolites in the ethanol extract of breadfruit leaves. However, these color-, precipitation-, and foam-based tests should be regarded only as preliminary qualitative screening methods. They indicate the possible presence or absence of particular phytochemical groups but do not establish the specific chemical identity of the individual constituents or their contribution to the observed antibacterial activity. Further phytochemical characterization using more specific analytical techniques would be required to confirm the compounds represented by these qualitative reactions and clarify their potential role in the antibacterial effect.

Analysis using the FTIR test

In this study, qualitative analysis was performed through phytochemical screening and FTIR spectroscopy. Phytochemical screening was used to provide preliminary indications of the presence of several classes of secondary metabolites, while FTIR spectroscopy was used as a qualitative analytical technique to identify the characteristic functional groups and chemical bonds present in the ethanolic breadfruit leaf extract. The identification was based on the characteristic absorption bands observed in the FTIR spectrum. The FTIR spectrum of the ethanolic breadfruit leaf extract is shown in Figure 2.

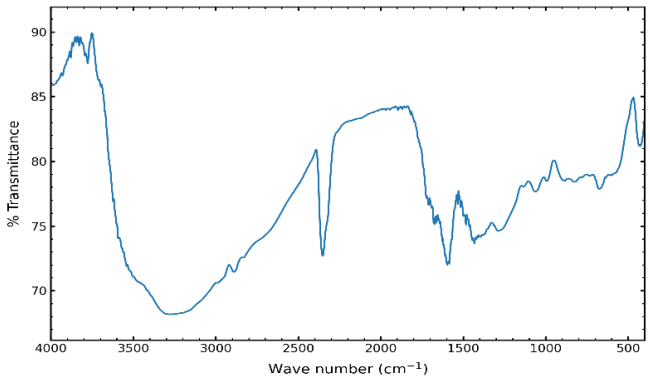

Figure 2. FTIR spectrum of the ethanolic extract of breadfruit leaves.

Based on Figure 2, the FTIR spectrum of breadfruit (*Artocarpus altilis*) leaf extract showed several absorption regions corresponding to different functional groups. A broad absorption band observed in the range of 3200–3500 cm^{-1} was associated with O–H stretching vibrations, which are commonly found in hydroxyl-containing compounds such as phenolics, flavonoids, tannins, and alcohols. Absorption in the 2850–2950 cm^{-1} region was attributed to aliphatic C–H stretching vibrations of CH_2 and CH_3 groups, which may be associated with terpenoid, steroid, or lipid-related structures. The absorption region around 1700–1650 cm^{-1} was assigned to C=O stretching vibrations, indicating the presence of carbonyl-related functional groups that may occur in flavonoids, esters, organic acids, or other carbonyl-containing compounds. Bands observed between 1600 and 1500 cm^{-1} were associated with aromatic C=C stretching vibrations, whereas the 1450–1400 cm^{-1} region was related to C–H bending and O–H deformation. These absorption regions are commonly associated with aromatic and hydroxyl-containing organic compounds.

In the fingerprint region, absorption between 1300 and 1000 cm^{-1} was assigned to C–O and C–O–C stretching vibrations, which may be related to compounds containing alcohol, ether, glycosidic, or phenolic structures. Absorption observed in the 900–700 cm^{-1} region was associated with aromatic C–H bending vibrations, while bands within the 600–500 cm^{-1} region represented skeletal vibrations in the fingerprint region. These results are shown in Table 4.

Overall, the FTIR profile provides qualitative information regarding the functional groups present in the breadfruit leaf extract. The observed absorption bands may be associated with several classes of plant secondary metabolites; however, FTIR analysis alone does not provide definitive identification of individual compounds or specific metabolite classes. Therefore, the assignments presented in Table 4 should be interpreted as possible functional-group associations rather than confirmation of particular secondary metabolites.

Table 4. FTIR Peak Assignment and Functional Group Identification of Breadfruit (*Artocarpus altilis*) Leaf Extract

No.	Wave number (cm^{-1})	FTIR Assignment	Functional Group/Vibration
1	3200–3500	Broad absorption band	O–H stretching vibration (hydroxyl group)
2	2850–2950	Medium absorption band	Aliphatic C–H stretching vibration (CH_2/CH_3)
3	1700–1650	Carbonyl absorption region	C=O stretching vibration
4	1600–1500	Aromatic absorption region	C=C stretching vibration of aromatic rings
5	1450–1400	Bending vibration region	C–H bending and O–H deformation
6	1300–1000	Fingerprint region	C–O and C–O–C stretching vibrations
7	900–700	Aromatic fingerprint region	Aromatic C–H bending vibration
8	600–500	Fingerprint region	Skeletal vibrations of organic molecules

CONCLUSION

In conclusion, ethanol extract of breadfruit (*Artocarpus altilis*) leaves demonstrated potential antibacterial activity against *Vibrio* sp. and contained several bioactive secondary metabolites that may contribute to its inhibitory effect. Maceration extraction using 70% ethanol produced a thick extract with a yield of 5.4%, indicating that ethanol was effective in extracting polar and semi-polar bioactive compounds from breadfruit leaves. The antibacterial assay revealed that the extract exhibited concentration-dependent inhibitory activity, with the highest activity observed at 100% extract concentration, producing an inhibition zone of 10.275 ± 0.106 mm, classified as strong activity. However, the effect remained lower than the tetracycline positive control (21.550 ± 1.131 mm). Statistical analysis confirmed a significant difference among extract concentrations (Kruskal–Wallis, $p = 0.048$) and demonstrated a very strong positive correlation between extract concentration and inhibition zone diameter (Spearman correlation coefficient = 0.950, $p < 0.001$).

Phytochemical screening revealed the presence of alkaloids, flavonoids, steroids, saponins, and tannins. FTIR analysis confirmed the presence of functional groups associated with hydroxyl (O–H), carbonyl (C=O), aromatic (C=C), and C–O bonds, which are characteristic

of phenolic compounds, flavonoids, tannins, and other bioactive metabolites. These secondary metabolites may contribute to antibacterial activity through mechanisms involving bacterial membrane disruption, enzyme inhibition, protein denaturation, and interference with essential cellular processes. Therefore, breadfruit leaf extract (*Artocarpus altilis*) has potential as a natural antibacterial agent against *Vibrio* sp., although further studies involving increased replication, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and molecular identification of active compounds are recommended to validate its antibacterial potential.

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