

Antibacterial Efficacy of Aqueous and Ethanolic Extracts of Pear (*Pyrus communis*) Leaves Against Bacterial Pathogens Isolated from Infected African Catfish (*Clarias gariepinus*)

RESEARCH ARTICLE

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ABSTRACT

The increasing prevalence of antibiotic-resistant pathogens in aquaculture has heightened the need for natural alternatives. This study investigates the antibacterial activity of both aqueous and ethanolic extracts of pear (*Pyrus communis*) leaves against bacterial isolates obtained from infected African catfish (*Clarias gariepinus*). Diseased fish were collected from multiple farms in Ondo State, Nigeria. Pathogens such as *Aeromonas hydrophila*, *Streptomyces spp.*, and *Flavobacterium spp.* were isolated from infected tissues. Aqueous and ethanolic extracts were prepared and tested using the agar well diffusion method. The ethanolic extract exhibited significantly higher antibacterial activity across all concentrations (100, 200, and 400 mg/mL), with inhibition zones ranging from 12.2 ± 0.2 mm to 24.7 ± 0.2 mm. *Streptomyces* showed the highest sensitivity to both extracts, while *Flavobacterium* displayed the lowest. The results affirm the potential of *Pyrus communis* leaf extracts as effective antimicrobial agents in aquaculture and support the integration of plant-based therapies for sustainable fish health management.

Study Design Agar well diffusion method testing aqueous and ethanolic extracts at concentrations of 100, 200, and 400 mg/mL	Key Pathogens <i>Aeromonas hydrophila</i> , <i>Streptomyces spp.</i> , and <i>Flavobacterium spp.</i> isolated from infected catfish	Main Finding Ethanolic extract showed superior antimicrobial activity with inhibition zones up to 24.7 mm
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Keywords: Antimicrobial activity, Ethanolic extract, *Streptomyces*, *Clarias gariepinus*, *Flavobacterium*.

INTRODUCTION

Clarias gariepinus (African catfish) is widely farmed in Africa, but its productivity is often hampered by bacterial infections such as those caused by *Aeromonas hydrophila*, *Flavobacterium* spp., and *Streptomyces* spp. These pathogens result in conditions like fin rot, ulceration, and haemorrhagic septicemia, with *A. hydrophila* being particularly aggressive (Caruso et al., 2024). Due to over-reliance on synthetic antibiotics, resistant strains have emerged (Pepi & Focardi, 2021), prompting a need for safe, cost-effective alternatives (Abdel-Latif et al., 2025).

The pear tree, scientifically known as *Pyrus communis*, is predominantly cultivated in temperate climates for its commercially valuable fruits. Recent scientific investigations have increasingly focused on its leaves, which are recognised for their rich phytochemical profile (Kruczyńska, 2020). The biochemical constituents of pear leaves include various biologically active compounds that demonstrate a plethora of pharmacological properties, including antioxidant, antimicrobial, anti-inflammatory, and cytotoxic effects (Kumar et al., 2021).

01	02	03
Aquaculture Challenge	Antibiotic Resistance	Plant-Based Solutions
African catfish farming faces significant losses from bacterial pathogens, including <i>Aeromonas hydrophila</i> , leading to fin rot, ulceration, and haemorrhagic septicemia, which affect productivity.	Over-reliance on synthetic antibiotics has led to the emergence of resistant bacterial strains, creating an urgent need for natural alternatives in fish health management.	<i>Pyrus communis</i> leaves contain rich phytochemical profiles with demonstrated antimicrobial properties, offering promising natural therapeutic alternatives for aquaculture.

Numerous studies conducted over the past decade have provided substantial evidence supporting the therapeutic potential of *Pyrus communis* leaves. For instance, in vitro analyses have revealed that the extracts obtained from pear leaves exhibit significant antioxidant activity, effectively scavenging free radicals and mitigating oxidative stress (Kruczyńska, 2020). Furthermore, these leaves have shown promising antimicrobial activity against a range of pathogenic microorganisms, suggesting their potential as natural preservatives or therapeutic agents in treating infections (Kot et al., 2019).

Beyond their intrinsic antimicrobial properties, the use of *Pyrus communis* in aquaculture may offer additional benefits. Natural plant extracts can help alleviate stress responses in fish, boost immune function, and promote overall health (Riyaz et al., 2019). Incorporating such natural treatments into aquacultural practices supports the principles of sustainable aquaculture by reducing reliance on chemically synthesised antibiotics and fostering an ecosystem-focused approach to fish farming.

The findings from this study will provide valuable insights into the effectiveness of pear leaf extracts in inhibiting the growth of bacterial pathogens isolated from catfish. This research will

contribute to evaluating their potential as natural antimicrobial agents. Recognising this organic alternative could promote more sustainable aquaculture practices by reducing reliance on chemical treatments and addressing the risks associated with antibiotic resistance.

MATERIALS AND METHODS

Study Location

The study was carried out in the Fisheries and Aquaculture Laboratory of Wesley University, Ondo. The University is located at Km 3, Ondo - Ife Road, Ondo City, Ondo-West Local Government Area.

Collection of Infected Fish Samples

Diseased fish samples were randomly collected from three fish farms in Ondo-West Local Government Area of Ondo State. The farms will not be mentioned for the sake of confidentiality. The farms have earthen pond facilities. The collected samples were transported alive, while the dead ones were taken in an ice chest to the Fisheries and Aquaculture Laboratory, Department of Agriculture, Wesley University, Ondo, for observation and microbial analyses.

The infected fish were physically examined using physical descriptors described by Matter et al. (2018). The size of the fish was also considered to ascertain that the pathogen had passed its incubation, latent, and prodromal periods on the fish.

Collection of Plant Materials

Fresh and young pear leaves were collected from the ethnobotanical garden of Wesley University, Ondo. The leaves were taken to the university's botanist for identification. The samples were examined for any infection, spores, damage, discolouration, or distortion. The undamaged leaves were thoroughly washed with tap water, then rinsed with distilled water to remove any dirt or contaminants (Baliyan et al., 2022) before they were allowed to dry at room temperature (36°C) for 3 weeks. The weights of the leaves were constantly monitored to affirm their dryness level before extraction was carried out.

Sample Collection

- Three fish farms in Ondo-West LGA
- Fresh and young pear leaves from ethnobotanical garden
- Proper identification and quality control

Preparation Methods

- Aqueous and ethanolic extraction techniques
- 72-hour extraction period with intermittent shaking
- Centrifugation and concentration procedures

Preparation of Plant Extracts

Aqueous and ethanolic extraction methods were used to obtain the extracts from the plants. Ethanol and distilled water were obtained from the chemistry laboratory of Wesley University, Ondo. The midrib and stalk of the dried leaves were removed before grinding them into fine particles using a Speedo 3.0 kitchen electric blender to prepare the extracts.

To prepare the pure water extract, 10 grams of the powdered pear leaves were added to 125 millilitres of distilled water. For the ethanol extract, 10 grams of the powdered pear leaves were added to 125 millilitres of 50% ethanol. Each mixture was placed in an extraction jar, sealed, and placed on a slab at room temperature for 72 hours. During this period, the jar was intermittently shaken to ensure thorough extraction of the bioactive compounds.

After 72 hours, the contents of each jar were filtered using Whatman filter paper and transferred to a conical flask. The extracts were centrifuged and concentrated using a water bath to obtain a clearer filtrate, and stored until needed for subsequent experiments.

Isolation of Pathogens from Infected Fish

The samples for bacteriological examination were taken from the infected parts of the fish. These infected parts included the dorsal fin, the tail, and mouth. Swabs were also collected from gross lesions, including skin ulcers, fin rot, skin discolouration, and collapsed eyes, at each sampling location. The infected parts were rinsed with distilled water and crushed with a mortar and pestle in the Fisheries and Aquaculture Laboratory of Wesley University, Ondo. After crushing the infected parts, a clean swab stick was dipped into the crushed parts and inoculated onto Nutrient agar and Potato Dextrose agar, respectively, and incubated at 28°C for 48 hours to isolate the bacterial and fungal pathogens.

The distinct isolated colonies observed from the growth of mixed culture colonies after the incubation of the isolates were sub-cultured into a new nutrient agar to obtain pure isolates using the streak plate method (Osuntoku et al., 2020), while the fungal isolates were also sub-cultured on PDA. The isolated colonies were counted using a colony counter and documented. After sub-culturing, the pure isolates were stored on slants of Nutrient agar in a refrigerator at a temperature of 3°C.

The isolates from the different parts of the fish were labelled for proper identification as follows: Skin-SK; Dorsal Fin-DF; Liver-LV; Gills-GL; and Tail-TL.

Macroscopic Identification of Bacteria Isolates

Morphological examination of the isolates on the cultured plate were observed macroscopically and recorded. Different parameters such as colonial appearances, shape, edge, colour, and opacity were used to identify the organisms.

Biochemical Identification of Isolated Pathogens

The isolated pathogens were further tested biochemically to affirm the genus of the isolated pathogens. The tests carried out include Gram-staining, catalase, oxidase, glucose fermentation, motility, nitrate reduction, indole production, citrate utilisation, urease and hydrogen sulphide production tests.

Antimicrobial Activity of Plant Extracts

Agar well diffusion method was used to perform the sensitivity test of the plant extracts. 15 mL of sterilised Nutrient Agar (NA) were poured into their respective labelled plates and allowed to solidify. Bacterial and fungal isolates obtained previously were evenly spread across the NA plates using a sterile swab stick.

Under aseptic conditions, wells measuring approximately 5 mm in diameter were cut out at four points on each agar plate using a sterile stainless cork borer. Each well was then filled with various concentrations (mL) of the aqueous and ethanolic plant extracts as follows: 100mg/mL, 200mg/mL, 400mg/mL respectively. An anti-sensitivity disc (Ciprofloxacin) was also placed on a plate containing the different organisms. Water was also put in one of the wells to serve as control.

The agar plates were allowed to sit for proper diffusion of the extracts and then incubated upright at 37°C for 72 hours for bacterial isolates and at 25°C for 72 hours for fungal isolates. After the specified incubation periods, the agar plates were examined for zones of inhibition around each well. The diameter of each zone of inhibition was measured using a caliper. Larger zones of inhibition indicate greater antimicrobial activity of the extracts against the bacterial isolates.



Pathogen Isolation

Bacterial isolates obtained from infected fish parts using standard microbiological techniques and streak plate method.



Extract Testing

Agar well diffusion method with three concentrations (100, 200, 400 mg/mL) against isolated pathogens.



Quality Control

Ciprofloxacin as positive control and sterile water as negative control for validation of results.

RESULTS

Clinical Signs of Infected Fish

In this study, fish were specifically chosen based on observable physiological changes indicative of infection. The infected specimens exhibited a range of clinical signs that suggest varying degrees of infection, including skin ulcerations, frayed fins, fin congestion, lesions on the body surface, and deep ulcerations. These clinical manifestations were primarily noted on the skin, oral structures, tissues, and ocular regions of the infected catfish obtained from multiple farms within the Ondo West Local Government Area of Ondo State. Illustrative representations of the various clinical symptoms observed in the collected fish samples are presented in Plates 1 - 2.

Isolation of Bacteria from Infected Fish

The results pertaining to the bacterial isolates obtained from infected fish samples sourced from various farms in the Ondo West Local Government Area are summarised in Table 1. This table delineates the morphological characteristics of the different bacterial organisms isolated from distinct anatomical regions of the fish. Only selected organisms were employed in the assessment of the antibacterial activity of pear leaves.

Plate 1: Fish showing skin ulcers and frayed fins





Plate 2: Fish showing bloody ascites and ulcers

Table 1: Morphological Observations of Cultured Isolates

	FISH ORGAN	MORPHOLOGICAL CHARACTERISTICS	NO. OF COLONIES	GRAM STAINING	POSSIBLE ORGANISM
FARM 1	SKIN	Filamentous, GIM, Light yellow, dry		Gram-Positive	<i>Streptomyces</i>
FARM 2	DORSAL FIN	Filamentous, GIM, Light green, dry	24	Gram- Negative	<i>Pseudomonas</i>
		Irregular, flat, erose, transparent/shiny, mucoid	15	Gram- Negative	<i>Aeromonas hydrophilla</i>
	GILLS	Filamentous, flat, smooth, white, moist			<i>Flavobacterium</i>
		Filamentous, raised, smooth, light green, dry	16	Gram- Negative	<i>Pseudomonas</i>
	TAIL	Filamentous, raised, white, dry	11		<i>Fungi</i>
		filamentous, flat, smooth, black, dry			<i>fungi</i>
	LIVER	Filamentous, flat, black, dry			<i>Fungi</i>
FARM 3	SKIN	Filamentous, raised, black, dry	4		<i>fungi</i>
		Irregular, GIM, Erode, Opaque, Moist		Gram-Negative	<i>Aeromonas hydrophilla</i>
		Circular, convex, smooth, opaque, shiny, moist		Gram-Negative	<i>Aeromonas hydrophilla/Edwardsiella</i>
		circular, convex, smooth, white, shiny, moist		Gram-Negative	<i>Aeromonas hydrophilla</i>
	LIVER	Filamentous, flat, black, dry			<i>Fungi</i>

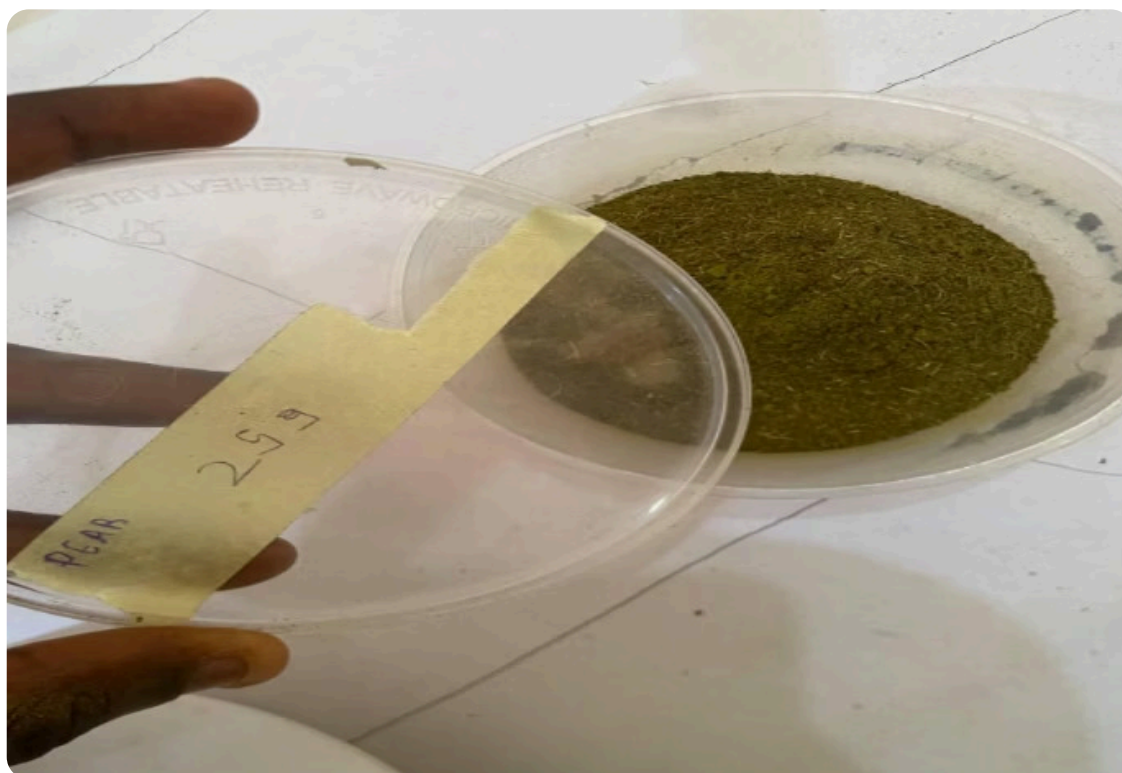


Plate 3: Blended pear leaves powder

Table 2: Biochemical Characteristics of Bacteria Isolated from Infected Catfish

Biochemical Test	<i>Streptomyces spp.</i>	<i>Aeromonas hydrophila</i>	<i>Flavobacterium spp.</i>
Gram Stain	Positive (filamentous)	Negative	Negative
Shape	Filamentous rods	Rods	Rods
Catalase	Positive	Positive	Positive
Oxidase	Positive	Positive	Positive
Motility	Variable (non-motile or slow)	Motile	Non-motile
Glucose fermentation	Negative (oxidative only)	Positive	Negative
Nitrate reduction	Negative	Positive	Negative
Indole production	Negative	Positive	Negative
Citrate utilization	Positive	Positive	Positive
Urease	Negative	Negative	Negative
H ₂ S production	Negative	Positive	Negative

Source: Author's biochemical analysis

Antibacterial Activity of Aqueous and Ethanolic Extracts

Table 3 represents the antibacterial activity of the aqueous and ethanolic extracts of pear (*Pyrus communis*) leaves against bacterial isolates obtained from infected *Clarias gariepinus*. The antimicrobial activity was evaluated by measuring the zones of inhibition in millimetres (mm) using the agar well diffusion method, and Ciprofloxacin was used as the standard (Control) antibiotic.

Table 3: Antibacterial Activity Results

Organism	Aqueous Extract (mg/ml)			Ethanolic Extract (mg/ml)			Control	
	100	200	400	100	200	400	Ciprofloxacin	Distilled water
<i>Aeromonas</i>	9.3±0.3	13.6±0.5	17.3±0.4	12.2±0.2	15.4±0.3	19.6±0.5	22.0±0.3	-
<i>Streptomyces</i>	12.5±0.4	16.3±0.4	18.4±0.3	14.3±0.5	18.1±0.3	24.7±0.2	27.4±0.5	-
<i>Flavobacterium</i>	6.6±0.5	8.4±0.4	13.6±0.3	7.3±0.2	10.3±0.4	17.2±0.4	20.5±0.2	-

Source: Author's antimicrobial assay results

24.7

Maximum Inhibition

Highest zone of inhibition (mm) for ethanolic extract against *Streptomyces*

400

Optimal Concentration

Most effective extract concentration (mg/mL) showing maximum antimicrobial activity

3

Pathogens Tested

Number of major bacterial species isolated from infected catfish



DISCUSSION

This study evaluated the antimicrobial properties of *Pyrus communis* leaf extracts against bacterial pathogens isolated from infected *Clarias gariepinus*. The data showed that both aqueous and ethanolic extracts possessed measurable antibacterial activity, with ethanolic extracts demonstrating superior efficacy across all tested concentrations. This finding aligns with earlier reports that ethanol is a more efficient solvent for extracting flavonoids, alkaloids, and phenolic compounds (Grzelczyk et al., 2020; Ribeiro et al., 2025).

Several studies have investigated the antimicrobial potential of pear leaf extracts. Zbikowska et al. (2025) reported that both aqueous and ethanolic extracts of *Pyrus communis* leaves showed inhibitory effects against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. The antimicrobial action is largely attributed to arbutin and phenolic acids, which disrupt microbial membranes. Their combined actions on bacterial cell walls, protein synthesis machinery, and nucleic acid metabolism contribute to their overall effectiveness as antimicrobial agents. For instance, flavonoids and phenolic acids are prominent constituents in pear that contribute to their antibacterial properties by disrupting bacterial membranes and inhibiting enzymatic activities crucial for bacterial survival (Ghasemzadeh et al., 2012).

Extract Superiority

Ethanolic extracts showed significantly higher antimicrobial activity compared to aqueous extracts, likely due to better extraction of bioactive phenolic compounds.

Pathogen Sensitivity

Streptomyces demonstrated highest sensitivity to both extracts, while *Flavobacterium* showed lowest sensitivity, indicating species-specific responses.

Concentration Effect

Antimicrobial activity increased with concentration, with 400 mg/mL showing maximum inhibition zones across all tested organisms.

Understanding the mechanisms of action of plant extracts in combating fish diseases is essential for harnessing their therapeutic potential in aquaculture settings. By leveraging these natural compounds, aquaculture practitioners can develop sustainable disease management strategies that mitigate the risk of antibiotic resistance and environmental pollution associated with conventional antibiotics. Continued research into plant-derived antimicrobial agents holds promise for expanding treatment options, enhancing food safety, and promoting ecological sustainability in global aquaculture practices (Caruso et al., 2024).

Abraham et al. (2022) and Castro et al. (2008) have reported significant antimicrobial properties of ethanol and aqueous extracts from various plants. These studies highlight the potential of plant-derived substances in combating microbial infections, indicating that the choice of plant material, extraction methods, and concentrations are critical factors influencing the outcomes.

The ethanol extracts in this study showed significantly better results compared to the aqueous extracts, aligning with the understanding that ethanol is a more efficient solvent for extracting bioactive compounds such as flavonoids, alkaloids, and phenolic acids. These compounds have been recognised for their potent antimicrobial properties (Ribeiro et al., 2025; Patel and Williams, 2021). The effectiveness demonstrated suggests that the concentration of bioactive compounds was sufficient to inhibit the tested bacterial isolates.

The differential susceptibility of bacterial isolates to the plant extracts observed in this study aligns with previous research. Studies by Thiptara et al. (2025) and Castro et al. (2008) demonstrated significant inhibition zones with similar extracts, emphasising the variability in responses due to different bacterial strains, plant material sources, and extraction procedures. This variability underscores the importance of standardised experimental conditions to achieve consistent and reliable results.

This study's implications for aquaculture are profound. Natural extracts like those from *Pyrus communis* offer promising avenues for disease control, especially in regions where the overuse of synthetic antibiotics is prevalent. However, variability in results across studies underscores the need for standardised extract preparation and optimised application techniques (Jones & Brown, 2020).

Future directions should involve in vivo studies to assess the systemic effects of these extracts on fish physiology and survival, as well as phytochemical characterisation to isolate specific antimicrobial constituents. Additionally, investigating synergistic effects with conventional antibiotics could provide enhanced treatment protocols for aquaculture diseases.

In conclusion, this study supports the antibacterial potential of *Pyrus communis* leaf extracts, especially ethanol-based preparations, against catfish pathogens. This highlights the need for further research to standardise their use and integrate them effectively into sustainable aquaculture management strategies. In addition, bioactive compounds found in *Pyrus communis* leaves represent a promising strategy to combat the challenges posed by antibiotic resistance in aquaculture. Further research into the specific mechanisms of action, optimal concentrations for efficacy, and long-term effects on fish health will be crucial in establishing guidelines for the integration of pear leaf extracts into aquaculture management practices. This initiative has the potential to enhance sustainability and food security within the aquaculture industry while promoting the welfare of farmed species.

Standardisation Needs

Develop standardised protocols for extract preparation, concentration determination, and application methods to ensure consistent antimicrobial efficacy.

In Vivo Studies

Conduct comprehensive fish trials to evaluate systemic effects, safety profiles, and optimal dosing regimens for practical aquaculture applications.

Commercial Integration

Investigate synergistic effects with existing treatments and develop cost-effective formulations for sustainable aquaculture disease management.

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Not Applicable

CONFLICTS OF INTEREST

The authors declare no conflict of interest

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