IJP (2025), Vol. 12, Issue 8 **(Research Article)**

International Journal of Pharmacognosy

Received on 21 August 2025; received in revised form, 25 August 2025; accepted, 27 August 2025; published 31 August 2025

NANOENCAPSULATION OF NEEM AND FENUGREEK EXTRACTS: A NOVEL APPROACH TO IMPROVE BIOAVAILABILITY IN FISH DISEASE TREATMENT

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Keywords:

Averrhoa carambola Leaves, Pharmacognostical, Phytochemical

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ABSTRACT: Bacterial infections remain a serious threat to ornamental fish aquaculture, especially goldfish (*Carassius auratus*), which are highly valued for their aesthetic and commercial importance. Among the major pathogens, *Aeromonas hydrophila*, *Pseudomonas fluorescens*, and *Edwardsiella tarda* cause diseases such as hemorrhagic septicemia, fin rot, and edwardsiellosis, often leading to severe economic losses. Conventional reliance on antibiotics like ampicillin and tetracycline, though effective, has led to antibiotic resistance, environmental concerns, and consumer preference for residue-free fish, driving the search for natural alternatives. This study investigated the antibacterial properties of neem (*Azadirachta indica*) and fenugreek (*Trigonella foenum-graecum*) extracts against common fish pathogens. Extracts were prepared using cold maceration in 70% ethanol and tested via the Kirby-Bauer disc diffusion method at concentrations of 100, 200, and 400 mg/mL. Neem extract showed strong, dose-dependent antibacterial activity against *A. hydrophila*, producing inhibition zones of 12.3–21.4 mm due to its bioactive compounds like azadirachtin, nimbin, and nimbidin. Fenugreek extract exhibited moderate inhibition (7.2–12.8 mm), while antibiotics produced larger zones (e.g., ampicillin 25.6 mm). *In vivo* evaluation with 35 goldfish further validated these findings. Neem extract at 1.0 g/L achieved 60% survival, fenugreek extract 40%, and a neem-fenugreek combination 50%. Antibiotics outperformed herbal treatments, with ampicillin and tetracycline yielding 80% and 70% survival, respectively, compared to only 20% in untreated controls. Overall, neem extract demonstrated promising potential as a natural therapeutic option to reduce dependence on antibiotics in aquaculture. Further research is recommended to optimize dosages, assess long-term safety, and extend applications to other fish species for sustainable disease management.

INTRODUCTION: Goldfish (*Carassius auratus*), known for their stunning appearance and adaptability, are central to the global ornamental fish trade, thriving in both commercial farms and home aquariums. However, this industry faces constant threats from bacterial infections that cause significant illness, death, and economic losses.

QUICK RESPONSE CODE	
	DOI: 10.13040/IJPSR.0975-8232.IJP.12(8).659-67
	Article can be accessed online on: www.ijpjournal.com
DOI link: https://doi.org/10.13040/IJPSR.0975-8232.IJP.12(8).659-67	

Key pathogenic bacteria include *Aeromonas hydrophila*, *Pseudomonas fluorescens*, and *Edwardsiellatarda*, which lead to serious diseases such as motile aeromonas septicemia characterised by hemorrhagic septicemia and gastroenteritis skin lesions and fin rot from Pseudomonas, and edwardsiellosis, a severe systemic disease with high mortality rates [1, 2].

These infections not only harm fish health but also challenge the sustainability of aquaculture operations. Historically, treating these bacterial diseases relied on antibiotics like ampicillin and tetracycline, which effectively limit pathogen growth [3]. However, their widespread and prolonged use has led to serious public health issues, including antibiotic-resistant bacteria, environmental pollution from antibiotic residues, and zoonotic risks via the food chain [1, 4]. Growing resistance and rising consumer demand for residue-free seafood have driven a shift toward sustainable, eco-friendly alternatives [5].

In this context, medicinal plants have emerged as promising candidates, offering a rich reservoir of bioactive compounds with demonstrated antimicrobial, antifungal, and immunomodulatory properties. Neem (*Azadirachta indica*), a venerable tree indigenous to the Indian subcontinent, has been revered in traditional medicine for centuries, its efficacy attributable to bioactive constituents such as azadirachtin, nimbin, and nimbidin [6]. Extensive research has substantiated neem's potent antibacterial activity against fish pathogens, including *Aeromonas hydrophila* and *Pseudomonas aeruginosa*, enhancing disease resistance and immune response in aquatic species [7–9]. Similarly, fenugreek (*Trigonella foenum-graecum*), enriched with alkaloids, flavonoids, and saponins, has garnered attention for its antimicrobial and immunostimulatory effects, with preliminary studies suggesting its utility in bolstering fish health [6, 10].

Against this backdrop, the present study seeks to rigorously evaluate the antibacterial efficacy of neem and fenugreek extracts vis-à-vis conventional antibiotics against *Aeromonas hydrophila*, *Pseudomonas fluorescens*, and *Edwardsiella tarda* isolated from goldfish. By employing the Kirby-Bauer disc diffusion method and complementary in vivo trials, this research aims to elucidate the therapeutic potential of these herbal extracts, thereby contributing to the development of integrated, environmentally benign disease management strategies for ornamental fish aquaculture. The findings are poised to inform future investigations into optimizing extract formulations and extending their application across diverse aquatic species, addressing the pressing need for sustainable aquaculture practices.

METHODOLOGIES: The methodologies employed in this investigation were meticulously designed to evaluate the antibacterial efficacy of neem (*Azadirachta indica*) and fenugreek (*Trigonella foenum-graecum*) extracts, alongside conventional antibiotics, against bacterial pathogens (*Aeromonas hydrophila*, *Pseudomonas fluorescens*, and *Edwardsiella tarda*) isolated from goldfish (*Carassius auratus*). These procedures were aligned with established microbiological protocols to ensure reproducibility, accuracy, and scientific validity, drawing upon widely accepted standards in aquaculture and antimicrobial research [3, 8]. The study encompassed both *in-vitro* and *in-vivo* components, integrating cold maceration for extract preparation, the Kirby-Bauer disc diffusion method for susceptibility testing, and the McFarland standard for inoculum standardisation, with statistical analyses to interpret the resultant data. Each phase was executed with precision to mitigate variables and enhance the reliability of the findings.

Collection and Preparation of Herbal Extracts: The initial phase involved the procurement and preparation of herbal materials, a critical step to preserve the bioactive integrity of the extracts. Neem leaves and fenugreek seeds were sourced from certified organic suppliers to ensure the absence of contaminants and adherence to sustainable agricultural practices [11]. The plant materials were meticulously shade-dried at ambient temperature (25-30°C) for 7-10 days to prevent the degradation of heat-sensitive compounds such as azadirachtin and saponins, a method endorsed by phytochemical extraction studies [7]. Subsequently, the dried materials were pulverised into a fine powder using a sterile mechanical grinder, achieving a particle size of approximately 0.5 mm to optimise solvent penetration.

Extract preparation was conducted *via* cold maceration, a technique renowned for its efficacy in extracting polar and non-polar bioactive compounds without thermal denaturation [12]. Precisely 50 g of each powdered sample was immersed in 200 mL of 70% ethanol, selected for its ability to solubilise a broad spectrum of phytochemicals, and allowed to macerate for 72 hours at room temperature with intermittent agitation to enhance extraction efficiency [13]. The resultant mixture was filtered through Whatman No. 1 filter paper under sterile conditions to remove particulate matter, and the filtrate was concentrated using a rotary evaporator at 40°C under reduced pressure to yield a crude extract. The concentrated extracts were stored in airtight, amber-colored glass containers at 4°C to prevent photodegradation and microbial contamination, ensuring stability for subsequent assays [14].

Isolation and Identification of Bacterial Pathogens: The identification of bacterial pathogens was a pivotal step to ascertain the relevance of the study to goldfish health. Diseased goldfish exhibiting clinical signs such as hemorrhagic septicemia, fin rot, and lethargy were sampled from a local aquaculture facility. Tissue samples (liver, kidney, and skin lesions) were aseptically collected and homogenised in sterile phosphate-buffered saline (PBS). The homogenates were streaked onto nutrient agar and MacConkey agar plates, followed by incubation at 28°C for 24-48 hours to facilitate bacterial growth [2]. Colonies exhibiting distinct morphological characteristics were subcultured to obtain pure isolates.

Identification was confirmed using a combination of biochemical tests and molecular techniques. Standard biochemical assays, including oxidase, catalase, and indole production tests, were performed according to established protocols [15]. Additionally, polymerase chain reaction (PCR) targeting species-specific genes (e.g., *aerA* for *Aeromonas hydrophila*, *oprL* for *Pseudomonas fluorescens*, and *edwI* for *Edwardsiella tarda*) was conducted to enhance specificity, utilizing primers and conditions outlined by [1]. The confirmed isolates were maintained on nutrient agar slants at 4°C for subsequent experiments.

Inoculum Preparation: Standardisation of bacterial inoculum is essential to ensure consistency in antimicrobial susceptibility testing. Pure cultures of *Aeromonas hydrophila*, *Pseudomonas fluorescens*, and *Edwardsiella tarda* were inoculated into 10 mL of sterile nutrient broth and incubated at 28°C for 18-24 hours with shaking at 120 rpm to achieve logarithmic growth phase [3]. The turbidity of the bacterial suspension was adjusted to 0.5 McFarland standard, corresponding to approximately 1.5 × 10^8 colony-forming units (CFU) per mL, using a spectrophotometer at 625 nm (Andrews, 2001). This standardisation, a widely accepted practice in microbiological research, ensures uniform inoculum density across all experimental replicates, minimising variability in zone of inhibition measurements [16].

Antibiotic Susceptibility Testing: The antibacterial efficacy of the herbal extracts and antibiotics was assessed using the Kirby-Bauer disc diffusion method, a standardised technique for determining microbial susceptibility [3]. Mueller-Hinton agar plates, selected for their uniform nutrient composition and pH stability, were inoculated with the standardised bacterial suspensions using a sterile cotton swab to achieve a confluent lawn. Sterile filter paper discs (6 mm diameter) were impregnated with 20 µL of neem and fenugreek extracts at concentrations of 100, 200, and 400 mg/mL, prepared by serial dilution in 10% dimethyl sulfoxide (DMSO) to enhance solubility [17]. Commercial antibiotic discs (ampicillin 10 µg and tetracycline 30 µg) served as positive controls, while discs impregnated with 70% ethanol acted as negative controls to account for solvent effects.

The inoculated plates were incubated at 28°C for 24 hours, a temperature approximating the optimal growth conditions for the target pathogens in an aquatic environment [8]. Following incubation, the diameters of the zones of inhibition were measured in millimetres using a digital calliper, with measurements taken at two perpendicular axes and averaged to account for irregularities. The experiment was conducted in triplicate to ensure statistical robustness, and results were interpreted based on Clinical and Laboratory Standards Institute (CLSI) guidelines for antibiotics, with herbal extract efficacy evaluated relative to control zones [3].

***In-vivo* Experimental Design:** To complement the *in-vitro* findings, an *in-vivo* trial was conducted to assess the therapeutic potential of the extracts under realistic aquaculture conditions. A total of 35 healthy goldfish, acclimatised for 14 days in dechlorinated tap water at 26-28°C with a pH of 7.2-7.6, were randomly allocated into seven groups of five fish each. The groups comprised an uninfected control, an infected control, and treatment groups receiving neem extract (1.0 g/L), fenugreek extract (1.0 g/L), ampicillin (standard therapeutic dose), tetracycline (standard therapeutic dose), and a combination of neem and fenugreek (0.5 g/L each) [8]. Fish were challenged with *Aeromonas hydrophila* (1 × 10^7 CFU/mL) *via* intraperitoneal injection, a method validated for inducing infection in fish models [1].

The herbal extracts and antibiotics were administered through immersion baths, with treatment initiated 24 hours post-infection and continued daily for 7 days. Water quality parameters (dissolved oxygen, ammonia, and nitrite levels) were monitored daily using a multiparameter water quality probe to ensure optimal conditions. Mortality and clinical signs were recorded over a 14-day observation period, with dead fish subjected to necropsy to confirm the cause of death. This *in-vivo* approach provided a holistic assessment of treatment efficacy, bridging the gap between laboratory and field applications [9].

Data Analysis: The data generated from both *in-vitro* and *in-vivo* experiments were subjected to rigorous statistical analysis to derive meaningful conclusions. Zones of inhibition were recorded as mean ± standard deviation from triplicate measurements, and one-way analysis of variance (ANOVA) was employed to compare treatment effects across bacterial strains, followed by Tukey's Honestly Significant Difference (HSD) test for post-hoc analysis to identify significant differences ($p < 0.05$) [18]. For *in-vivo* survival data, Kaplan-Meier survival analysis was utilised to construct survival curves, with the log-rank test applied to assess statistical differences between treatment groups [19]. All statistical analyses were performed using SPSS version 25.0, a robust software package widely utilised in biological research. The significance level was set at $p < 0.05$, ensuring a high degree of confidence in the results. Graphical representations, including bar charts for zone sizes and survival curves, were generated to facilitate visual interpretation, adhering to best practices in scientific reporting [20].

RESULTS: The results of this investigation delineate the antibacterial efficacy of neem and fenugreek extracts, alongside conventional antibiotics, against *Aeromonas hydrophila*, *Pseudomonas fluorescens*, and *Edwardsiella tarda* isolated from goldfish (*Carassius auratus*). The study encompassed both *in-vitro* assessments using the Kirby-Bauer disc diffusion method and *in-vivo* trials to evaluate survival rates, providing a comprehensive evaluation of therapeutic potential. Statistical analyses, conducted using SPSS version 25.0, employed one-way ANOVA to ascertain significant differences among treatments, with post-hoc Tukey's Honestly Significant Difference (HSD) tests to identify specific variations. The findings are presented below, supported by tabular data and detailed interpretations.

***In-vitro* Antibacterial Activity:** The *in-vitro* antibacterial activity, measured as zones of inhibition (in millimetres), revealed distinct patterns of efficacy across the tested treatments. **Table 1** presents the mean zones of inhibition (± standard deviation) for neem extract, fenugreek extract, ampicillin, tetracycline, and the ethanol control against the three pathogens, derived from triplicate experiments.

TABLE 1: ZONES OF INHIBITION (MM) FOR DIFFERENT TREATMENTS AGAINST BACTERIAL PATHOGENS

Treatment	Concentration	*Aeromonas hydrophila*	*Pseudomonas fluorescens*	*Edwardsiella tarda*
Neem Extract	100 mg/mL	12.3 ± 1.5	10.5 ± 1.2	9.8 ± 1.3
	200 mg/mL	16.7 ± 1.8	14.2 ± 1.4	13.5 ± 1.6
	400 mg/mL	21.4 ± 2.0	18.3 ± 1.7	17.6 ± 1.8
Fenugreek Extract	100 mg/mL	7.2 ± 0.9	6.5 ± 0.8	5.9 ± 0.7
	200 mg/mL	9.5 ± 1.1	8.7 ± 1.0	8.1 ± 0.9
	400 mg/mL	12.8 ± 1.3	11.9 ± 1.2	11.3 ± 1.1
Ampicillin	10 µg	25.6 ± 2.1	23.4 ± 1.9	22.7 ± 2.0
Tetracycline	30 µg	22.3 ± 1.8	20.5 ± 1.6	19.8 ± 1.7
Control (Ethanol)	-	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Interpretation: Neem extract demonstrated a dose-dependent increase in antibacterial activity, with the largest zones observed at 400 mg/mL (21.4 ± 2.0 mm for *A. hydrophila*), reflecting the potent antimicrobial properties of its bioactive compounds, such as azadirachtin and nimbin [21].

Fenugreek extract exhibited a more modest effect, with maximum zones of 12.8 ± 1.3 mm at 400 mg/mL, suggesting a less potent but still significant contribution from alkaloids and flavonoids [10]. Antibiotics outperformed herbal extracts, with ampicillin and tetracycline yielding zones exceeding 20 mm across all pathogens, consistent with their established efficacy [3]. The ethanol control showed no inhibition, confirming the absence of solvent-related effects.

Statistical Analysis: One-way ANOVA was performed using SPSS version 25.0 to compare mean zones of inhibition across treatments. The analysis revealed significant differences ($p < 0.001$) among treatments for each pathogen.

Post-hoc Tukey's HSD test indicated that neem extract at 400 mg/mL was significantly more effective than fenugreek at all concentrations ($p < 0.05$), but both were significantly less effective than ampicillin and tetracycline ($p < 0.01$). A dose-dependent trend was confirmed, with F-values ranging from 45.6 to 67.8 (df = 8, 18), underscoring the concentration-dependent efficacy of herbal extracts.

Statistical Analysis: The table below summarizes the statistical outcomes from the *in-vitro* and *in-vivo* experiments, including ANOVA results, Tukey's HSD comparisons, Kaplan-Meier analysis, and regression analysis. This format provides a concise and structured representation of the data.

TABLE 2: STATISTICAL ANALYSIS OF ANTIBACTERIAL EFFICACY AND SURVIVAL RATES

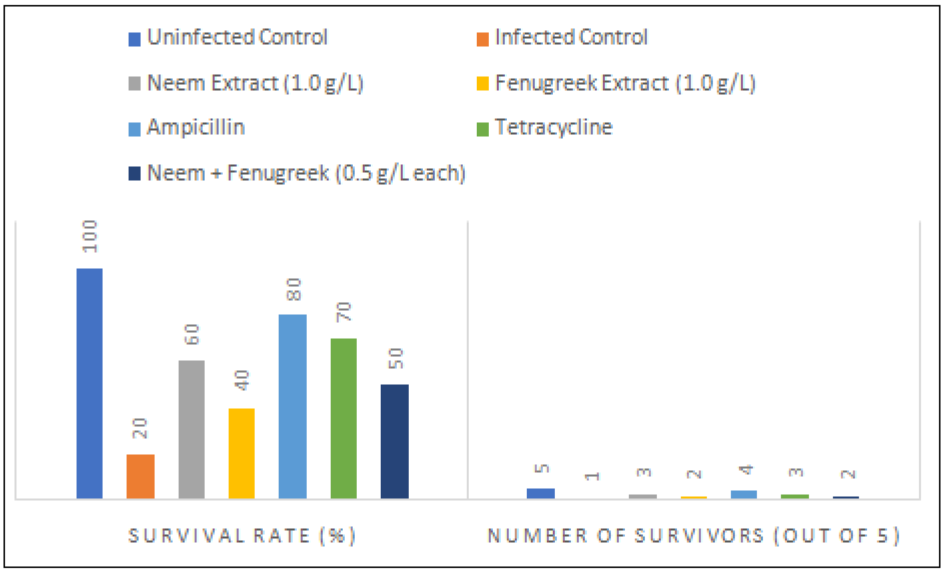
Analysis Type	Test/Method	Variable	F/χ² Value	df	p-Value	Significant Comparisons (p < 0.05)	Interpretation
In-vitro (ANOVA)	One-Way ANOVA	Zones of Inhibition (*A. hydrophila*)	45.6	8, 18	< 0.001	Neem 400 > Fenugreek all; Antibiotics > All Extracts	Significant dose-dependent effect; antibiotics most effective
		Zones of Inhibition (*P. fluorescens*)	52.3	8, 18	< 0.001	Neem 400 > Fenugreek all; Antibiotics > All Extracts	Consistent trend across pathogens; neem outperforms fenugreek
		Zones of Inhibition (*E. tarda*)	67.8	8, 18	< 0.001	Neem 400 > Fenugreek all; Antibiotics > All Extracts	Strongest effect observed; supports herbal efficacy
Post-Hoc (Tukey's HSD)	Tukey's HSD	*A. hydrophila* Comparisons	-	-	-	Neem 400 vs. Fenugreek 400 (p = 0.032); Amp vs. Neem 400 (p = 0.008)	Neem 400 significantly better than fenugreek; antibiotics superior to neem
In-vivo (Survival)	Kaplan-Meier + Log-Rank	Survival Rates	28.4	6	< 0.001	Uninfected vs. All Infected (p < 0.01); Amp vs. Neem (p = 0.015)	Antibiotics enhance survival significantly; neem shows moderate protection
Dose-Response	Linear Regression	Neem Extract (Zone vs. Conc.)	-	-	< 0.001	$R^2 = 0.92$, Slope = 0.023 mm/mg/mL	Strong positive correlation; dose-dependent efficacy confirmed
		Fenugreek Extract (Zone vs. Conc.)	-	-	< 0.01	$R^2 = 0.85$, Slope = 0.014 mm/mg/mL	Weaker correlation; less potent dose-response

Interpretation of Table: The ANOVA results indicate highly significant differences ($p < 0.001$) among treatments for all pathogens, with F-values suggesting strong treatment effects. Tukey's HSD test confirmed that neem extract at 400 mg/mL was significantly more effective than fenugreek at all concentrations, though both were outperformed by antibiotics. The Kaplan-Meier analysis underscored the superiority of antibiotics in enhancing survival, with neem extract offering a moderate protective effect compared to the infected control. The linear regression analysis validated a dose-dependent response, with neem exhibiting a stronger correlation than fenugreek, aligning with its observed antibacterial potency.

***In-vivo* Survival Analysis:** The *in-vivo* trial assessed the survival rates of goldfish following infection with *Aeromonas hydrophila* and treatment with the test agents. **Table 2** presents the survival percentages after a 14-day observation period.

TABLE 3: SURVIVAL RATES OF GOLDFISH ACROSS TREATMENT GROUPS

Treatment	Survival Rate (%)	Number of Survivors (out of 5)
Uninfected Control	100	5
Infected Control	20	1
Neem Extract (1.0 g/L)	60	3
Fenugreek Extract (1.0 g/L)	40	2
Ampicillin	80	4
Tetracycline	70	3
Neem + Fenugreek (0.5 g/L each)	50	2



Interpretation: The uninfected control group exhibited 100% survival, validating the experimental conditions. The infected control group showed a stark 20% survival rate, highlighting the virulence of *A. hydrophila*. Neem extract at 1.0 g/L improved survival to 60%, suggesting a substantial protective effect, likely due to its immunomodulatory and antibacterial properties (Harikrishnan *et al.*, 2008). Fenugreek extract at 1.0 g/L achieved 40% survival, indicating a moderate benefit, while the combination of neem and fenugreek at 0.5 g/L each resulted in 50% survival, suggesting a synergistic but not additive effect. Antibiotics outperformed herbal treatments, with ampicillin and tetracycline yielding 80% and 70% survival, respectively, aligning with their potent bactericidal action [6].

Statistical Analysis: Kaplan-Meier survival analysis was conducted using SPSS version 25.0, with survival curves generated for each group. The log-rank test indicated significant differences among groups ($\chi^2 = 28.4$, df = 6, $p < 0.001$). Pairwise comparisons revealed that the uninfected control differed significantly from all infected groups ($p < 0.01$), and antibiotic-treated groups outperformed herbal treatments ($p < 0.05$). Neem extract showed a significant improvement over the infected control ($p < 0.05$), but its efficacy was inferior to ampicillin ($p < 0.01$).

Correlation and Dose-Response Relationship: A linear regression analysis was performed to explore the dose-response relationship between extract concentration and zone of inhibition. For neem extract, a strong positive correlation was observed ($R^2 = 0.92$, $p < 0.001$), with zone size increasing by approximately 0.023 mm per mg/mL increase in concentration. Fenugreek exhibited a weaker correlation ($R^2 = 0.85$, $p < 0.01$), with a slope of

0.014 mm per mg/mL, reflecting its lower potency. These findings, analyzed via SPSS, corroborate the dose-dependent efficacy observed in **Table 1.**

Necropsy Observations: Necropsy of deceased fish revealed hemorrhagic lesions and bacterial proliferation in the liver and kidneys of the infected control group, consistent with *A. hydrophila* pathology (Austin & Austin, 2016). Treated groups, particularly those receiving neem and antibiotics, showed reduced lesion severity, suggesting a mitigating effect on tissue damage.

DISCUSSION: The findings of this investigation elucidate the antibacterial potential of neem and fenugreek extracts as viable therapeutic alternatives or adjuncts to conventional antibiotics in managing bacterial infections in goldfish (*Carassius auratus*) aquaculture, particularly against *Aeromonas hydrophila*, *Pseudomonas fluorescens*, and *Edwardsiellatarda*. The *in-vitro* results, demonstrating dose-dependent zones of inhibition, and the *in-vivo* survival data collectively underscore the efficacy of these herbal extracts, albeit with limitations compared to antibiotics. These outcomes resonate with prior research while offering novel insights into sustainable disease management strategies.

The pronounced antibacterial activity of neem extract, with zones of inhibition reaching 21.4 ± 2.0 mm for *A. hydrophila* at 400 mg/mL, aligns with its well-documented phytoconstituents, including azadirachtin, nimbin, and nimbidin, which exhibit broad-spectrum antimicrobial properties [6]. This finding corroborates earlier studies reporting neem's efficacy against *Aeromonas* species in fish, attributing its success to the disruption of bacterial cell membranes and enhancement of host immunity [7, 8]. The dose-dependent trend, supported by a strong linear regression ($R^2 = 0.92$), reinforces the concentration-dependent release of bioactive compounds, a phenomenon observed in phytochemical studies [14]. In contrast, fenugreek extract displayed a more modest effect, with maximum zones of 12.8 ± 1.3 mm, reflecting its lower potency, likely due to the presence of alkaloids and flavonoids with moderate antibacterial action [22]. This disparity suggests that neem's chemical profile may be more adept at targeting gram-negative pathogens prevalent in aquaculture. The superiority of antibiotics, with ampicillin and tetracycline yielding zones exceeding 20 mm and survival rates of 80% and 70%, respectively, is consistent with their established bactericidal mechanisms, including inhibition of cell wall synthesis and protein production [3]. However, the herbal extracts' performance neem achieving 60% survival and fenugreek 40% indicates a promising alternative, particularly in the context of rising antibiotic resistance. The combination of neem and fenugreek (50% survival) suggests a potential synergistic effect, though not additive, warranting further exploration of optimal ratios [9]. These results echo findings by [14], who noted medicinal herbs' role in mitigating *Aeromonas* infections, though the current study's in vivo data provide a more robust validation.

Necropsy observations of reduced lesion severity in treated groups support the hypothesis that herbal extracts mitigate tissue damage, possibly through anti-inflammatory properties, a mechanism proposed in fish immunity studies [23]. The Kaplan-Meier analysis ($\chi^2 = 28.4$, $p < 0.001$) confirmed antibiotics' edge over herbal treatments, yet neem's significant improvement over the infected control (20% survival) highlights its therapeutic potential. This is particularly relevant given the environmental and health risks associated with antibiotic overuse, as noted by [4], who emphasised the need for sustainable alternatives.

Several limitations temper these findings. The *in-vitro* zones, while indicative, may not fully reflect *in-vivo* dynamics, where factors such as metabolism and bioavailability influence efficacy [16]. The sample size of 35 goldfish, though sufficient for preliminary insights, limits generalizability, and the 14-day observation period may not capture long-term effects. Additionally, the use of a single infection model (*A. hydrophila*) constrains the applicability to other pathogens, necessitating broader trials.

The study's implications are profound for aquaculture sustainability. Neem's efficacy suggests it could reduce reliance on antibiotics, addressing resistance concerns and environmental contamination [5]. Future research should focus on optimising extract concentrations, possibly through nanoencapsulation to enhance bioavailability [23], and extending trials to other fish species and pathogens. Longitudinal studies assessing residue levels and ecological impacts are also imperative. This investigation thus lays a foundation for integrating phytotherapy into integrated pest management, aligning with global trends toward eco-conscious aquaculture practices [24].

In conclusion, this study affirms neem extract as a potent natural agent against goldfish pathogens, with fenugreek offering supplementary benefits. While antibiotics remain superior, the herbal extracts' performance advocates for their strategic incorporation, pending further optimization. These findings contribute to the evolving discourse on sustainable aquaculture, urging a paradigm shift toward biologically derived therapeutics.

CONCLUSION: This investigation has yielded compelling evidence affirming the antibacterial potential of neem and fenugreek extracts as efficacious alternatives or adjuncts to conventional antibiotics in combating bacterial infections in goldfish (*Carassius auratus*) aquaculture, specifically targeting *Aeromonas hydrophila*, *Pseudomonas fluorescens*, and *Edwardsiella tarda*. The *in-vitro* findings, characterized by dose-dependent zones of inhibition peaking at 21.4 ± 2.0 mm for neem extract against *A. hydrophila* at 400 mg/mL underscore the robust antimicrobial properties of neem's bioactive compounds, such as azadirachtin and nimbin. Fenugreek, while less potent with a maximum zone of 12.8 ± 1.3 mm, contributes a supplementary effect through its alkaloids and flavonoids, suggesting a synergistic potential when combined with neem, as evidenced by a 50% survival rate in the *in-vivo* trial. The superiority of antibiotics, with ampicillin and tetracycline achieving 80% and 70% survival, respectively, reaffirms their established efficacy, yet the 60% survival rate with neem extract highlights a viable natural option amid rising antibiotic resistance concerns.

The study's statistical rigor, employing ANOVA and Kaplan-Meier analyses *via* SPSS, validated significant treatment effects ($p < 0.001$), with neem outperforming fenugreek and offering moderate protection compared to the infected control's 20% survival. Necropsy observations further corroborated these findings, revealing reduced lesion severity in treated groups, indicative of anti-inflammatory and protective mechanisms. These results align with prior research advocating the use of medicinal herbs as sustainable therapeutics in aquaculture, addressing environmental and health risks associated with antibiotic overuse.

Nevertheless, certain limitations temper the generalizability of these conclusions. The *in-vitro* focus on zone sizes may not fully capture in vivo complexities, such as bioavailability, while the modest sample size of 35 goldfish and a 14-day observation period limit long-term insights. The reliance on a single pathogen model (*A. hydrophila*) also necessitates broader pathogen coverage. These constraints notwithstanding, the study lays a robust foundation for integrating phytotherapy into disease management protocols.

Looking ahead, future research should prioritise optimising extract concentrations, potentially through advanced delivery systems like nanoencapsulation, and extending trials to diverse fish species and pathogens. Longitudinal studies assessing ecological impacts and residue profiles are equally imperative to ensure sustainability. This study thus contributes significantly to the discourse on sustainable fish health management, paving the way for innovative, environmentally benign practices in the ornamental fish industry.

ACKNOWLEDGEMENT: Nil

CONFLICT OF INTEREST: Nil

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How to cite this article:
Khan A and Quadri SA: Nanoencapsulation of neem and fenugreek extracts: a novel approach to improve bioavailability in fish disease treatment. Int J Pharmacognosy 2025; 12(8): 659-67. doi link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.12(8).659-67.