ISSN 2518–2021 (Print) 2415–4474 (Electronic)

Fundamental and Applied Agriculture

Journal home page: https://www.f2ffoundation.org/faa/index.php/home

Vol. 9(4), pp. 247 – 255: 2024, doi: https://doi.org/10.5455/faa.221087

AQUACULTURE | ORIGINAL ARTICLE

Antibacterial Effects of *Terminalia arjuna* (Roxb.) Leaf and *Curcuma longa* (L.) Bulb Extracts on the Fish pathogen *Acinetobacter rudis*

Sourav Acharjee, Towsif Iqbal, Kartik Chandra Saha, Naima Sultana, Tamanna Tabassum, Salman Shahriar Nibir, Tanvir Rahman ✉

Department of Aquaculture, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

ARTICLE INFO

Article history
Received: 11 Sep 2024
Accepted: 20 Dec 2024
Published online: 31 Dec 2024

Keywords
Terminalia arjuna, Extraction, *Curcuma longa*, Infection, *Acinetobacter rudis,* Health management

Correspondence
Tanvir Rahman
✉: tanvir.aq@bau.edu.bd

ABSTRACT

Studies were conducted to determine the antibacterial effects of arjun, *Terminala arjuna* (Roxb.) leaf and turmeric, *Curcuma longa* (L.) bulb extracts against pathogenic *Acinetobacter rudis* infection in pangas, *Pangasianodon hypophthalmus* and rohu, *Labeo rohita*. The arjun leaf and turmeric bulb were collected from the adjacent area of Bangladesh Agricultural University (BAU), Mymensingh, dried and powdered finely using an automated grinder. Laboratory stocked fish pathogen *A. rudis*, isolated previously from the body lesions of pangas, was used for challenge test. Biosafety test was performed by aqueous extracts of arjun leaf and turmeric bulb to determine the tolerance of fish. Pangas (7.194±1.09 g) and rohu (3.46±1.12 g) both could tolerate 120 ppm dip bath of arjun extracts. In case the of turmeric extract, pangas tolerated 120 ppm but rohu could tolerate upto 90 ppm. An immersion challenge test with 70 *P. hypopthalmas* and 70 *L. rohita* was performed in laboratory using *A. rudis* with pre-fixed doses of 4.07×10^6 and 4.07×10^7 CFU/mL. After the experimental infection, aqueous extract of turmeric was found to have distinguished effects where 87.5% of infected fish were recovered with prolonged immersion treatment. Water extracts of arjun leaf exhibited 67.5% survival of fish. *In-vitro* test by acetone extracts of both arjun and turmeric showed inhibition zones against *A. rudis* at different dosage in comparison with frequently used antibiotics. Maximum inhibition zone was 7 mm at 125 µL of the acetone extract of arjun while turmeric extract showed 5 mm at the same concentration against *A. rudis*. Thin layer chromatography confirmed the presence of at least one component in the acetone extracts which might be responsible for the antibacterial activity. This preliminary investigation revealed the potential antibacterial effects of *T. arjuna* leaf and *C. longa* bulb extracts against *A. rudis* infection in fish and can be suggested for further detailed studies to establish these medicinal plants for alternative fish health management.

1. Introduction

The contribution of aquaculture sector for the livelihood and food security is significant, providing millions of people with food, nutrition, income and employment (FAO, 2024). Infectious microbial diseases of fish is one of the most significant threats to successful aquaculture of Bangladesh and every year both culture and wild fish production are averted by these disease incidence which declines fish production affecting aquatic protein supply as well as national economy of Bangladesh (Munirazzaman, 2004). Thus, proper medication is needed to maintain animal health and manage fish production. The routine practice of applying the antimicrobial agents as a means of preventing and treating diseases, as well as promoting growth is an important factor in the emergence of antibiotic resistant and unacceptable residues in aquaculture and environment. The resistant bacteria are subsequently transferred to human through the food chain (Salehi et al., 2005; WHO, 2000). Increasing global demand for the preservation of eco-friendly environments, the indiscriminate application of antibiotics, which are notorious for increasing antibiotic-resistant pathogens and inducing environmental deterioration, is being questioned (Samanidou and Evaggelopoulou, 2007; He et al., 2016, Uchida et al., 2016). Sometimes aquaculture farmers or labors are severely affected by the pathogenic bacteria due to exposure to infected fish (Butt et al., 2004; Wang et al., 2007; Weir et al., 2012; Ziarati et al., 2022) which have not always been reported.

Since plant-based drugs cause much lower incidence of adverse reactions compared to synthetic pharmaceuticals (Sharif et al., 2006), scientist felt the urgency to develop an alternative approach of herbal medication towards management of fish disease. Many countries have already

Cite This Article

Acharjee S, Iqbal T, Saha KC, Sultana N, Tabassum T, Nibir SS, Rahman T. 2024. Antibacterial Effects of *Terminalia arjuna* (Roxb.) Leaf and *Curcuma longa* (L.) Bulb Extracts on the Fish pathogen *Acinetobacter rudis*. *Fundamental and Applied Agriculture*, 9(3): 247–255. https://doi.org/10.5455/faa.221087

banned the use of several antibiotics since their prolonged exposure to environment is associated with harmful side effects and is a concern of public health (Cuhna, 2000). Among the medicinal plants *Terminalia arjuna* (Family: Combertaceae), commonly known as 'Arjuna', Bengali name, 'Arjun' is a large and evergreen tree grown abundantly throughout the South Asian region, particularly on the bank of the rivers, streams and dry watercourses (Dwivedi, 2007). Different parts of this plant like root, stem, bark and leaf have the medicinal value. Another plant, well known for its medicinal properties, *Curcuma longa,* belongs to Zingiberaceae family is commonly known as 'turmeric' and is widely used as spice and coloring agent in food (Prasad and Aggarwal, 2011). Turmeric is also well known as a traditional remedy for human illness. Meena et al., 2024 conducted a research on *L. rohita* to know the efficacy of *T. arjuna* bark powder against bacterial pathogens and as a potential immunomodulator of this fish. They revealed that *T. arjuna* cured the infection caused by the bacteria and enhanced the immunity of this fish. Both aqueous and ethanolic extracts of *C. longa* were also tried for the sensitivity test against *A. hydrophila*, *P. aeruginosa*, *P. fluorescens*, *E. coli*, *E. tarda* and *F. columnare* isolated from fish. In case of every test, inhibition zone were recorded and proved that *C. longa has* antibacterial property against number of fish pathogenic bacteria (Sahu et al., 2005).

Among lots of fish pathogenic bacteria *Acinetobacter* species are, generally found in abundance in nature including soil, marine fish, freshwater fish and vegetables (Gennari and Stegagno, 1986). But there is still not much information on the role of *Acinetobacter* sp. in fish pathology. An association of various *Acinetobacter* sp. *viz., A. junii*, *A. pittii, A. johnsonii, A. lwoffii* etc. with pathological conditions of various fish species in some countries, such as India, China, Croatia, and Turkey, was considered in the last decade (Kozińska et al., 2014; Malick et al., 2020; Laltlanmawia et al., 2023; Zhang et al., 2023;). *A. johnsonii* and *A. lwoffii* have been reported as emerging fish pathogens isolated from diseased rainbow trout, *Oncorhynchus mykiss* and carps in Poland (Kozińska et al., 2014). Li et al. (2017) reported on the prospective threat by *A. pittii*, a multidrug-resistant pathogen for freshwater fish farming. However, as a fish pathogenic bacteria, *Acinetobacter* sp. has not yet been reported clearly from the catfish or carp culture of Bangladesh.

During routine diagnosis of bacterial infections in catfish, a Gram-negative short rod bacterium was isolated from the deeper lesions of skin, fin, liver and kidney of infected farmed *P. hypophthalmus* (average weight 672.03±0.4 g) that exhibited non-wrinkled and slightly whitish colony on tryptone soya agar from 8 to 42ºC. Biochemical and molecular identification revealed for the first time in Bangladesh that the emerging causative bacterium was *Acinetobacter rudis* (Shams, 2020). Herbal medicines, including crude herbs, herbal compound, herbal crude extracts, and herbal effective components, are widely known for their unique scent, flavor, or therapeutic properties. A number of the bioactive components from medicinal plants, such as polysaccharides, flavonoids, polyphenols, saponins, alkaloids, essential oils, and terpenoids, have been widely used in prevention and control of viral, bacterial, parasitic, and fungal diseases in fish (Zhang, 2022). But still, the research on using medicinal plants in fish health management in Bangladesh is under experimental stage. Considering the situation, the aim of this preliminary investigation was to determine the antibacterial efficacy of two herbal preparations from *T. arjuna* leaf and *C. longa* bulb against newly emerging *Acinetobacter rudis* under *in-vitro* and *in-vivo* condition on striped catfish, *Pangasianodon hypophthalmus* and rohu, *Labeo rohita*.

2. Materials and Methods

2.1. Collection of medicinal plants

The experiment was conducted at Fish Disease Laboratory, Faculty of Fisheries and Agricultural Chemistry Laboratory, Faculty of Agriculture, Bangladesh Agricultural University (BAU), Mymensingh 2202. Bulk volume of *T. arjuna* leaf and *C. longa* bulb was collected from the adjacent area of BAU campus, Mymensingh. After washing with clean water, the leaves and bulbs were dried under direct sunlight, cut into small pieces, finely powdered by an automated grinder machine and sieved using 1/32-inch mesh sized sieve for better extraction. The *T. arjuna* leaf and *C. longa* bulb powder was dried again using an oven at 80ºC for 4 hours before extraction and weighed to determine the volume of moisture free powder.

2.2. Preparation of aqueous extracts of *T. arjuna* leaf and *C. longa* bulb

Aqueous extracts of *T. arjuna* leaf and *C. longa* bulb were prepared following slightly modified method of Bussmann et al. (2010) for the biosafety test the and dip bath treatment for artificial infection of *P. hypophthalmus* and *L. rohita*. Completely dried powder of *T. arjuna* leaf and *C. longa* bulb were weighted and required volume plant materials were submerged in every 1 liter of normal tap water separately and left to macerate for 48 hours. After maceration, the plant materials were filtered using clean cotton cloths and the extracts of 180, 150, 120 and 90 ppm for both plants were prepared separately. About 4-liter water extracts of above mentioned concentrations were prepared and kept into selected aquarium for biosafety tests.

2.3. Preparation of Acetone extracts of *T. arjuna* leaf and *C. longa* bulb

One hundred grams of the dried *T. arjuna* leaf powder and another 100 grams of *C. longa* powder were taken into two different thimbles and set inside Soxhlet apparatus with addition of 700 mL of acetone respectively. Extractions were continued for 48 hours and the two extracted materials were taken out and filtered, followed by kept into two plastic bottles. Volume of two extracts were concentrated under reduced pressure by rotary evaporator (HAHNSHIN, HS-2005S-N, Korea) at 60°C. Those extracts were kept in refrigerator at 10ºC for further works.

2.4. Thin layer chromatography (TLC)

Thin layer chromatographic technique was applied to ascertain the number of compounds present in both of the *T. arjuna* leaf and *C. longa* bulb extracts to determine the retention factor (R_f) of the compounds present. The chromatographic plates were prepared by spreading a suspension of finely powdered silica gel on glass plates of suitable sizes.

Silica gel was thoroughly mixed with distilled water in a beaker at 1:2 ratio and manually spread very thinly on clear glass plates. Firstly, the plates were completely dried in room temperature for 24 hours and then at 70ºC in hot air oven for 1 hour. A number of solvent chambers were prepared by solvents from low to high polarity by mixing solvents of expected polarities. To obtain a chromatogram, few drops of acetone extracts of *T. arjuna* leaf and *C. longa* bulb were applied with a capillary tube on a bare line along the bottom of the plate. Those plates were kept in a TLC tank containing a selected carrier solvent and mixed solvents. The samples exhibited spots on the TLC plate in ordinary conditions. After solvents running, plates were dried and sprayed with freshly prepared iodine reagents which were used to detect the bands on the TLC plates. The movement of the active compound was expressed by its retention factor (R_f) values and were calculated for different samples. The retention factor (R_f) values of acetone extraction of *T. arjuna* leaf extract and *C. longa* bulb extract were calculated by using the following formula:

$$R_f = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent front TLC plates}}$$

2.5. Determination of biosafety effects

Biosafety effects of aqueous extracts of *T. arjuna* leaf and *C. longa* bulb were tested using *P. hypophthalmus* and *L. rohita,* separately to determine the possible harmful effects on the experimental fish as well as the time and tolerable concentration of the extracts for fish. Healthy *P. hypophthalmus* weighing 7.194±1.09 g and *L. rohita* weighing 3.46±1.12 g were collected from a private fish hatchery, acclimatized in aquaria for seven days and checked for disease before using in the challenge test. Ten fish of both species were immersed in 180, 150, 120 and 90 ppm of the herbal suspensions, separately in the glass aquaria. Control fish were kept in another aquarium and did not receive any herbal extraction for treatment. Water temperature (°C) was recorded.

2.6. Experimental bacteria

Laboratory stocked *A. rudis* was previously isolated from a diseased farmed *P. hypophthamus* (Shams, 2020). The strain was previously confirmed by our research team as *A. rudis* strain P3A1b 16S ribosomal RNA gene, partial sequence (size of polynucleotide analyzed = 1049 bp), GenBank accession: MZ712942.1. Pure culture of *A. rudis* showed dense growth of slightly whitish, smooth colony on Trypton Soya Agar (TSA, Himedia) (Figure: 1A). Gram staining exhibited the presence of Gram-negative, slightly rod-shaped *A. rudis* bacteria (Figure: 1B). The bacterium was stored at –20°C in Trypton Soya Broth (TSB, BD) supplemented with 25% glycerol.

2.7. Immersion challenge

Seventy *P. hypophthalmus* and seventy *L. rohita* were divided into 7 aquaria (10 pangas + 10 rohu/) per aquarium prior to 48h of the experiment to heal the skin injury caused by netting before immersion challenge. For challenge, test fish were kept in 40-liter plastic buckets separately, to which the bacterial suspensions of two different doses, 4.07×10^6 and 4.07×10^7 CFU/mL, of *A. rudis* were added. Ten fish, each of two species were immersed separately in clean water without the bacterial suspension for negative control. After immersion for 40 min, fish were transferred again to the respective rectangular aquaria equipped with continuous aeration. The fish received no feed for 7 days of the experimental period. Water temperature was measured regularly. Gross pathological changes and moribund fish were checked daily.

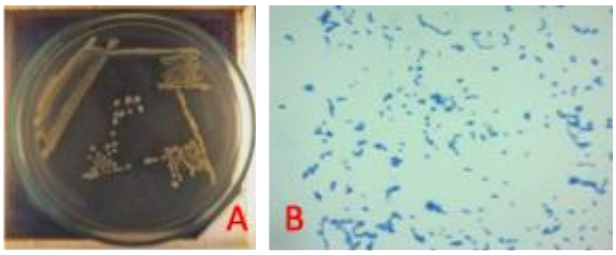

Figure 1. (A) Colony of A. rudis isolated from diseased pangas. Slightly off-whitish, rough, flat edge appearance colony grown on TSA media at 37°C for 24 hrs. (B) Photomicrographs of A. rudis stained with methylene blue at 100× magnification

2.8. Dip bath treatment with herbal extracts

About 4 liter of *T. arjuna* leaf extracts (120 ppm) and 4 liter of *C. longa* bulb extracts (90 ppm) were prepared and kept into separate aquarium for dip bath treatment. Then two groups of challenged fish were dipped into two herbal extracts for thirty seconds and then again kept into separate aquaria. Same process was done for consecutive 4 times. Around 70% of water was exchanged regularly. One aquarium from each challenge group received no herbal dip bath treatment and kept as positive control.

2.9. Antibiogram studies

2.9.1. Antibiotic sensitivity test

Pathogenic *A. rudis* was tested for its antibiotic sensitivity by disc diffusion method on TSA. The antibiotic discs (Hi-media, India) tested included azithromycin (10 µg), doxycycline (30 µg), erythromycin (15 µg), tetracycline (10 µg), streptomycin (25 µg), cefotaxime (30 µg), chloramphenicol (30 µg), ciprofloxacin (10 µg), gentamycin (10 µg), kanamycin (30 µg) and ampicillin (10 µg). Zones of inhibition were measured after 24 h and

again after 48 h of incubation at 37°C. The isolates were classified as sensitive (S), moderately resistant (MR) and resistant (R) based on the size of the zone of bacterial growth inhibition.

2.9.2. Determination of antibacterial sensitivities of herbal extracts

A. rudis was cultured in TSA at 27ºC for 24 h and then suspended in 0.87% physiological saline and spread on TSA plates. About 6 mm diameter wells were prepared using a sterile cork borer in the TSA plates (4 wells in a plate). About 50, 75, 100, 125 µl of acetone *T. arjuna* leaf extracts and *C. longa* bulb extracts were dropped using micropipette, dried and incubated by upside down for 24 h at 27ºC. The inhibition zone around the wells indicated absence of bacterial growth and was reported as positive. Absence of zone was indicated as negative.

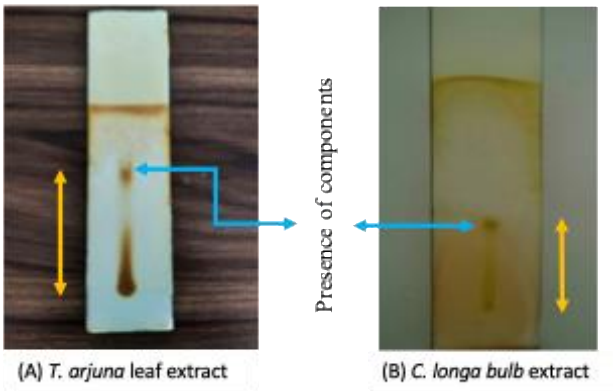

Figure 2. TLC of (A) *T. arijuna* leaf, and (B) *C. longa* bulb extract

2.10. Data analysis

Data on survivability of treated and control fishes were analyzed by using Microsoft Excel 2016 software.

3. Results

3.1. Thin layer chromatography of acetone extracts

Acetone extracts of *T. arjuna* leaf (Figure 2A) and *C. longa* bulb (Figure 2B) showed spot on TLC plate which ensured the presence of components. The retention factor (R_f) values were determined for *T. arjuna* leaf extract and *C. longa* bulb extract (Table 1).

3.2. Antibiogram study

3.2.1. Antibiotic sensitivity

Antibiotic susceptibility test indicated that *A. rudis* was sensitive to azithromycin, doxycycline, erythromycin, tetracycline, streptomycin, ciprofloxacin, gentamycin, chloramphenicol. The results also showed that *A. rudis* sample was resistant (R) to ampicillin and moderately resistant (MR) to kanamycin and cefotaxime (Figure 3 and Table 2).

3.2.2. Herbal sensitivity

The applied herbal extracts exposed their inhibitory effect for pathogenic bacteria *A. rudis* on TSA agar plate. It is observed that, acetone extracts of *T. arjuna* leaf showed more inhibition zone where *C. longa* bulb shows small inhibition zone or resistance (Figure 4A & 4B and Table 3).

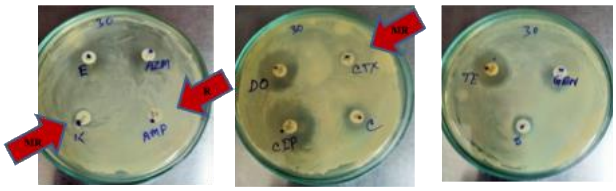

Figure 3. Antibiogram study of A. rudis using different antibiotic discs. (*A. rudis*: Laboratory code = P3A1b, GenBank accession: MZ712942.1)

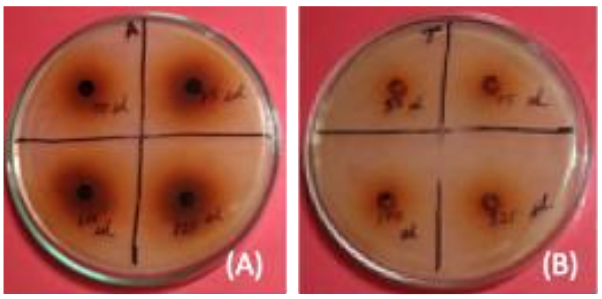

Figure 4. Antibiogram study of *A. rudis* using (A) *T. arjuna* leaf extracts, and (B) *C. longa* bulb extracts

3.2.3. Biosafety test results

After preparing aqueous extracts of *T. arjuna* leaf and *C. longa* bulb, biosafety test was conducted to determine the time and concentration that fish can tolerate. Pangas and rohu could tolerate *T. arjuna* extract of 120 ppm for 5 min and 3 min respectively. On the other hand, pangas could tolerate *C. longa* extraction of 120 ppm for 3 min at where rohu could tolerate 90 ppm for 3 min (Fig. 5 and 6). The average temperature was 23.1 ± 1.5ºC.

3.3. Pathogenicity of *A. rudis* for immersion challenge with herbal therapy

A. rudis showed effective result when applied into pangas and rohu in immersion challenge test. After experimental infection (challenge dose: 4.07×10^6 CFU/fish and 4.07×10^7 CFU/ fish), aqueous extracts were found to have distinguished effects where 55-90% fish were survived with the application of dip immersion treatment (Table 4). *C. longa* bulb extract showed better effects to recover challenged fish rather than *T. arjuna* leaf extract. But mass infection and mortality rate was found for untreated fish. Average water temperature was recorded 22.5±0.75ºC.

Table 1. R_f value of extracted sample

Extracting solvent	TLC solvent system	R_f value	Probable compound present in the crude extract
Acetone extract of *T. arjuna*	Benzene : Ethyl Acetate = 1 : 9	5.4/8.3 = 0.65	1
Acetone extract of *C. longa*	Benzene : Ethyl Acetate = 1 : 2	3.6/9.3 = 0.38	1

Table 2. Sensitivity of A. rudis to various antimicrobial agents

Short Name	Antimicrobial agents	Disc content (µg)	Sensitivity
AZM	Azithromycin	10	S
DO	Doxycycline	30	S
E	Erythromycin	15	S
T	Tetracycline	10	S
S	Streptomycin	25	S
CTX	Cefotaxime	30	MR
C	Chloramphenicol	30	S
CIP	Ciprofloxacine	10	S
GEN	Gentamycin	10	S
K	Kanamycin	30	MR
AMP	Ampicillin	10	R

S = sensitive; R = resistant; MR = moderately resistant

Table 3. Sensitivity *A. rudis* to acetone herbal extracts

Acetone herbal extracts		Sensitivity	Radius of inhibition zone (mm)
T. arjuna	50 µl	S	3
	75 µl	S	4
	100 µl	S	5
	125 µl	S	7
C. longa	50 µl	R	0
	75 µl	MR	1
	100 µl	S	3
	125 µl	S	5

R = resistant, MR = moderately resistant, S = sensitive

Table 4. Recovery of challenged fish (P = pangas, R = rohu)

Bacteria	Challenge method	Challenge dose (CFU/ mL or fish)	Herbal treatment (Aqueous extracts)	No. of dead fish during the periods of (N= 10)						Mortality (%)	
				0-1 d		2-3 d		4-7 d		P	R
				P	R	P	R	P	R		
A. rudis	Immersion	4.07×10^7	Treated with *T. arjuna*	1	2	1	1	0	2	20%	50%
			Treated with *C. longa*	0	0	0	0	1	1	10%	10%
		4.07×10^6	Treated with *T. arjuna*	0	1	1	2	1	1	20%	40%
			Treated with *C. longa*	1	1	0	1	0	0	10%	20%
Positive Control		4.07×10^7	No treatment given	0	0	2	3	5	5	70%	80%
		4.07×10^6		0	0	1	2	4	5	50%	70%
Negative Control		Not challenged with bacteria	No treatment given	0	0	0	0	0	0	0%	0%

Average weight (g) of challenged fish: pangas = 7.531 ± 1.17, rohu = 3.64 ± 1.25; Average water temperature = 22.5+0.75ºC

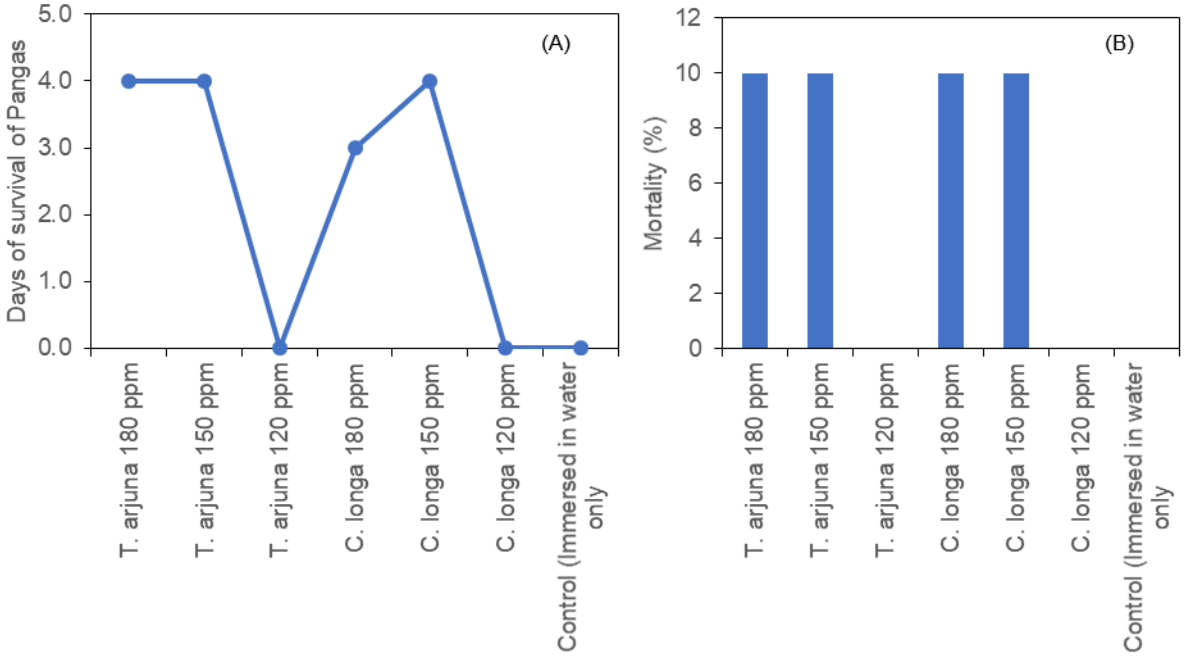

Figure 5. (A) Days of survival, (B) percent mortality of Pangas (*Pangasianodon hypophthalmus*) as affected by *Terminalia arjuna* leaf and *Curcuma longa* bulb extracts. Average weight (g) of challenged pangas = 7.194 ± 1.09 and average water temperature = 23.1 ± 1.5ºC

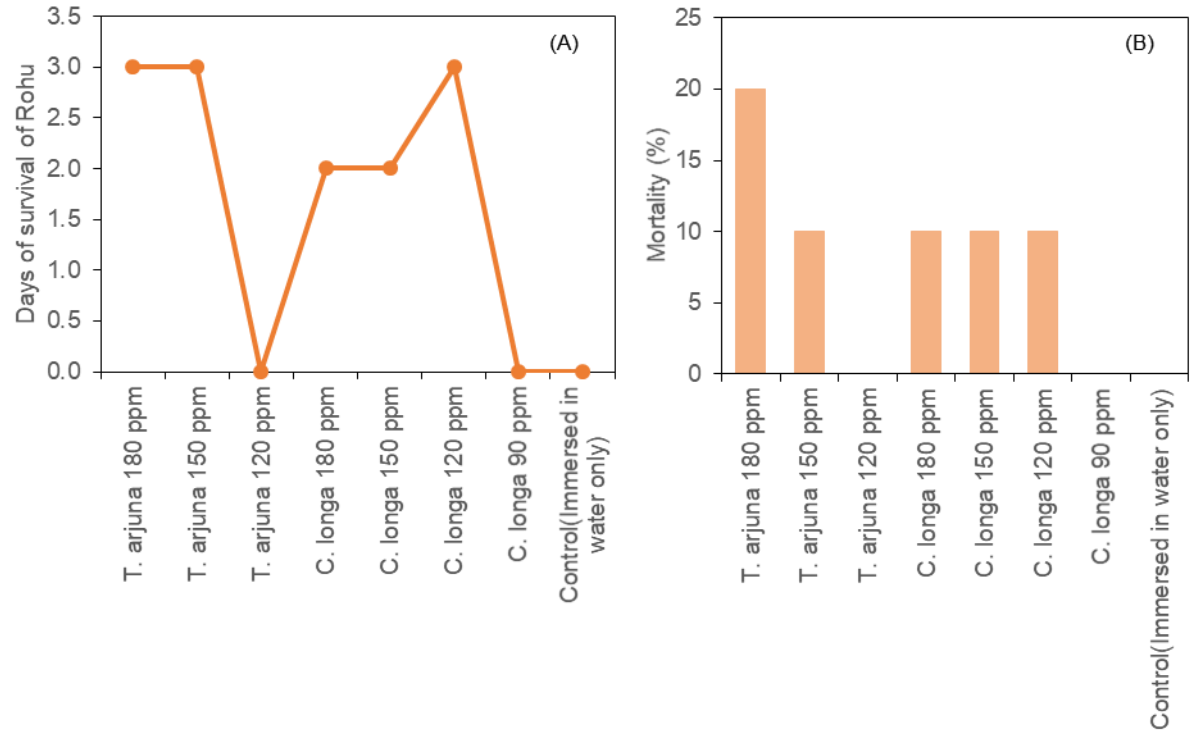

Figure 6. (A) Days of survival, (B) percent mortality of Rohu (*Labeo rohita*) as affected by *Terminalia arjuna* leaf and *Curcuma longa* bulb extracts. Average weight (g) of challenged rohu = 3.46 ± 1.12; average water temperature = 23.1 ± 1.5ºC

4. Discussion

Pangas (*P. hypophthalmus*) and rohu (*L. rohita*) are the most commonly used aquaculture species in Bangladesh's freshwater ponds (Ali et al., 2018, Kabir et al., 2017). The stress level of farmed fish has increased due to the rapid growth of intensive aquaculture. In recent years the incidence and severity of numerous fish diseases have increased as well (Song et al*.,* 2014). Fish mortality may occur as a result of extreme oxygen depletion, or they may become prone to various bacterial diseases (Siti-Zahrah et al*.,* 2008). Therefore, antibiotics have become essential medicine for animal health and welfare (Romero et al., 2012). Chemists and representatives of several pharmaceutical companies usually advise the fish farmers to utilize their medicines for managing diseases, however, most of the farmers of Bangladesh indiscriminately use such chemicals without knowing their requirements, efficacies or method of application (Kawsar et al., 2022) and hence, antibiotic usages develop antimicrobial resistance (Rahman et al., 2009).

Natural origins have enormous chemical and structural diversity among antimicrobial products, it is often difficult for bacteria to oppose the action of natural products and plants are one of the main sources of antimicrobial natural products (Warnke et al., 2009; Pisoschi et al., 2018), additionally, plant-based drugs cause much lower incidence of adverse reactions compared to synthetic pharmaceutical (Sharif et al., 2006), various primary or secondary plant metabolites have been shown to exert antibiotic activities (Lelario et al., 2018). Therefore, in the present study, investigation on the herbal medication by *T. arjuna* leaf and *C. longa* bulb extracts tried to establish an eco-friendly fish health management approach against *A. rudis*, an emerging opportunistic fish pathogenic bacteria for the first time in Bangladesh. Resistance of this bacterium to multiple antibiotics is also shown in this study.

Bio-safety effect of *T. arjuna* leaf and *C. longa* bulb was tested using *P. hypophthalmus* and *L. rohita* separately to determine the possible harmful effects on fish. Aqueous extracts of both *T. arjuna* leaf and *C. longa* were used for this test. The test showed that both pangas and rohu can survive longer time at aqueous extraction of *T. arjuna* but less time at *C. longa*. Pangas and rohu can tolerate *T. arjuna* leaf extraction at 120 ppm for 5 min and 3 min respectively. On the other hand, pangas can tolerate *C. longa* bulb extraction for 5 min at 120 ppm whereas rohu can tolerate 3 min at 90 ppm. The reason behind not tolerating turmeric extraction for longer period might be due to the spicy nature of turmeric that could create uncomfortable situation in gaseous exchange in experimental fish and resulted erratic movement during biosafety test. *In-vivo* challenge test resulted 32.5% and 12.5% (average) mortality of the infected fish whereas the untreated infected fish showed 67.5% mortality. Water extract of *T. arjuna* leaf and *C. longa* bulb were able to heal the injuries caused by *A. rudis* infection in both fish.

Curcumin has already shown anti-oxidant, anti-mutagenic, anti-carcinogenic, anti-coagulant, and anti-infective effects, additionally, curcumin possesses anti-inflammatory and wound-healing properties (Reshad et al., 2021). *C. longa* extract corresponds to an intermediate stage of cell disruption, plasmolysis and partial disappearance of the cytoplasmic membrane of bacteria (Prasad et al., 2011). So, this may help in reducing mortality of challenged fish in *C. longa* treated group against *A. rudis*. On the other hand, the active components of *T. arjuna* are tannins, triterpenoid saponin (arjunic acid, arjunolic acid, arjungenin, and arjunglycosides), flavonoids, garlic acid, oligomeric proanthocyanidines (OPCs), phytosterols, calcium, magnesium, zinc and copper (Islam et al., 2024, Ali et al., 2003, Sultana et al., 2007). The active compounds present in the extract of curcumin and arjun are reported to interfere with the synthesis of microbial cell proteins and is essential for the treatment of tissues that are ulcerated or inflamed (Hussain et al*.,* 2007). Some of this component might have adverse effects on fish, that's why the mortality rate was higher in *T. arjuna* leaf treated group than turmeric it may probably the component(s) or the concentration of the component(s) in *T. arjuna* leaf that are not effective for the healing of *A. rudis* infection like *T. arjuna* bark. Further comparative study is necessary to establish the fact.

Again, *In-vitro* study showed that *A. rudis* was resistant against ampicillin and moderately resistant against cefotaxime, kanamycin. It is known that *T. arjuna* has antibacterial activity (Gupta and Kumar, 2017). Earlier, it was found that biomolecules isolated from bark extracts of *T. arjuna* possessed antibacterial activity, which can specifically act against *Salmonella* sp. (Islam et al*.*, 2024). In this study, acetone extracts of *T. arjuna* leaf was found sensitive against *A. rudis* at different doses like 50 µL, 75 µL, 100 µL, and 125 µL. The inhibition zones were 3, 4, 5, and 7 mm respectively. On the other hand, acetone extracts of *C. longa* bulb were found less sensitive where the inhibition zones were 0, 1, 3, and 5 mm for 50 µL, 75 µL, 100 µL, and 125 µL doses respectively. So, it can be said that the higher the dose of acetone extraction, the higher its antibacterial effects. This resistance may also be associated with either the lipid bilayer of the outer membrane of the bacterium preventing the extracts specially the antioxidants from entering into the cell that prevent the oxidation of other molecules. The bacterium may also have been able to generate enzymes that can alter the extracts chemically, thereby interfering with the extract's activity, rendering it ineffective (Zaika and Kissinger, 1981). As we found inhibition zones, TLC was done to identify if any component(s) was/were present in those extractions. Both the acetone extracts of arjun leaf and turmeric showed at least one spot on TLC plate at different solvent ratio. The ideal range of retention factor (R_f) value is 0.3-0.7 (Lynch et al., 2023). R_f values were found 0.65 and 0.38 for arjun leaf and turmeric respectively in this study.

5. Conclusion

The present study is a clinical validation of the traditionally used medicinal plants which indicates that pangas and rohu can tolerate definite concentration of plant extracts from *T. arjuna* leaf and *C. longa* bulb which have already used in this study as natural alternatives to synthetic antibiotics to control ampicillin resistant *A. rudis* bacteria. However, further in-depth studies should be conducted to ensure fish safety, isolation and identification of herbal

components, and making aqua drugs in modern treatment forms by using targeted component (s) followed by large scale field trials to elucidate possible development of medicinal plants therapy in aquaculture

Ethical approval

This study was conducted in accordance with the Ethical Standard of Research Committee of Bangladesh Agricultural Research System (BAURES), Bangladesh Agricultural University, Mymensingh-2202, Bangladesh.

Conflict of Interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Ali A, Kaur G, Hamid H, Abdullah T, Ali M, Niwa M, Alam M. 2003. Terminoside A, a new triterpene glycoside from the bark of *Terminalia arjuna* inhibits nitric oxide production in murine macrophages. Journal of Asian Natural Products Recourses, 5: 137–142. doi: 10.1080/1028602031000066834.

Ali H, Rahman MM, Murshed-e-Jahan K, Dhar GC. 2018. Production economics of striped catfish (*Pangasianodon hypophthalmus*, Sauvage, 1878) farming under polyculture system in Bangladesh. Aquaculture, 491: 381-390.

Butt AA, Aldridge KE, Sanders CV. 2004. Infections related to the ingestion of seafood Part I: Viral and bacterial infections. Lancet Infectious Diseases, 4: 201–212.

Bussmann RW, Malca-García G, Glenn A, Sharon D, Chait G, Díaz D, Pourmand K, Jonat B, Somogy S, Guardado G, Aguirre C, Chan R, Meyer K, Kuhlman A, Townesmith A, Effio-Carbajal J, Frías-Fernandez F, Benito M. 2010. Minimum inhibitory concentrations of medicinal plants used in Northern Peru as antibacterial remedies. Journal of Ethnopharmacology, 28: 132(1): 101–108. doi:10.1016/j.jep.2010.07.048.

Cuhna BA. 2000. The diagnostic significance of relative bradycardia in infectious disease. Clinical Microbiological Infection, 6(12): 633-634.

DoF 2021: Department of Fisheries, Bangladesh: Ministry of Fisheries and Livestock, Government of the People's Republic of Bangladesh.

Dwivedi S. 2007. *Terminalia arjuna* Wight & Arn. - a useful drug for cardiovascular disorders. Journal of Ethnopharmacology, 114(2): 114–129. doi:10.1016/j.jep.2007.08.003.

FAO, 2024. Fishery and Aquaculture Statistics – Yearbook 2021. FAO Yearbook of Fishery and Aquaculture Statistics. Rome. https://doi.org/10.4060/cc9523en.

Gennari M, Stegagno F. 1986. Isolation and characterization of *Acinetobacter* from raw, washed and frozen vegetables. Archivio Veterinario Italiano, 37(3): 131137.

Gupta D, Kumar M. 2017. Evaluation of in vitro antimicrobial potential and GC–MS analysis of *Camellia sinensis* and *Terminalia arjuna*. Biotechnology Reports, 13: 19-25.

He X, Deng M, Wang Q, Yang Y, Nie X. 2016. Residues and health risk assessment of quinolones and sulfonamides in cultured fish from Pearl River Delta, China. Aquaculture, 458: 38-46.

Hussain S, Siddiqui SU, Khalid S, Jamal A, Qayyum A, Ahmad Z. 2007. Allelopathic potential of senna (*Cassia angustifolia*) on germination and seedling characters of some major cereal crops and their associated grassy weeds. Pakistan Journal of Botany, 39 (11): 45-53.

Islam S, Saha MKC, Sultana N, Rahman MR, Raihan J, Farjana K, Raihan RB, Tabassum T, Acharjee S, Shovon SS, Nibir SS, Kubra KT, Rahman T. 2024. Efficacy of *Terminalia arjuna* (Roxb.) bark extracts against *Salmonella* sp. isolated from aquaculture ponds. Research in Agriculture Livestock and Fisherie,. 11(2): 255-269.

Kabir MA, Rahman MS, Begum M, Faruque MH. 2017. Genetic diversity by RAPD in four populations of Rohu *Labeo rohita*. Croatian Journal of Fisheries, 75(1): 12-17.

Kawsar MA, Alam MT, Pandit D, Rahman MM, Mia M, Talukdar A, Sumon TA. 2022. Status of disease prevalence, drugs and antibiotics usage in pond-based aquaculture at Narsingdi district, Bangladesh: A major public health concern and strategic appraisal for mitigation. Heliyon, 8(3): e09060.

Kozińska A, Paździor E, Pękala A, Niemczuk W. 2014. *Acinetobacter johnsonii* and *Acinetobacter lwoffii* - the emerging fish pathogens. Bulletin of the Veterinary Institute in Pulawy, 58(2): 193-199. doi: 10.2478/bvip-2014-0029

Lelario F, Scrano L, De Franchi S, Bonomo MG, Salzano G, Milan S, Bufo SA. 2018. Identification and antimicrobial activity of most representative secondary metabolites from different plant species. Chemical and Biological Technologies in Agriculture, 5(1): 1-12.

Li J, Cao J, Wang X, Liu N, Wang W, Luo Y. 2017. *Acinetobacter pittii*, an emerging new multi-drug resistant fish pathogen isolated from diseased blunt snout bream (*Megalobrama amblycephala* Yih) in China. Applied Microbiology and Biotechnology, 101(16): 6459-6471.

Lynch J, Sherwood J, McElroy CR, Murray J, Shimizu S. 2023. Dichloromethane replacement: towards greener chromatography via Kirkwood–Buff integrals. Analytical methods, 15(5): 596-605.

Malick RC, Bera AK, Chowdhury H, Bhattacharya M, Abdulla T, Swain HS, Baitha R, Kumar V, Das BK. 2020. Identification and pathogenicity study of emerging fish pathogens *Acinetobacter junii* and *Acinetobacter pittii* recovered from a disease outbreak *in Labeo catla* (Hamilton, 1822) and *Hypophthalmichthys molitrix* (Valenciennes, 1844) of freshwater wetland in West Bengal, India. Aquaculture Research, 51(2):1–11. https://doi.org/10.1111/are.14584.

Meena DK, Das BK, Sahoo AK, Sahu NP, Srivastava PP, Borah Ss. 2024. *Terminalia arjuna* Bark Powder as a Potential Immunomodulator in *Labeo rohita*: Enhanced Hematological, Adaptive, and Humoral Responses against Bacterial Pathogens and Concordant Liver Histomorphology. Pathogens, 13(4): 295. doi: 10.3390/pathogens13040295.

Muniruzzaman M. 2004. Etiopathology and control measures of ulcer diseases in the cultured fishes of Bangladesh. A Ph. D. Thesis, submitted to the Department of Aquaculture, Bangladesh Agricultural University, Mymensingh, Bangladesh. 302 pp.

Pandey P, Awasthi R, Dhiman N, Sharma B, Kulkarni GT. 2022. Ethnopharmacological reports on herbs used in the management of tuberculosis. In Herbal Medicines, Academic Press, pp. 501-523. doi: 10.1016/B978-0-323-90572-5.00024-X.

Pisoschi AM, Pop A, Georgescu C, Turcus V, Olah NK, Mathe E. 2018. An overview of natural antimicrobials role in food. European Journal of Medicinal Chemistry, 143: 922–935.

Prasad S, Aggarwal BB. 2011. Turmeric, the Golden Spice: From Traditional Medicine to Modern Medicine. In: Benzie IFF, Wachtel-Galor S, editors. Herbal Medicine: Biomolecular and Clinical Aspects. 2nd edition. Boca Raton (FL): CRC Press/Taylor & Francis; Chapter 13. https://www.ncbi.nlm.nih.gov/books/NBK92752/

Rahman M, Huys G, Kühn I, Rahman M, Möllby R. 2009. Prevalence and transmission of antimicrobial resistance among *Aeromonas* populations from a duckweed aquaculture based hospital sewage water recycling system in Bangladesh. Antonie Van Leeuwenhoek, 96: 313-321.

Rahman MM, Chowdhury MBR. 1996. Isolation of bacterial pathogen causing an ulcer disease in farmed carp fishes of

Mymensingh. Bangladesh Journal of Fisheries, 19: 103-110.

Reshad RAI, Alam S, Raihan HB. 2021. In silico investigations on curcuminoids from *Curcuma longa* as positive regulators of the Wnt/β-catenin signaling pathway in wound healing. Egyptian Journal of Medical Human Genetics, 22: 65. doi: 10.1186/s43042-021-00182-9.

Romero J, Feijoó CG, Navarrete P. 2012. Antibiotics in aquaculture–use, abuse and alternatives. Health and Environment in Aquaculture, 159(1): 159-198.

Sahu S, Mishra BK, Pradhan J, Das BK. 2005. Antibacterial activity of curcuma longa on fish microbial pathogens. Journal of Aquaculture, 13 (2005): 9-16.

Salehi TZ, Mahzounieh M, Saeedzadesh A. 2005. Isolation of Antibiotic-resistant *Salmonella* from intestine and liver of poultry in Shiraz of Iran. International Journal of Poultry Science, 4(5): 320-322.

Samanidou VF, Evaggelopoulou EN. 2007. Analytical strategies to determine antibiotic residues in fish. Journal of Separation Science, 30: 2549-2569. doi: 10.1002/jssc.200700252.

Shams A. 2020. Identification and Pathogenicity Analyses of Bacterial Pathogens Isolated from Infected Farmed Catfish. MS Thesis, submitted to the Department of Aquaculture, Bangladesh Agricultural University, Mymensingh. 89p.

Shariff N, Sudarshana MS, Umesha S, Hariprasad P. 2006. Antimicrobial activity of *Rauvolfia tetraphylla* and *Physalis minima* leaf and callus extracts. African Journal of Biotechnology, **5:** 946- 950.

Singh DR, Chandra R, Bose M, Luthra P. 2002. Antibacterial activity of *Curcuma longa* rhizome extract on pathogenic bacteria. Current Science, 83(6): 737-740.

Siti-Zahrah A, Padilah B, Azila A, Rimatulhana R, Shahidan H. 2008. Multiple streptococcal species infection in cage-cultured red tilapia but showing similar clinical signs. Diseases in Asian Aquaculture VI. Manila: Fish Health Section, Asian Fisheries Society, 6: 313-320.

Song X, Zhao J, Bo Y, Liu Z, Wu K, Gong C. 2014. *Aeromonas hydrophila* induces intestinal inflammation in grass carp (*Ctenopharyngodon idella*): An experimental model. Aquaculture, 434: 171-178.

Sultana BF, Anwar, Przybylski R. 2007. Antioxidant activity of phenolic components present in barks of *Azadirachta indica*, *Terminalia arjuna*, *Acacia nilotica*, and *Eugenia jambolana* Lam. trees. Food Chemistry, 104: 1106–1114.

Uchida K, Konishi Y, Harada K, Okihashi M, Yamaguchi T, Do MHN, Thi Bui L, Nguyen TD, Nguyen PD, Thi Khong D, Thi Tran H, Nguyen TN, Viet Le H, Chau VV, Thi Van Dao K, Thi Ngoc Nguyen H, Kajimura K, Kumeda Y, Tran Pham K. Ngoc Pham K, Trong Bui C, Quang Vien M, Le NH, Van Dang C, Hirata K, Yamamoto Y. 2016. Monitoring of antibiotic residues in aquatic products in urban and rural areas of Vietnam. Journal of Agricultural Food Chemistry, 64:6133-6138.

Warnke PH, Becker ST, Podschun R, Sivanantha, S, Springer IN, Russo PA, Wiltfang J, Fickenscher H, Sherr, E. 2009. The battle against multi-resistant strains: Renaissance of antimicrobial essential oils as a promising force to fight hospital-acquired infections. Journal of Cranio-Maxillofacial Surgery, 37: 392–397.

Wang CYC, Shie HS, Chen SC, Huang JP, Hsieh IC, Wen MS, Lin FC, Wu D. 2007. *Lactococcus garvieae* infections in humans: Possible association with aquaculture outbreaks. International Journal of Clinical Practice, 61:68–73.

Weir M, Rajic A, Dutil L, Cernicchiaro N, Uhland FC, Mercier B, Tusevljak N. 2012. Zoonotic bacteria, antimicrobial use and antimicrobial resistance in ornamental fish: a systematic review of the existing research and survey of aquaculture-allied professionals. Epidemiology and Infection, 140(2): 192–206.

World Health Organization Report. 2000. Navarro, Vicente. The Lancet, Volume 356, Issue 9241, 1598 – 1601.

Zaika LL, Kissinger JC. 1981. Inhibitory and stimulatory effect of Oregano on *lactobacillus plantarum* and *Pediococcus cerevisiae*. Journal of Food Science, 46 (12): 05-10.

Ziarati M, Zorriehzahra MJ, Hassantabar F, Mehrabi Z, Dhawane M, Sharun K, Emran TB, Dhama K, Chaicumpa W, Shamsi S. 2022. Zoonotic diseases of fish and their prevention and control. Veterinary Quarterly, 42 (1): 95–118

Zhang W, Zhao J, Ma Y, Li J, Chen X. 2022. The effective components of herbal medicines used for prevention and control of fish diseases. Fish & Shellfish Immunology, 126: 73-83.

Zhang M, Dou Y, Xiao Z, Xue M, Jiang N, Liu W, Xu C, Fan Y, Zhang Q, Zhou Y. 2023. Identification of an *Acinetobacter lwoffii* strain isolated from diseased hybrid sturgeon (*Acipenser baerii*♀× *Acipenser schrenckii*♂). Aquaculture, 574: 739649. https://doi.org/10.1016/j.aquaculture.2023.739649.