



Antibacterial and Antioxidant Activities of *Uncaria cordata* Root Extract Against Fish Pathogenic Bacteria

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Abstract: Bacterial infections significantly hinder aquaculture, causing high mortality and economic losses. This study evaluates the antibacterial and antioxidant activities of *Uncaria cordata* (Lour.) Merr. root extract (locally known as Kaik-Kaik). Antibacterial assays against *Aeromonas hydrophila*, *Pseudomonas fluorescens*, and *Edwardsiella ictaluri* were performed using the Kirby-Bauer method (200, 400, 600, and 800 ppm). Antioxidant potential was assessed via DPPH assay across ethanolic, n-hexane, and ethyl acetate fractions. The 800 ppm concentration showed the strongest inhibition zones (21.0–21.7 mm) across all strains. The ethanolic fraction exhibited the highest antioxidant capacity (IC_{50} = 49.22 ppm) and total flavonoid content (9.08 mg QE/g). Phytochemical screening confirmed the presence of flavonoids, phenolics, and alkaloids. These preliminary results highlight *U. cordata* as a potential source of natural bioactive compounds, though further *in vivo* studies are required to establish its safety and efficacy in aquaculture systems.

Keywords: *Uncaria cordata*, antibacterial activity, antioxidant, natural product, fish pathogenic bacteria

Atividades Antibacterianas e Antioxidantes do Extrato de Raiz de *Uncaria cordata* contra Bactérias Patogênicas de Peixes. Resumo: Atividades antibacteriana e antioxidante do extrato de raiz de *Uncaria cordata* contra bactérias patogênicas de peixes. As infecções bacterianas representam um dos principais entraves na aquicultura, causando elevadas taxas de mortalidade e perdas econômicas significativas. Este estudo teve como objetivo avaliar as atividades antibacteriana e antioxidante do extrato de raiz de *Uncaria cordata* (Lour.) Merr., conhecida localmente como Kaik-Kaik. Os ensaios antibacterianos foram realizados contra *Aeromonas hydrophila*, *Pseudomonas fluorescens* e *Edwardsiella ictaluri* utilizando o método de difusão em disco (Kirby-Bauer) nas concentrações de 200, 400, 600 e 800 ppm. O potencial antioxidante foi avaliado pelo método DPPH em diferentes frações (etanólica, n-hexano e acetato de etila). A concentração de 800 ppm apresentou as maiores zonas de inibição (21,0–21,7 mm) para todas as bactérias testadas. A fração etanólica demonstrou a maior atividade antioxidante (IC_{50} = 49,22 ppm) e o maior teor de flavonoides totais (9,08 mg QE/g). A triagem fitoquímica confirmou a presença de flavonoides, compostos fenólicos e alcaloides. Estes resultados preliminares indicam que *U. cordata* possui potencial como fonte de compostos

bioativos naturais, embora estudos in vivo adicionais sejam necessários para confirmar sua segurança e eficácia em sistemas de aquicultura.

Palavras-chave: *Uncaria cordata*, atividade antibacteriana, antioxidante, produtos naturais, bactérias patogênicas de peixes.

Introduction

Bacterial diseases pose a significant threat to the aquaculture industry, leading to substantial economic losses as a result of high fish mortality rates and reduced productivity. In most cases, bacterial infections in fish occur due to complex interactions among the host, the pathogen, and environmental conditions rather than through direct infection alone (Li et al., 2024; Suguna, 2020). Environmental stressors, poor water quality, and overcrowding can weaken the immune defense system of fish, making them more susceptible to opportunistic bacterial pathogens. Among the most prevalent and economically important pathogens in freshwater aquaculture are *Aeromonas hydrophila*, *Pseudomonas fluorescens*, and *Edwardsiella ictaluri*. These bacteria are responsible for systemic infections such as Motile Aeromonas Septicemia, Pseudomonas disease, and Enteric Septicemia of Catfish, which often result in high mortality and significant economic losses (Austin et al., 2016; Rasul et al., 2025; Maryani et al., 2013).

Disease outbreaks caused by these bacteria can rapidly spread through aquaculture systems, particularly under poor husbandry conditions, thereby threatening fish health, food security, and the sustainability of aquaculture production. Conventional disease control strategies rely heavily on the use of antibiotics and synthetic chemotherapeutic agents. However, the frequent and sometimes indiscriminate use of antibiotics has led to the emergence of multidrug-resistant bacterial strains and the accumulation of antibiotic residues in aquatic environments. These issues have prompted increasing concern regarding their ecological impact and human health risks, as well as the urgent need for eco-friendly and sustainable alternatives (Abdul et al., 2022; Okubo et al., 2025). Consequently, researchers and aquaculture practitioners are now exploring plant-derived bioactive compounds as potential substitutes for synthetic antimicrobials.

Medicinal plants have long been recognized as a valuable source of biologically active compounds with antimicrobial, antioxidant, and immunostimulant properties. One such genus with promising pharmacological potential is *Uncaria*, a mem-

ber of the Rubiaceae family, which has been traditionally used in ethnomedicine throughout Southeast Asia for treating various ailments. Several species within this genus, including *Uncaria cordata* (Lour.) Merr., *Uncaria longiflora*, *Uncaria gambir* Roxb., *Uncaria nervosa*, and *Uncaria tomentosa*, have been reported to contain important secondary metabolites such as alkaloids, flavonoids, phenolics, terpenoids, and saponins (Erwin, 2020; Maulina et al., 2019). These compounds are known to exhibit potent antibacterial and antioxidant activities, making them potential candidates for development as natural therapeutics in aquaculture.

In Kalimantan (Borneo), *Uncaria cordata* locally known as “Kaik-Kaik” or “Bajakah” is an indigenous medicinal plant that has gained increasing attention due to its rich phytochemical composition and traditional use by the Dayak community. The roots of *Uncaria cordata* are often utilized in traditional remedies to promote general health, wound healing, and resistance against infections. Preliminary phytochemical screenings have identified flavonoids, phenolics, terpenoids, and alkaloids as the major bioactive constituents responsible for its pharmacological activity (Deswita et al., 2022; Syafriana et al., 2024). Moreover, extracts from various parts of *Uncaria cordata* have been reported to demonstrate antioxidant and antimicrobial properties in human pathogenic bacteria. However, studies focusing on its antibacterial activity against aquatic pathogens, especially fish pathogenic bacteria, remain scarce.

The utilization of such native medicinal plants not only offers a promising natural alternative to synthetic antibiotics but also aligns with sustainable aquaculture practices. This approach supports biodiversity conservation, the use of renewable biological resources, and the incorporation of indigenous knowledge systems into modern aquaculture health management (Rachmatiah, 2020). Evaluating the antibacterial activity of *Uncaria cordata* against fish pathogens is therefore of scientific and practical importance. Furthermore, the combined evaluation of its antibacterial and antioxidant properties is crucial, as the latter supports host immune function and mitigates oxidative stress during infection. It provides insights into potential biotherapeutic agents that can

help reduce antibiotic dependency and promote environmentally responsible aquaculture systems.

Despite its traditional use and well-documented phytochemical composition, the specific antibacterial activity of *Uncaria cordata* (Lour.) Merr root extract against major fish pathogens such as *Aeromonas hydrophila*, *Pseudomonas fluorescens*, and *Edwardsiella ictaluri* has not yet been scientifically investigated. This knowledge gap highlights the need for systematic evaluation to validate its potential as a natural therapeutic agent in aquaculture. To the best of our knowledge, this study is the first to comprehensively examine the dual function potential of the *U. cordata* root extract, simultaneously evaluating its in vitro antibacterial efficacy and its corresponding antioxidant capacity specifically against these key pathogenic bacteria affecting freshwater cultured fish. Therefore, this study aims to evaluate the effectiveness of *Uncaria cordata* (Lour.) Merr root extract as an antimicrobial agent against *A. hydrophila*, *P. fluorescens*, and *E. ictaluri*, and to determine which bacterial species are most susceptible to inhibition by the extract.

Materials and methods

Materials. The materials used in this study included the root of *Uncaria cordata* (Lour.) Merr (Kaik-Kaik), bacterial strains of *Aeromonas hydrophila*, *Pseudomonas fluorescens*, and *Edwardsiella ictaluri*, as well as culture media such as Nutrient Agar (NA), Tryptic Soy Agar (TSA), and Mueller Hinton Agar (MHA). Additional materials used were distilled water, ethanol (96%), and sterile cotton swabs.

Preparation of Media. The agar media were prepared as bacterial growth substrates following standard microbiological protocols. Nutrient Agar (NA) medium was prepared by dissolving 8 g of NA powder in 400 mL of distilled water. The mixture was heated while being stirred on a hot plate until fully dissolved. The prepared medium was then sterilized in an autoclave at 121 °C for 15 minutes. After sterilization, the medium was cooled to 40–50 °C and poured into sterile Petri dishes. The plates were allowed to solidify at room temperature for 24 hours before being stored in a refrigerator until further use.

Preparation of *Uncaria cordata* Root Extract. Collection, Authentication, and Preparation of Plant Material. Fresh roots of *Uncaria cordata* (Lour.) Merr, locally known as "Kaik-Kaik" or "Bajakah," were collected using a purposive sampling method from representative locations within the peat swamp and riverine swamp forest areas in East Barito, Central Kalimantan. The sampling locations were geo-

graphically situated around the coordinates of 1.8°–2.2° South Latitude and 114.8°–115.2° East Longitude. Only mature stems showing the characteristic morphological features were selected. Additional samples were procured from traditional herbal vendors in Palangka Raya. All materials were immediately placed into sterile, labeled bags and transported under controlled conditions to prevent degradation.

The plant species was tentatively identified as *Uncaria cordata* (Lour.) Merr based on established local morphological characteristics. Its identity was further supported by preliminary phytochemical screening, which confirmed the presence of characteristic secondary metabolites (flavonoids, phenolics, alkaloids) consistent with the *Uncaria* genus.

The fresh roots of *U. cordata* were washed thoroughly to remove adhering debris, air-dried under shade with indirect sunlight (covered by a black cloth), and subsequently ground into fine powder using a blender.

Extraction Procedure. The extraction was conducted using the maceration method. A total of 100 g of powdered root was immersed in 750 mL of 96% ethanol in a dark glass container. The mixture was kept at room temperature for three days with occasional stirring every 8 hours. After maceration, the mixture was filtered, and the filtrate was collected as the first extract. The residue was re-macerated with 250 mL of fresh 96% ethanol for one day and then filtered again to obtain the second extract. Both filtrates were combined and left overnight to allow sedimentation.

The ethanol solvent was evaporated using a rotary evaporator under reduced pressure until a concentrated crude extract was obtained (Palupi and Hidayatullah, 2024). The resulting concentrated crude extract weighed 4.92 g, yielding an extraction efficiency of 4.92% (w/w dry basis).

The resulting *Uncaria cordata* root extract was subjected to preliminary phytochemical and UV-Vis spectrophotometric analyses to confirm the presence of major secondary metabolites. The appearance of a yellow-orange coloration in the magnesium–HCl test indicated the presence of flavonoids, confirming the presence of bioactive components known for their antibacterial and antioxidant properties (Deswita et al., 2022; Syafriana et al., 2024).

Preparation of Bacterial Suspension. Each bacterial strain (*A. hydrophila*, *P. fluorescens*, and *E. ictaluri*) was subcultured on Nutrient Agar plates and incubated at 30 °C for 24 hours. A loopful of fresh culture

was then suspended in 10 mL of sterile 0.85% NaCl solution and homogenized to obtain a uniform suspension. The turbidity of each suspension was adjusted to match the 0.5 McFarland standard, corresponding to an approximate bacterial density of 10^8 CFU/mL (Park et al., 2020).

Antibacterial Activity Assay. The antibacterial activity of *Uncaria cordata* (Kaik-Kaik) root extract was evaluated using the Kirby Bauer disk diffusion method. Sterile cotton swabs were used to evenly spread standardized bacterial suspensions of *Aeromonas hydrophila*, *Pseudomonas fluorescens*, and *Edwardsiella ictaluri* onto Mueller–Hinton agar plates. Sterile paper discs (6 mm in diameter) were impregnated with 20 μ L of *Uncaria cordata* (Kaik-Kaik) root extract at concentrations of 200, 400, 600, and 800 ppm and placed on the inoculated agar surface. A commercial chloramphenicol disc (30 μ g) served as the positive control to validate bacterial susceptibility and the effectiveness of the assay, while sterile distilled water was used as the negative control to confirm that any observed inhibition was due to the extract itself. Plates were incubated at 30 °C for 24 hours, after which the diameters of the inhibition zones were measured using a digital caliper. The antibacterial activity of *Uncaria cordata* (Kaik-Kaik) root extract was assessed by comparing the inhibition zones of each extract concentration with the positive and negative controls, allowing evaluation of its dose-dependent effect against all three tested bacterial strains.

Experimental Design and Statistical Analysis. The experiment followed a Randomized Block Design (RBD) with three bacterial species (*Aeromonas hydrophila*, *Pseudomonas fluorescens*, and *Edwardsiella ictaluri*) serving as blocks, and five extract concentrations as treatments. Each treatment was performed in triplicate.

The treatments for statistical analysis (ANOVA) were as follows:

- P1: 0 ppm (Negative Control)
- P2: Kaik-Kaik root extract 200 ppm
- P3: Kaik-Kaik root extract 400 ppm
- P4: Kaik-Kaik root extract 600 ppm
- P5: Kaik-Kaik root extract 800 ppm

Validation Control: A commercial Chloramphenicol disc (30 μ g) was run simultaneously against all bacterial species as a positive validation control (internal standard), separate from the P1–P5 groups used for the ANOVA. Data on inhibition zone diameters were analyzed using one-way analysis of variance (ANOVA). This was followed by Least Significant Difference (LSD) post hoc test-

ing at a significance level of $\alpha = 0.05$ to determine significant differences among the extract treatments (P1–P5).

Phytochemical Screening. Qualitative phytochemical screening of the *Uncaria cordata* (Kaik-Kaik) root extract was carried out to identify the presence of major bioactive compounds using standard methods (Wathan et al., 2024). The analysis revealed the presence of several important classes of secondary metabolites, each confirmed through specific chemical reactions. Specifically, flavonoids were detected by the Shinoda test, in which the addition of magnesium turnings followed by concentrated hydrochloric acid produced a distinct yellow orange coloration, indicating a positive reaction. In addition, the presence of alkaloids was confirmed using Mayer's reagent, where the formation of a creamy white precipitate signified a positive result. Furthermore, saponins were identified through the frothing test, and the persistence of foam formation after vigorous shaking was considered evidence of their presence.

Similarly, terpenoids and steroids were detected using the Liebermann–Burchard reaction, as demonstrated by the appearance of a reddish-brown or violet ring. Finally, the presence of phenolic compounds was confirmed by adding a ferric chloride solution, which resulted in a characteristic dark green or bluish-black coloration. Taken together, these observations confirmed that the ethanolic root extract of *Uncaria cordata* contains multiple classes of bioactive compounds, including flavonoids, alkaloids, saponins, terpenoids, steroids, and phenolics, all of which are known to contribute to the extract's antibacterial and antioxidant activities.

Determination of Total Flavonoid Content (TFC). The total flavonoid content (TFC) of the extract was quantified using the aluminum chloride (AlCl_3) colorimetric method, with quercetin as the standard reference (Silvia et al., 2022). Serial dilutions of quercetin (10–100 μ g/mL) were prepared to construct a calibration curve. For each sample, 1 mL of the extract solution was sequentially mixed with 4 mL of distilled water, 0.3 mL of 5% NaNO_2 , and after 5 minutes, 0.3 mL of 10% AlCl_3 was added. Following another 6 minutes, 2 mL of 1 M NaOH was added, and the final volume was adjusted to 10 mL. The absorbance of the pink-colored solution was measured at 510 nm using a UV–Vis spectrophotometer. The flavonoid content was expressed as milligrams of quercetin equivalent per gram of dry extract (mg QE/g). All measurements were performed in triplicate, and the results were expressed as mean $\pm$ standard deviation.

Results and Discussion

The results obtained from the series of laboratory experiments conducted in this study provide clear evidence of the antibacterial potential of Kaik-Kaik root extract (*Uncaria cordata* (Lour.) Merr) against major fish pathogenic bacteria. The presentation of results in this section begins with the evaluation of inhibition zone diameters as an indicator of antibacterial activity, followed by statistical analysis, phytochemical correlation, and mechanistic interpretation of the observed effects. Furthermore, the antioxidant capacity and total flavonoid content are discussed to establish the relationship between bioactive compound profiles and biological activities.

Antibacterial Activity of Kaik-Kaik Root Extract (*Uncaria cordata* (Lour.) Merr). The extract concentrations used in this study consisted of five treatments, namely P1 (0 ppm, control), P2 (Kaik-Kaik root extract at 200 ppm), P3 (Kaik-Kaik root extract at 400 ppm), P4 (Kaik-Kaik root extract at 600 ppm), and P5 (Kaik-Kaik root extract at 800 ppm). Each concentration was tested against *Aeromonas hydrophila*, *Pseudomonas fluorescens*, and *Edwardsiella ictaluri* using the disk diffusion method.

The inhibition zones of *U. cordata* root extract against these bacteria are shown in Table I. The visual observation revealed that the clear zones (inhibition zones) around the discs increased proportionally with the extract concentration. The inhibition zone formed around the 800 ppm extract disc (P5) appeared noticeably larger than those of P2, P3, and P4, while no inhibition zone was observed in the control group (P1). This clearly indicates a concentration-dependent antibacterial activity of the extract. The antibacterial efficacy was evaluated by measuring the diameter of the inhibition zone (Table I). The negative control (sterile distilled water) did not show any inhibitory effect against the tested bacteria, confirming the lack of antimicrobial activity in the solvent. Furthermore, the positive control (Chloramphenicol 30 µg) exhibited strong inhibition against all tested strains, confirming the viability and susceptibility of the pathogenic bacteria and validating the robustness of the assay.

As shown in Table I, the highest average diameter of inhibition zones was observed in treatment P5 (800 ppm concentration) with *A. hydrophila* (21.7 mm), followed by *E. ictaluri* (21.3 mm) and *P. fluorescens* (21.0 mm). In contrast, the control group (P1) produced no inhibition zone (0 mm).

The analysis of variance (ANOVA) demonstrated that both bacterial species and extract concentrations had a significant effect on inhibition

zone diameters ($p < 0.05$). Moreover, a significant interaction between bacterial type and extract concentration was observed, indicating that the antibacterial response varied depending on the bacterial species. Consequently, it can be concluded that the Kaik-Kaik root extract (*Uncaria cordata* (Lour.) Merr) had a statistically significant inhibitory effect on the growth of *A. hydrophila*, *P. fluorescens*, and *E. ictaluri*.

Table I. Average Diameter of the Inhibition Zone for *A. hydrophila*, *Pseudomonas fluorescens* And *E. ictaluri*

Bacteria	Concentration	Average Diameter (mm)
<i>Aeromonas hydrophila</i>	0 (control negative)	0.0±0.0
	200 ppm	15.3±0.5
	400 ppm	17.3±0.6
	600 ppm	20.0±0.3
	800 ppm	21.7±0.1
<i>Pseudomonas fluorescens</i>	0 (control)	0.0±0.0
	200 ppm	13.7±0.7
	400 ppm	15.5±0.6
	600 ppm	17.7±0.3
	800 ppm	21.0±0.3
<i>Edwardsiella ictaluri</i>	0 (control)	0.0±0.0
	200 ppm	13.0±0.5
	400 ppm	14.3±0.8
	600 ppm	16.7±0.4
	800 ppm	21.3±0.2

Note: The strength of antibacterial activity was categorized according to Chabán et al. (2019): Weak (≤ 5 mm), Moderate (6–10 mm), Strong (11–20 mm), Very Strong (≥ 21 mm).

Further analysis using Duncan's Multiple Range Test revealed that treatments P2, P3, P4, and P5 had similar inhibitory effects, which were all significantly different from the control treatment (P1). This finding indicates that even the lowest tested concentration (200 ppm) already exhibited measurable antibacterial activity, and that higher concentrations enhanced this effect consistently.

Overall, the results demonstrated that Kaik-Kaik root extract at all tested concentrations was capable of inhibiting the growth of the three pathogenic bacteria. The size of the inhibition zone increased with the concentration of the extract, confirming a dose-dependent antibacterial response. These findings are consistent with those of Sugiantina et al. (2022) and Praptiwi et al. (2021), who reported that higher extract concentrations provide

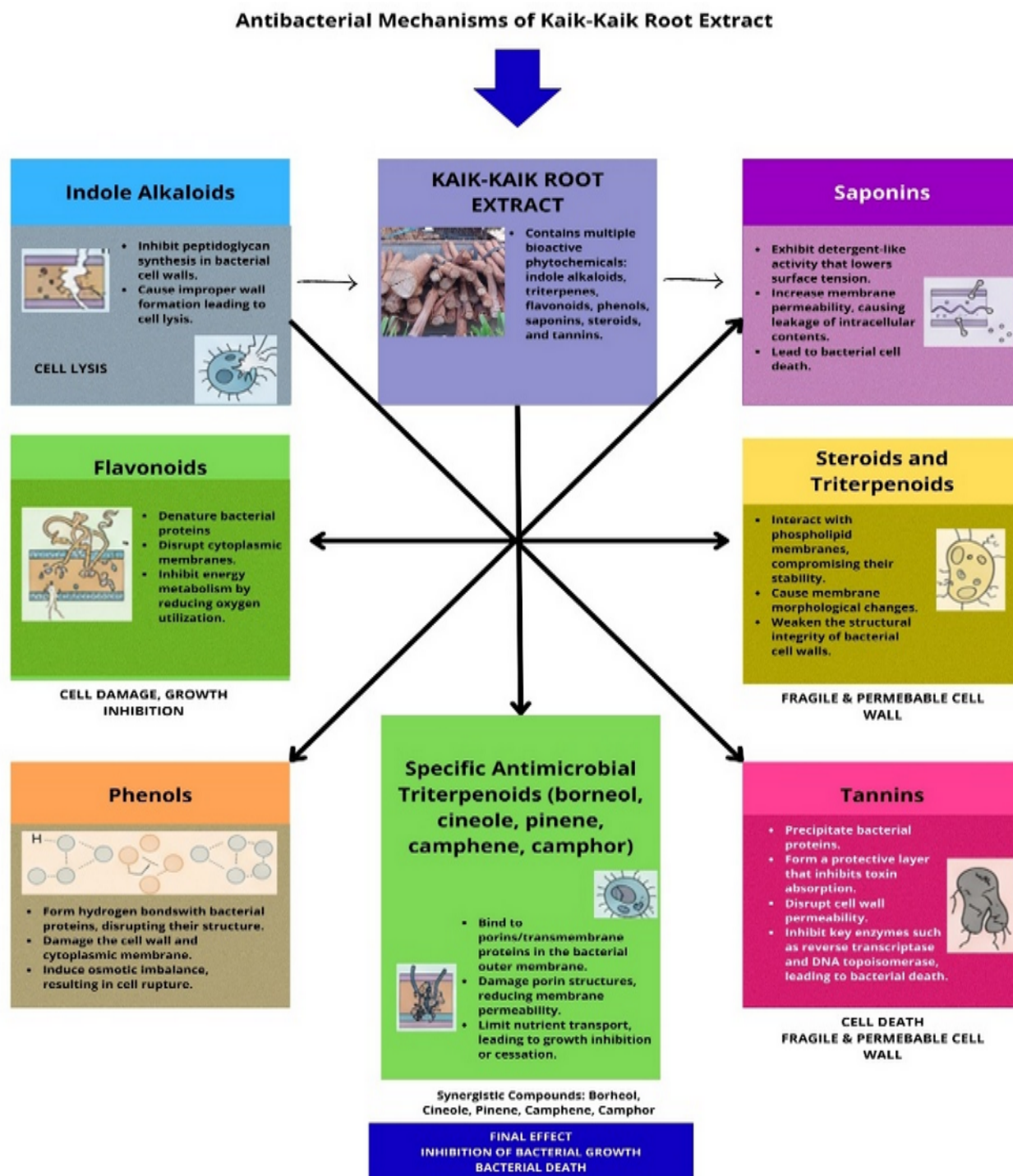


Figure 1. Multitargeted antibacterial mechanisms of *Uncaria cordata* (Kaik-Kaik) root extract.

stronger antibacterial effects due to increased availability of active compounds.

Mechanisms of Antibacterial Action. The potent antibacterial activity observed is attributed to the Kaik-Kaik root extract's rich phytochemical composition, which acts via multitargeted mechanisms as illustrated in Figure 1. These active compounds, including indole alkaloids, triterpenes, flavonoids, phenols,

and phenylpropanoids, function synergistically to inhibit bacterial growth through multiple, complementary pathways (Abdul et al., 2022; Dai et al., 2023).

The extract components collectively compromise both the structural integrity and functional processes of the bacterial cell (Harborne, 2006; Taher et al., 2021; Krati et al., 2024). Cell Wall and

Membrane Disruption commences as alkaloids interfere with the synthesis of peptidoglycan in bacterial cell walls, preventing proper formation and ultimately causing cell lysis. Concurrently, Flavonoids and Phenolic compounds damage cytoplasmic membranes and form hydrogen bonds with bacterial proteins, respectively (Maryani et al., 2013; Huang, 2022). This leads to structural disruption, compromised cell wall integrity, osmotic imbalance, and eventual cell rupture. Furthermore, Saponins exhibit detergent-like properties that damage membrane permeability, causing the leakage of intracellular contents and subsequent bacterial death. Steroid and triterpenoid compounds augment this damage by interacting with phospholipid membranes, compromising their stability and rendering the cell wall fragile and permeable (Prasad et al., 2024; Barlow et al., 2020).

In terms of Metabolic and Protein Interference, Flavonoids also inhibit energy metabolism through the suppression of oxygen utilization by bacterial cells (Ngazizah & Romaidha, 2022; Hans et al., 2022). Tannins contribute by precipitating bacterial proteins and interfering with bacterial enzymes such as reverse transcriptase and DNA topoisomerase, leading to cell death (Rachmatiah, 2020).

A final pathway involves nutrient uptake inhibition mediated by triterpenoids. These compounds are known to interact with porins or transmembrane proteins on the outer bacterial membrane, forming strong polymeric bonds that damage porin structures. This action reduces membrane permeability and limits nutrient uptake, subsequently inhibiting or halting bacterial growth entirely. Several triterpenoids with known antimicrobial activity, including borneol, cineole, pinene, camphene, and camphor, play a synergistic role in the observed antibacterial effect (Wulansari et al., 2020; Zielińska-Błajet, 2020; Leite-Sampaio et al., 2021; Kamil et al., 2023).

Overall, these findings demonstrate that the Kaik-Kaik root extract possesses a unique antibacterial profile arising from multiple, complementary mechanisms that simultaneously target the structural and functional integrity of bacterial cells.

Biological Implications and Safety Considerations. Although the 800 ppm extract concentration exhibited the strongest in vitro antibacterial activity, this observation should be interpreted with caution. High antibacterial potency does not necessarily imply safety for host tissues. It is possible that the concentration effective against bacteria may also exhibit cytotoxic effects on fish cells.

This concern aligns with the findings of Maryani et al. (2024), who reported that terpenoid compounds isolated from *Uncaria cordata* displayed strong cytotoxicity with IC₅₀ values as low as 2.57 µg/mL. Similarly, Wathan (2024) found that the ethanolic extract of *U. cordata* stem contained 3.6 ± 0.086% total flavonoid (as quercetin equivalent) and exhibited very strong antioxidant activity with an IC₅₀ value of 9.159 ppm.

These findings underscore the importance of conducting further cytotoxicity assays to evaluate the extract's safety profile before recommending its application in aquaculture. Thus, while Kaik-Kaik root extract holds promise as a natural antibacterial agent, comprehensive in vivo studies and toxicity evaluations remain essential to ensure its efficacy and safety for sustainable aquaculture use.

Antioxidant Activity. The antioxidant potential of the Kaik-Kaik (*Uncaria cordata* (Lour.) Merr) root extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Both extracts were fractionated with solvents of varying polarity ethanol, n-hexane, and ethyl acetate to determine their free radical inhibition capacity. The results of the DPPH assay are presented in Table II.

Table II. Antioxidant Activity IC₅₀ of *U. cordata* Root Extract Fractions

Solvent Fraction	IC ₅₀ Value (ppm)
Ethanol	49.22
n-Hexane	108.22
Ethyl Acetate	130.30

Note: Antioxidant activity was classified based on the IC₅₀ value, where: Very Strong (< 50 ppm), Strong (50-100 ppm), Moderate (100-150 ppm), and Weak (> 150 ppm).

Based on the DPPH assay, the ethanol fraction of *Uncaria cordata* demonstrated the highest antioxidant activity, with an IC₅₀ value of 49.22 ppm, categorized as very strong according to the classification by Blois (1958). The n-hexane and ethyl acetate fractions exhibited moderate antioxidant activity, with IC₅₀ values of 108.22 ppm and 130.30 ppm, respectively. These differences in activity are closely related to the polarity of the solvent and the solubility of antioxidant compounds, where ethanol, being a polar solvent, effectively extracts phenolic and flavonoid constituents that serve as potent free radical scavengers.

The strong antioxidant activity observed in the ethanol extract correlates with the presence of

flavonoids and phenolic compounds identified during phytochemical screening. These compounds are known to possess hydroxyl groups that can donate hydrogen atoms to neutralize DPPH radicals, stabilizing reactive species and preventing oxidative damage (Deswita et al., 2022; Wathan et al., 2024). The presence of these antioxidants supports the hypothesis that *U. cordata* contains bioactive compounds capable of both antibacterial and antioxidant functions.

Furthermore, the antioxidant mechanism may synergistically enhance the antibacterial activity of the extract by mitigating oxidative stress and maintaining cellular homeostasis. Reactive oxygen species (ROS) play a dual role in microbial infection processes; thus, the capacity of *U. cordata* to scavenge free radicals could indirectly contribute to improved host immunity and protection against oxidative damage. These findings reinforce the potential of *Uncaria cordata* as a dual-function herbal agent with strong antibacterial and antioxidant activities, supporting its application in sustainable aquaculture health management.

Total Flavonoid Content and Its Correlation with Antibacterial Activity. The total flavonoid content (TFC) of *U. cordata* root extract was determined using the aluminum chloride colorimetric method, with quercetin as the standard reference compound. The TFC values for each solvent fraction are presented in Table III below.

Table III. Total Flavonoid Content (TFC) of *U. cordata* Root Extract Fractions (mg QE/g)

Solvent Fraction	Total Flavonoid Content (mg QE/g sample)
Ethanol	9.08
n-Hexane	5.21
Ethyl Acetate	3.54

The ethanol fraction exhibited the highest flavonoid content (9.08 mg QE/g), followed by n-hexane (5.21 mg QE/g) and ethyl acetate (3.54 mg QE/g). This finding aligns with the pattern observed in antioxidant activity, where the ethanol fraction also demonstrated the strongest radical scavenging ability.

The strong correlation between total flavonoid content, antioxidant potential, and antibacterial activity suggests that flavonoids play a central role as the key bioactive components in *U. cordata* root extract. Flavonoids are known to exert antibacterial effects by damaging bacterial cell membranes, inactivating enzymes, chelating metal ions, and inhibit-

ing nucleic acid synthesis (Deswita et al., 2022; Syafriana et al., 2024).

In addition to their direct antibacterial role, flavonoids also act as powerful antioxidants, protecting fish cells from oxidative stress and thereby enhancing the immune response. This dual functionality underscores the potential of *U. cordata* root extract as a natural bio-therapeutic agent that provides both antimicrobial and cytoprotective benefits in aquaculture systems.

Moreover, previous studies have demonstrated that crude plant extracts containing a combination of flavonoids, alkaloids, saponins, and terpenoids often display stronger biological activity than isolated pure compounds. This is because multiple phytochemicals can interact synergistically, enhancing membrane penetration and broadening antimicrobial spectra (Ngazizah & Romaidha, 2022). Therefore, the multifaceted phytochemical composition of the Kaik-Kaik root extract likely contributes to its remarkable antibacterial and antioxidant potency.

While this study establishes the dose-dependent antibacterial and antioxidant activity of the crude extract, it is limited by the absence of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values. Furthermore, while phytochemical screening and TFC suggest that flavonoids and phenolics are key drivers of these bioactivities, specific bioactive compounds were not isolated. Future studies should prioritize bioassay-guided fractionation and *in vivo* safety assessments in fish models to bridge the gap between *in vitro* results and practical aquaculture application.

Conclusion

The Kaik-Kaik root extract (*Uncaria cordata* (Lour.) Merr) demonstrated notable antibacterial activity against *Aeromonas hydrophila*, *Pseudomonas fluorescens*, and *Edwardsiella ictaluri*. At a concentration of 800 ppm, the extract produced very strong inhibition zones, with mean diameters of 21.7 mm for *A. hydrophila*, 21.0 mm for *P. fluorescens*, and 21.3 mm for *E. ictaluri*, indicating a significant concentration-dependent antibacterial effect. These findings suggest that *U. cordata* root extract possesses potent bioactive compounds capable of suppressing the growth of major fish pathogenic bacteria.

However, the current study was limited to *in vitro* antibacterial evaluations, and therefore the practical applicability of the extract in aquaculture systems remains to be established. While the extract shows promising antibacterial potential, further re-

search is essential to ensure its safety for host organisms. In particular, cytotoxicity assays should be conducted to assess the possible toxic effects of the extract on fish cells. This step is critical to confirm that the strong antibacterial activity observed at high concentrations does not compromise host cell viability. Subsequent *in vivo* trials are also recommended to validate the extract's efficacy and safety under real aquaculture conditions.

Ethical statement

This study did not involve live animals or human participants. All bacterial isolates used in this study were obtained from the culture collection of the Aquaculture Study Program Laboratory, Department of Fisheries, Faculty of Agriculture, Forestry and Fisheries, University of Palangka Raya, Indonesia. Plant materials collected from their natural habitat and obtained from local herbal vendors in Palangka Raya, Central Kalimantan, Indonesia, in accordance with institutional procedures and applicable regulations. Therefore, no specific ethical approval for animal experimentation was required.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Acknowledgment

The authors would like to thank the Institute for Research and Community Service (Lembaga Penelitian dan Pengabdian kepada Masyarakat / LPPM), University of Palangka Raya, for supporting this research. This work was supported by the Institute for Research and Community Service (Lembaga Penelitian dan Pengabdian kepada Masyarakat / LPPM), University of Palangka Raya.

Author contributions

Maryani: Manuscript writing, Content review & editing, Conceptualization, Investigation, Data collection & analysis, Result interpretation, Project administration.

Mohamad Rozik: Manuscript writing, Content review, and editing, Resources, Methodology, Formal analysis, Data curation, Supervision.

Ricky Djauhari: Investigation, Visualization, Data analysis, Manuscript writing, Content review & editing, Supervision.

Muhammad Aldair Mukstofa Carda: Manuscript writing, Content review & editing, Methodology, Supervision.

Zakiah: Manuscript writing, Content review & editing, Conceptualization, Project administration.

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Received: October 2025

Accepted: April 2026

Published: July 2026