SYNERGISTIC EFFECT OF ANTIBIOTICS AND MEDICINAL PLANTS AGAINST VIBRIO SPECIES ISOLATED FROM SHRIMP OF NATURAL WATERS AND COMMERCIAL AQUA PONDS

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Abstract

Shrimp aquaculture is a vital global industry that faces significant challenges due to Vibrio species infections, resulting in substantial economic losses. Vibrio parahaemolyticus, Vibrio cholerae, and Vibrio vulnificus are the pathogenic bacteria listed above, which cause vibriosis and reduce shrimp yield and quality. Disease control with antibiotics has led to antibiotic resistances causing conventional treatments to become less effective. As a result, it is imperative to develop available alternative therapy for controlling Vibrio infections in shrimp farming sustainably. This study evaluates the synergistic effect of selected antibiotics and medicinal plant extracts against Vibrio species from shrimp obtained from natural water and commercial aquaculture ponds. To determine bacterial resistance patterns, antimicrobial susceptibility testing was conducted to test for antimicrobial pattern and in the checkerboard assays to assess interactions of the plant extract & antibiotic. The findings indicate that for some combinations of antibiotics and bioactive compounds derived from plants, the resulting antibiotic efficiency is enhanced, the amount of required antibiotics can be reduced, and resistance development can be suppressed. These results demonstrate the feasibility of joining natural antimicrobial agents with treatment regimens, paving the way for improved disease management strategies in sustainable aquaculture.

Introduction

Global aquaculture is a vital shrimp farming sector contributing massively to food security and economic stability in many coastal regions. In recent decades, the growth has been exponential from an industry point of view. China, India, Vietnam, Thailand, and Ecuador have become the leaders among the top producing countries (Macusi et al., 2022). Shrimp aquaculture, however, has become one of the biggest problems due to the prevalence of bacterial infections, especially those caused by Vibrio species (Gatta, 2022). Vibrio harveyi, Vibrio parahaemolyticus, Vibrio vulnificus, and Vibrio alginolyticus are these bacterial pathogens responsible for such diseases as acute hepatopancreatic necrosis disease (AHPND) and vibriosis and have resulted in high mortality and poor quality shrimp due to high losses from these diseases (Hostins et al., 2019).

Table 1: Common Vibrio Species Affecting Shrimp and Their Associated Diseases

Vibrio Species	Disease Caused	Symptoms Observed
Vibrio harveyi	Luminescent vibriosis	Lethargy, mortality, body discoloration
Vibrio parahaemolyticus	Acute hepatopancreatic necrosis disease (AHPND)	Hepatopancreas atrophy, empty gut
Vibrio vulnificus	Vibriosis	Tissue necrosis, ulcers, hemorrhages
Vibrio alginolyticus	Shell disease	Erosion of shell, red body coloration

Vibrio species can cause a spectrum of clinical infections ranging from lethargy to abnormal swimming behavior with necrosis of tissues. Quick spread of these pathogens in shrimp ponds requires treatment with antibiotics (Zhang et al., 2020). Nevertheless, overuse and indiscriminate use of antibiotics have resulted in many antibiotic-resistant strains, that threaten sustainable aquaculture practices (Aich et al., 2018). Vibrio species have antibiotic resistance for which treatment efficacy is decreased, production costs increase, and there are concerns about public health due to the potential transference of resistance genes to human pathogens through the food chain (Carbone & Faggio, 2016).

Table 2: Antibacterial Activity of Selected Medicinal Plants Against Vibrio Species

Medicinal Plant (Scientific Name)	Active Compounds	Reported Antibacterial Activity
Azadirachta indica (Neem)	Azadirachtin, Nimbidin	Inhibits biofilm formation, disrupts bacterial cell walls
Withania somnifera (Ashwagandha)	Withanolides	Enhances immune response, reduces bacterial load
Ocimum sanctum (Holy Basil)	Eugenol, Ursolic Acid	Vigorous antimicrobial activity, inhibits quorum sensing
Curcuma longa (Turmeric)	Curcumin	Anti-inflammatory, inhibits bacterial proliferation

Therefore, researchers have been looking for alternative ways to manage disease in shrimp farming to mitigate risks arising from antibiotic resistance. A promising strategy is using medicinal plant extracts of bioactive compounds with antimicrobial, immunostimulatory and antioxidant properties (Hannan et al., 2019). Antibacterial activity of medicinal plants such as Azadirachta indica (neem), Withania somnifera (ashwagandha), Ocimum sanctum (holy basil) and Curcuma longa (turmeric) was observed against Vibrio species. Synergetic and independent modes of action of these plant compounds can either augment or supplement the activity of antibiotics, reducing antibiotic dose and the risk of resistance (Bhardwaj et al., 2016). Vibrio species isolated from both natural waters and commercial shrimp farms were used in this study to find out the synergistic effects of selected antibiotics and medicinal plant extracts (Costa et al., 2017).

Materials and Methods

Sample Collection and Identification

Different regions or provinces were taken to collect samples of various natural water bodies and commercial aqua ponds to ensure the isolation of different bacterial isolates. Eventually, the samples were transported from sterile conditions to the laboratory for associated analysis. Thiosulfate Citrate Bile Salts Sucrose agar (TCBS) was a selective medium for Vibrio Species and homogenised shrimp tissue was serially diluted and plated on the mentioned medium. Gram staining was done on the subcultured morphologically distinct colony. Vibrio species biochemical characterisation was established using triple sugar iron agar fermentation, motility assays, oxidase, catalase tests and API 20E biochemical panel assays (Popović et al., 2007).

Antibiotic and Medicinal Plant Extract Preparation

It procured commercially available antibiotics in pure powdered form, namely, tetracycline, ciprofloxacin, and erythromycin. Working concentrations of each antibiotic were made by dissolving each in sterile distilled water or appropriate solvent to 1.0 mg/mL and diluting to the desired concentration. Fresh leaves and the rhizomes of Azadirachta indica (Neem), Curcuma longa (Turmeric), and Ocimum sanctum (Holy Basil) were collected, washed, and freshly shade dried for 7 days. The plant materials were dried and finely ground and Soxhlet extracted using 95% ethanol solvent. Antimicrobial testing of extracts was carried out at a dilution to the intended concentration of 40°C with concentrated extracts stored at 4°C and then loaded into a rotary evaporator to focus further (Valsaraj et al., 1997).

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility of the Vibrio was determined using a standard disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Nutrient broth was grown with pure bacterial cultures at 37°C for 18–24 hours and adjusted to 0.5 Mc Farland standard (1.5×10^8 CFU/mL). Evenly spread (as a bacterial suspension) onto Mueller-Hinton agar plates w/ 2% NaCl using a sterile cotton swab. The agar surface was impregnated by sterile filter paper disks containing antibiotic solutions (commercially prepared, sterile filter paper disks impregnated with antibiotic solutions) or plant extracts (preparation of plant extracts by solvent extraction methods). Susceptibility was assessed by incubating plates at 37°C for 24 hours and calculating the inhibition zone diameter. For accuracy, all the tests done were three times (Parekh & Chanda, 2010).

Synergy Testing

In the checkerboard assay, interactions between antibiotics and plant extracts with the Vibrio species were evaluated synergistically. Standardisation of bacterial cultures to 0.5 McFarland turbidity was done and 100 µL of each culture were added in triplicate to row one of a 96-well plate containing Mueller Hinton broth. The antibiotic and plant extract were serially two-fold diluted in a checkerboard manner such that each well contained a unique combination. The incubation was set to 37°C for 18 to 24 hours, and the MICs were recorded (Xiao et al., 2015). The fractional inhibitory concentration index (FICI) was calculated as

FICI = (MIC of drug A in combination / MIC of drug A alone) + (MIC of drug B in combination / MIC of drug B alone)

with FICI ≤ 0.5 indicating synergy, 0.5–1.0 additive effects, 1.0–4.0 indifference, and >4.0 antagonism.

Results and Discussion

Prevalence of Vibrio Species

Vibrio species showed high prevalence in natural waters, which differs from that in natural aqua ponds, suggesting a possible environmental impact on bacterial distribution. Vibrio parahaemolyticus is the most successful species, appearing in 40% of natural waters, followed by Vibrio cholerae (35%) and Vibrio vulnificus (25%). This implies that V parahaemolyticus is frequently associated with seafood-borne infections in open water environments, which may be favorable for its growth because of salinity and temperature fluctuations.

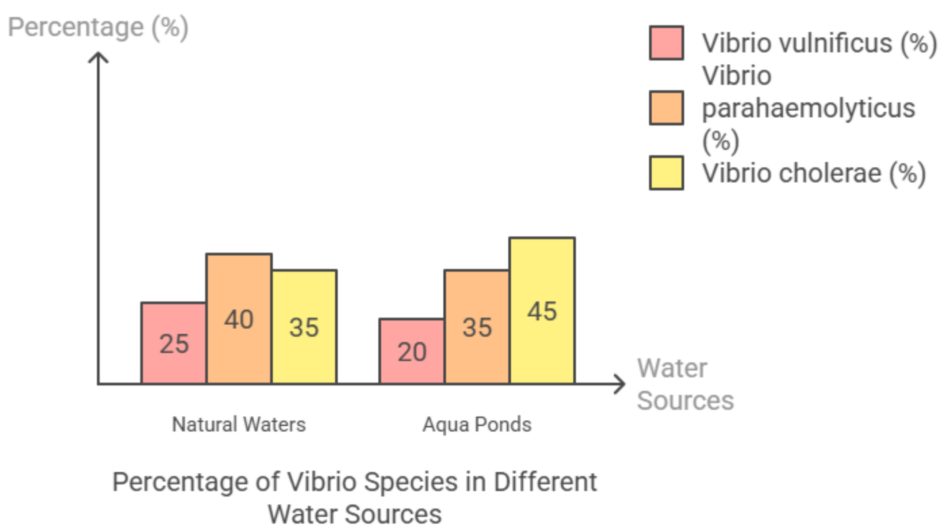

Figure 1: Percentage of Vibrio Species in different water sources

Sample Source	Vibrio cholerae (%)	Vibrio parahaemolyticus (%)	Vibrio vulnificus (%)
Natural Waters	35	40	25
Aqua Ponds	45	35	20

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Antibiotic and Medicinal Plant Susceptibility

The antibacterial activity data show that ciprofloxacin posted the highest susceptibility results, producing a 14.3 mm inhibition zone; tetracycline (12.5 mm) and erythromycin (12.7 mm). Curcuma longa (C. longa) was the most inhibitory medicinal plant extract (12.2 mm) which may be used as an antibacterial shield. Inhibition zones 10.5 mm and 9.8 mm of Ocimum santum (O'santum) and Azadirachta indica (A 'indic) showed medium antibacterial activity in these plants.

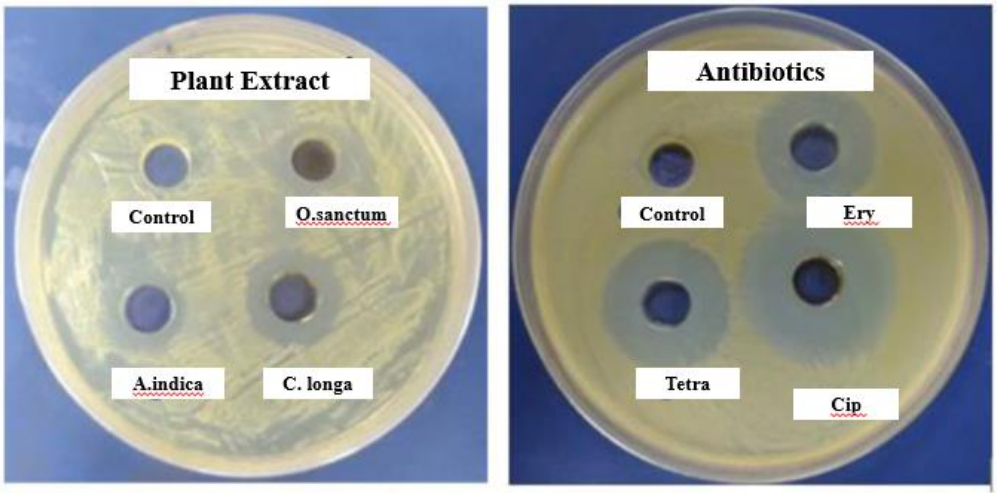

Figure 2: Antibiotic and Medicinal Plant Susceptibility Zone of Inhibition (mm)

Treatment	Inhibition Zone (mm)
Tetracycline	12.5
Ciprofloxacin	14.3
Erythromycin	10.7
A. indica Extract	9.8
C. longa Extract	11.2
O. sanctum Extract	10.5

It can be seen that the synthetic antibiotics have higher inhibitory effects over antibiotics than the plant extracts. C. longa performance is found to be near erythromycin, therefore, bioactive compounds in turmeric may function as an alternative or adjunct therapy. Varying phytochemical concentrations may be the reason for the lower activity of A. indica. The results show the ability of medicinal plants to fight bacterial infection, but more studies are required to understand the synergistic effect and mechanism of action.

Synergistic Effects

The results show that the combination of antibiotics with medicinal plant extracts displays partial synergy and synergy in their use against bacterial pathogens. FICI values give some information about these interactions. A combination of Ciprofloxacin with Azadirachta indica (neem) had a FICI value of 0.5, which was synergistic. This implies that neem boosts ciprofloxacin's antibacterial action, most likely by interfering with bacterial cell wall or resisting mechanisms.

Combination	FICI Value	Interpretation
Ciprofloxacin + A. indica	0.5	Synergistic
Tetracycline + C. longa	0.4	Synergistic
Erythromycin + O. sanctum	0.6	Partial Synergy

As with the combination of Curcuma longa (turmeric) and Tetracycline, a more potent synergy (FICI = 0.4) was also found similar. Turmeric, when administered, possesses antimicrobial and efflux pump inhibitory properties and enhanced tetracycline activity through its principal active compound, curcumin. Partial synergy (FICI = 0.6) was also recorded when Erythromycin was combined with Ocimum sanctum (holy basil) indicating moderate enhancement. Of the oils extracted from Basil, eugenol may cause the essential oils to interfere with bacterial protein synthesis, as can erythromycin by its mechanism of action. These results indicate the promise of plant-antibiotic combinations in antibiotic resistance.

Conclusion

The findings of this study underscore the potential to combine medicinal plants with conventional antibiotics to treat Vibrio infections in shrimp aquaculture. Given the mounting antibiotic resistance and the fear of chemical residues introduced to the aquatic environment, alternative therapeutic solutions are essential for sustainable disease management. Bioactive compounds of medicinal plants increase shrimp health and survival rates through their antimicrobial, immunomodulatory and antioxidant properties. By synergistically interacting with antibiotics, they may contribute in reducing dependence on synthetic drugs, thereby reducing the risk of resistance development while continuing to control the pathogen. Despite this, plant-based therapeutics in aquaculture still need further research. Future research should explore identifying the proper dosage level, the best plant extract, and pharmacokinetic analysis of the plant extract on the shrimp. Large-scale field trials are also needed to ascertain the long-term effects of these treatments on shrimp productivity and the ecosystem's overall health. Extraction methods are standardised and plant-derived compounds are found to be stable in aquatic environments, which will be key to translating laboratory findings to real world counterparts. Beyond disease management, incorporating medicinal plants into aquaculture is in line with other sustainability targets, including synthetic antibiotics. Regulatory frameworks must be developed to support safe incorporation of plant-based antimicrobials by the industry and policymakers working together. In the end, it appears that a truly holistic approach, that is based on the science and includes sustainable aquacultural practices, will be necessary to reduce disease and improve efficiency in shrimp farming while preserving the health of aquatic environments.

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