



## Antibacterial effects of mangrove ethanolic leaf extract against zoonotic fish pathogen *Salmonella arizonae*

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### Abstract

The emergence of multiple drug-resistant pathogens, affecting aquaculture and public health, has put the spotlight on alternative medicine research. This study was conducted to evaluate the *In vitro* and *In vivo* antibacterial activity of mangrove ethanolic leaf extract (MLEE) against *Salmonella arizonae* isolated from *Carassius auratus*. *In vitro*, antimicrobial activity of 10 mangrove species and 13 commercial antibiotics were determined using the agar diffusion method. MLEE with the highest antimicrobial activity were subjected to qualitative phytochemical tests and bioassay experiments. *In vivo* antibacterial activity of MLEE was assessed using *C. auratus* intraperitoneally injected with *S. arizonae*. Results showed that *Sonneratia alba* has the highest antimicrobial activity against *S. arizonae* followed by *Avicennia marina*, *A. officinalis*, *Sonneratia ovata*, *Rhizophora mucronata*, *Excoecaria agallocha*, and *Bruguiera cylindrica*. However, bacterial isolate was resistant to *A. rhumpiana*, *Scyphiphora hydrophyllacea*, and *Laguncularia racemosa*. Interestingly, *S. alba* has comparable antimicrobial activity with amoxicillin, trimethoprim, novobiocin, and cefixime. The activity of *S. alba* could be attributed to the presence of flavonoids, saponin, sterols, tannin, and terpenoids. Moreover, *S. alba* has reduced and delayed the onset of goldfish mortality infected with *S. arizonae*. Based on these findings, the *S. alba* MLEE, is a potential antimicrobial resource against *S. arizonae*.

**Keywords:** Antibacterial; *In vitro*; *In vivo*; mangroves; phytochemicals; zone of inhibition

### 1 | INTRODUCTION

*Salmonella enterica* subspecies *arizonae* is uncommon *Salmonella* species belonging to the family of Enterobacteriaceae. Caldwell and Ryerson (1939) initially consid-

ered *S. arizonae* as a pathogen for cold-blooded animals, especially snakes. Until Seligmann *et al.* (1944) reported the first *S. arizonae* human infection, with gastroenteritis as primary symptoms. Moreover, Jortner and Larsen

(1984) reported the *S. arizonae* infection in domestic poultry while Kodama *et al.* (1987) reported infection in *Arapaima gigas* pirarucu. The recent report of the occurrence of *S. arizonae* in farmed fish was also reported in Brazil (dos Santos *et al.* 2019). Hence, *S. arizonae* was able to mediate human, reptilian, avian, and fish diseases, and was considered zoonotic. In fish, *S. arizonae* leads to mortality secondary to septicemia (Kodama *et al.* 1987). In humans, most *S. arizonae* infections have a good prognosis and fewer complications (Lee *et al.* 2016), still, severe cases have been documented in children below seven years of age and immune-compromised adults (Smilack and Goldberg 1975; Carvalho *et al.* 1990; Mahajan *et al.* 2003; Di Bella *et al.* 2011), individuals with underlying diseases such as human immunodeficiency virus (HIV) infection (Casner and Zuckerman 1990) and even malignancy (Cortes *et al.* 1992). The most recent report of *S. arizonae* infection was reported in Nigeria, Japan, and Taiwan (Lee *et al.* 2016; Nishioka *et al.* 2017; Nuhu *et al.* 2017).

In the Philippines, *S. arizonae* infection in humans and other domestic animals has not been reported. In March 2018, five moribund goldfish *Carassius auratus* were brought to the College's Fish Health Laboratory from a local ornamental pet shop in Barotac Nuevo, Iloilo, Philippines. Researchers immediately examined the fish and performed Koch's postulate to establish a pathogen-disease relationship. One of the possible and potential causative agents of morbidity identified in the laboratory was *S. arizonae*.

At present, there is no standard treatment for *S. arizonae* infection, although development of nano-bodies for its detection was already initiated (Nishioka *et al.* 2017; Ahmed *et al.* 2020). In humans, several cases have been reported that *S. arizonae* is successfully treated with cephalosporins (Nowinski and Albert 2000; Hoag and Sessler 2005; Kolker *et al.* 2012). However, a published case in Japan reported that treatment with cephalosporins might not be enough leading to recurrence of pyelonephritis (Nishioka *et al.* 2017). This could be alarming both in public health and ornamental fish industry since some *Salmonella* strains are zoonotic and have developed resistance to cephalosporins, clavulanic acid, cotrimoxazole, gentamicin, nalidixic acid, norfloxacin, and other common antibiotics (Wang *et al.* 2017; Cameron-Veas *et al.* 2018). More so, that ornamental fish trade industry is one of the most common pathways of spreading of pathogens that are carried along during live fish transport. On top of that, the intermittent use of antibiotics as metaphylaxis in aquaculture has led to the emergence of antimicrobial resistance which has spread across the world and is considered as a very serious concern by the World Health Organization (WHO) and European Union (EU) (Watts *et al.* 2017; Santos and Ramos 2018; Miranda *et al.* 2018). Hence, paving the way for massive research on

discovering novel antimicrobial agents from a natural resource.

Mangroves are tree halophytes growing in the brackish and coastal areas (Kathiresan and Bingham 2001). Because of its long usage in folklore medicine, pharmaceutical chemists have acknowledged the presence of natural products in mangroves that has possible therapeutic effects (Nabeelah *et al.* 2019). Several studies have been published on the chemical compounds present in mangrove plants and their antimicrobial properties against human and animal pathogens (e.g. Chandrasekaran *et al.* 2009; Abeysinghe 2010; Ravikumar *et al.* 2011; Saad *et al.* 2012; Harizon *et al.* 2015; Audah *et al.* 2018; Behbahani *et al.* 2018; Eswarajah *et al.* 2019; Musa *et al.* 2019). Other potential pharmacological uses of mangrove plant extracts are also reported, including antiparasitic (Lopez *et al.* 2018), antidiabetic (Okla *et al.* 2019; Sachithanandam *et al.* 2019), antioxidants (Okla *et al.* 2019), and anti-cancer (Lopez *et al.* 2018).

Given the urgency of the problem and as part of the growing efforts of the college to provide technical support to the fisheries industry, this study was conducted. This could be used as a reference for alternative prophylaxis for ornamental fish should *S. arizonae* infection occur. The objective of this study was to evaluate the antibacterial activities of mangrove ethanolic leaf extracts against *S. arizonae*, *In vitro*, and *In vivo*. Specifically, to determine the antimicrobial activity of ethanolic leaf extracts from 10 mangrove species and 13 commercial antibiotics against *S. arizonae* isolated from *C. auratus*. Further preliminary qualitative phytochemical analyses and *In vivo* experiments were conducted for mangrove ethanolic extract with the highest antimicrobial activity to determine its anti-bactericidal effect against *S. arizonae* using *C. auratus* infection model. This study, with our thorough knowledge, is the first time to evaluate the antibacterial activity of mangrove ethanolic leaf extracts against *S. arizonae* - a zoonotic and uncommon fish pathogen.

## 2 | METHODOLOGY

### 2.1 Preparation of mangrove leaf ethanolic extracts (MLEE)

Leaves of 10 mangrove species were collected from coastal Barangays of Guintas and Sitio Lamintao, Talisay, Barotac Nuevo, Iloilo, Philippines on 5 April 2018. Fresh leaf samples were then brought to Fish Health Laboratory, College of Fisheries and Aquatic Sciences, Iloilo State College of Fisheries. Mangrove species were identified using the handbook of Primavera *et al.* (2004). Identified mangrove species include (1) *Avicennia marina*, (2) *A. officialis*, (3) *A. rhumpiana*, (4) *Sonneratia alba*, (5) *S. ovata*, (6) *Rhizophora mucronata*, (7) *Excoecaria agallocha*, (8) *Scyphiphora hydrophyllacea*, (9) *Lumnitzera racemosa* and, (10) *Bruguiera cylindrica*.

Leaves were then washed in distilled water to remove adherent soil and salt contaminants. Then leaves were air-dried for an hour, oven-dried at 70°C for 72 hours, and were powdered using bullet blender. The powdered leaves (100 g) were soaked in 300 ml of 80% ethyl alcohol and were stored in a dark room for 72 hours. The solution was filtered using the Buchner funnel lined with Whatman No. 2 filter paper. Extracts (100 ml) were water bath at 60°C until 20 ml concentrated extract was left, this is to remove 80% ethyl alcohol from the solution; thus, pure plant extract remains.

## 2.2 Presumptive bacterial identification

Standard procedures on the presumptive identification of bacterial isolates from fish samples were performed following the methods of Tonguthai *et al.* (1999) and Ruangpan and Tendencia (2004). Supernatant from viscera of moribund goldfish was streak on McConkey Agar and Salmonella-Shigella Agar (SSA) and incubated at 25°C for 48 hours. Series of presumptive analyses were then conducted such as gram staining, biochemical tests (indole, methyl-red, Voges-Proskauer, Simmons citrate, hydrogen sulfide, motility), and fermentation of sugars (glucose, sucrose, mannose, lactose, D-xylose, D-sorbitol, L-arabinose, Trehalose).

## 2.3 Test organism and culture

Pure cultures of *S. arizonae* isolated from moribund goldfish were maintained in nutrient broth and incubated at 25°C for 48 hours. Every three days, working cultures were transferred to fresh nutrient broth media to assure purity. Before subsequent experiments, a loopful *S. arizonae* culture was aseptically transferred to SSA. Colonies on SSA plates were aseptically transferred into 10 ml Tryptic Soy Broth (TSB) in replicate until bacterial suspension and turbidity reach  $1.5 \times 10^9$  CFU ml<sup>-1</sup> (used for *In vitro* tests) and  $3 \times 10^9$  CFU ml<sup>-1</sup> (used for *In vivo* tests) following 0.5 MacFarland Nephelometer Standard (Ruangpan and Tendencia 2004). Cultures incubated at 25°C for five hours were used for subsequent study.

## 2.4 Preparation of MacFarland nephelometer standards

A  $1.5 \times 10^9$  CFU ml<sup>-1</sup> MacFarland standard was prepared by mixing 5 ml of 1.175% barium chloride dihydrate (BaCl<sub>2</sub>·2H<sub>2</sub>O) with 95 ml 1% sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) in a test tube with constant stirring (Chapin and Lauderdale 2007). On the other hand,  $3 \times 10^9$  CFU ml<sup>-1</sup> MacFarland standard was prepared by mixing 10 ml 1.175% BaCl<sub>2</sub>·2H<sub>2</sub>O with 90 ml of 1% H<sub>2</sub>SO<sub>4</sub>.

## 2.5 Preparation of impregnated disc

Sterilized plates were prepared for the 10 different mangrove leaves extract in duplicate, following the disc immersion method (Ruangpan and Tendencia 2004). Sterilized 6 mm diameter filter paper discs in five compact

layers were impregnated with 10 ml MLEE by immersion technique in Petri plates. MLEE were oven-dried at 60°C until dried.

## 2.6 Susceptibility test

The standard disc diffusion method (Ruangpan and Tendencia 2004) was carried out to assess the susceptibility of *S. arizonae* on MLEE of 10 mangrove species and 13 common commercial antibiotics. Bacterial culture was lawn on Mueller Hinton agar (MHA) plates using a sterile cotton swab and were dried for 10 minutes. Then discs impregnated with different treatments: (1) MLEE for treatments, (2) antibiotics for positive control, and (3) blank disc for negative control. Discs were then placed on the MHA surface.

Antibiotics used as positive controls were (1) 2 g clindamycin, (2) 10 g gentamycin, (3) 5 g ciprofloxacin, (4) 10 units bacitracin, (5) 30 g vancomycin, (6) 10 g streptomycin, (7) 30 g chloramphenicol, (8) optochin, (9) 10 units penicillin G, (10) 5 g cefixime, (11) 2.5 g trimethoprim, (12) 25 g amoxicillin, and (13) 30 mcg novobiocin.

## 2.7 Preliminary phytochemical analyses

Species of mangrove with the highest zone of inhibition were subjected to preliminary qualitative phytochemical analyses. Methods of Khlif *et al.* (2015) were used to test the presence of alkaloids, flavonoids, saponin, steroids, tannin, and terpenoids.

## 2.8 *In vivo* experiments

**Experimental animal:** A total of 55 healthy, sub-adult goldfish, with an average weight of 25 g, were obtained from freshwater ornamental Biotech fish pet shop, Iloilo City, Philippines. Samples were divided into five fish per 20 L aquarium, provided with continuous aeration, and were acclimatized for five days before conducting the experiments.

**Experiment I: sub-acute bioassay test:** The MLEE with the highest zone of inhibition was used for this experiment. Healthy 25 sub-adult *C. auratus* were subdivided into five fish per 20 L aquarium. After acclimatization, fish samples were exposed to different concentrations of MLEE. Different treatment concentrations include: (1) 50 ppm, (2) 100 ppm, (3) 200 ppm, (4) 300 ppm, and (5) 500 ppm. Daily fish mortality was recorded to determine the maximum allowable concentration (MAC) of MLEE in *C. auratus*. MAC was used in the antibacterial test experiment.

**Experiment II: antibacterial test:** Fifteen goldfish were divided into three groups: (1) treated, (2) negative control and, (3) positive control group. Each group is consisting of five fish per aquarium in 20 L capacity aquarium. Treated and positive control groups were intraperitoneally (i.p) injected with 100 µl of *S. arizonae* ( $3 \times 10^9$  CFU fish<sup>-1</sup>). The

negative control group was i.p injected with 100  $\mu$ l distilled water. After 48-hour post-infection, goldfishes in the treated group were bath with 300 ppm *S. alba* ethanolic leaf extract. Daily observations of fish mortality were monitored for fifteen days to determine the antibacterial effect of the *S. alba* leaf extract infected goldfish. This experiment was conducted with two trials, employing the same methodologies.

## 2.9 Data analyses

Descriptive data analysis was performed. Unless specified, results were expressed as mean  $\pm$  standard deviation (SD) from two trial experiments. All data analyses were performed and encoded with a statistical analysis system in MS Office Excel 365.

## 3 | RESULTS

### 3.1 Presumptive identification of bacterial isolate

Results of presumptive identification including morphology, gram staining, growth in selective media, and biochemical characteristics of *S. arizonae* are shown in Table 1. The isolate had a rod shape, a gram-negative, and growth was observed in SSA and McConkey Agar at 25°C. Positive results were observed in methyl red, Simmons citrate, H<sub>2</sub>S production, motility, glucose (gas and acid), mannose, lactose, D-xylose, D-sorbitol, L-arabinose, Trehalose while the negative results were obtained from indole production, Voges–Proskauer and fermentation of sucrose.

**TABLE 1** Biochemical characteristics of *Salmonella arizonae* isolated from goldfish *Carassius auratus*.

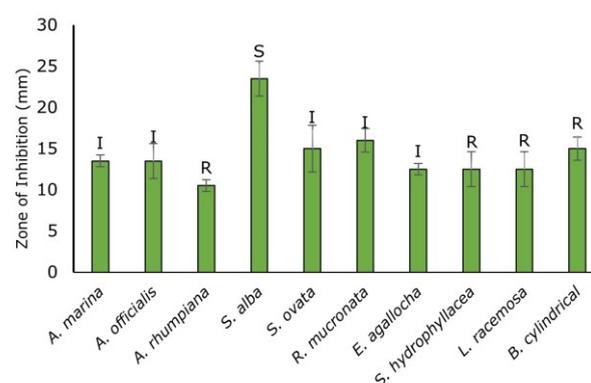
Parameters	Results
Morphology	Rod
Gram Staining	–
Growth in SSA	+
Growth in McConkey Agar	+
Indole Production	–
Methyl Red	+
Voges–Proskauer	–
Simmons Citrate	+
Hydrogen Sulfide production	+
Motility	+
Glucose, gas	+
Glucose, acid	+
Fermentation of	
Sucrose	–
Mannose	+
Lactose	+
D-xylose	+
D-sorbitol	+
L-arabinose	+
Trehalose	+

+, positive results; –, negative results.

### 3.2 Susceptibility test

The result of the study revealed that MLEE showed prom-

ising antibacterial activity against *S. arizonae*, *In vitro* (Figure 1). Among 10 mangrove species tested, *S. alba* leaf extract had the highest antimicrobial activity against *S. arizonae* with an average zone of inhibition of about  $23.5 \pm 2.12$  mm. This zone of inhibition was followed by *A. marina* ( $13.5 \pm 0.71$  mm), *A. officinalis* ( $13.5 \pm 2.12$  mm), *S. ovata* ( $15 \pm 2.83$  mm), *R. mucronata* ( $16 \pm 2.41$  mm), *E. agallocha* ( $12.5 \pm 0.71$  mm), and *B. cylindrica* ( $15 \pm 1.41$  mm) having an intermediate effect against the tested pathogen. However, *S. arizonae* was resistant to three of the other species, *A. rhumpiana*, *S. hydrophyllacea*, and *L. racemosa*.



**FIGURE 1** Susceptibility of *Salmonella arizonae* to 10 mangrove species ethanolic leaf extract. Each value is the mean zone of inhibition (mm)  $\pm$  computed standard deviations from two replicates. R, resistant ( $\le 13$  mm); I, intermediate (14–18 mm); S, susceptible ( $\ge 19$  mm).

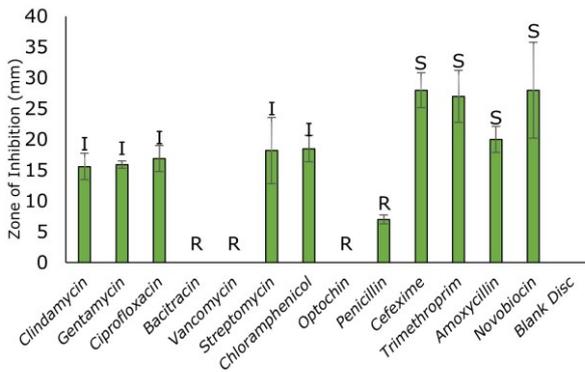
The antibacterial of *S. alba*, moreover, was comparable to common commercial antibiotics. The *S. arizonae* was inhibited by cefixime, trimethoprim, amoxicillin, and novobiocin with a 20 – 28 mm range zone of inhibition (Figure 2). The test pathogen was moderately inhibited by clindamycin, gentamycin, ciprofloxacin, streptomycin, and chloramphenicol, while resistant to bacitracin, vancomycin, optochin and penicillin, *In vitro*.

### 3.3 Preliminary phytochemical analyses

The presence of phytochemicals and possible unknown bioactive compounds could be attributed to the antimicrobial effect of medicinal plants on pathogenic microorganisms. In this study, the ethanolic leaf extract of *S. alba* leaves have flavonoids, saponin, sterols, tannin, and terpenoids; however, free of alkaloids (Table 2).

### 3.4 Sub-acute bioassay toxicity

The ethanolic extract of *S. alba* exhibited toxicity on *C. auratus* at Treatment V, with an 80 % mortality (Table 3). While all other remaining Treatments, I, II, III, and IV, were non-lethal to fish with no mortality. Hence, 300 ppm, is the maximum allowable concentration (MAC) of *S. alba* in *C. auratus*, was used in the next *In vivo* antibacterial assay.



**FIGURE 2** Susceptibility of *Salmonella arizonae* to common commercial antibiotics. Each value is the mean zone of inhibition (mm) ± computed standard deviations from two replicates. R, resistant (≤13 mm); I, intermediate (14–18 mm); S, susceptible (≥19 mm).

**TABLE 2** Phytochemical analyses of *Sonneratia alba* ethanolic leaf extract.

Phytochemical	Tests	Results
Alkaloids	Mayer’s reagent	–
Flavonoids	Shinoda, alkaline reagent	+
Saponin	Foam	+
Sterols	Chloroform and concentrated sulfuric acid	+
Tannin	10 ml of bromine	+
Terpenoids	Chloroform and concentrated sulfuric acid	+

+, positive results; –, negative results.

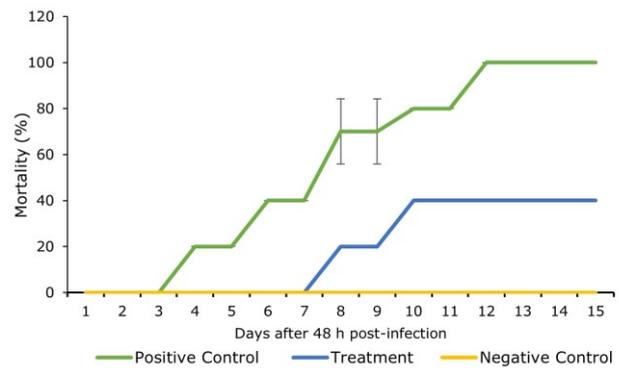
**TABLE 3** Mortality records of goldfish exposed to different concentrations of *Sonneratia alba* ethanolic leaf extract.

Days of exposure	Treatments (ppm)				
	50	100	200	300	500
1	–	–	–	–	–
2	–	–	–	–	–
3	–	–	–	–	–
4	–	–	–	–	–
5	–	–	–	–	–
6	–	–	–	–	1
7	–	–	–	–	1
8	–	–	–	–	1
9	–	–	–	–	1
10	–	–	–	–	–
Total	0	0	0	0	4
Mortality (%)	0	0	0	0	80

### 3.5 Antibacterial bioassay test

The *In vivo* antibacterial activity of *S. alba* leaf extract was examined using the *C. auratus* infection model. *Carassius auratus* were infected with *S. arizonae* through intraperitoneal injection. After 48-hour post-infection, treated group was exposed to 300 ppm, the non-lethal dose of *S. alba* extract. As shown in Figure 3, mortality from positive

control group started on day four with 20% mortality and has increased to 100% mortality in day fifteen. While treated group, has a late onset of mortality, beginning from 20% on the 8th day and maintains 40% mortality from 10th day until the 15th day of post-infection. The negative control group has zero mortality throughout the experimental period.



**FIGURE 3** Percentage of mortality ± standard deviation from two trial experiments of *Carassius auratus* i.p injected with *Salmonella arizonae* (n = 5).

## 4 | DISCUSSION

Worldwide, the ornamental fish business is developing and considered one of the most lucrative in the aquaculture industry (Galib and Mohsin 2010; Walczack *et al.* 2017; Muyot *et al.* 2019). However, the unintended transport of pathogens along with the pet fish could lead to spread and may cause disease to a susceptible host (Smith *et al.* 2012). The spread and emergence of antimicrobial resistance (AMR) pathogen have put research of natural resource antimicrobial in the spotlight in the past decade.

Mangroves have been used as traditional medicine and claimed to be effective against human diseases (Thatoi *et al.* 2016; Nabeelah *et al.* 2019). Several studies have been established on the antimicrobial activity of different parts of mangroves against resistant human and animal pathogens to address the growing problem of the emergence of multiple drug resistance and zoonotic bacterial strains (e.g. Chandrasekaran *et al.* 2009; Abeyasinghe 2010; Ravikumar *et al.* 2011). With this, it is significant to study the antibacterial effect of mangrove plants in the uncommon but drug-resistant pathogen, *S. arizonae*. Evaluating the antimicrobial properties of mangrove species, which is abundant in tropical regions is worthwhile, as it may lead to phytomedicine development against pathogenic microbes. In this study, the *In vitro* antibacterial activity of 10 mangrove species was tested against *S. arizonae* and was compared to 13 commercial antibiotics. Further *In vivo* experiments were conducted with *S. alba* ethanolic leaf extract using a piscine model infection.

The ethanolic leaf extract of *S. alba* had the highest zone of inhibition against *S. arizonae*, which is compara-

ble to *S. typhi* (Sahoo *et al.* 2012). Antibacterial properties of mangrove plants could be attributed to the presence of phytochemicals and some unknown bioactive compounds which might have a future clinical application (Bandaranayake 2002; Eswarajah *et al.* 2019). In this study, preliminary phytochemical analyses detected the presence of flavonoids, saponin, sterols, tannin, and terpenoids in *S. alba*. Flavonoids, for example, are known antimicrobial agents against a broad spectrum of microorganisms (Xie *et al.* 2015). Interestingly, plants synthesized flavonoids in response to microbial infection (Kumar and Pandey 2013; Mierziak *et al.* 2014). The flavonoid mechanism of action, leading to its antimicrobial properties involves disruption of microbial membranes, nucleic acid biosynthesis inhibition, and microbial adhesins enzyme inactivation (Kumar and Pandey 2013; Djouossi *et al.* 2015; Mishra *et al.* 2017). However, it should be noted that ethanolic leaf extracts from different mangrove tested has varying antimicrobial effects against *S. arizonae*. There might be some unknown compounds that may explain its antimicrobial properties and diverse mechanism of mangrove species actions on *S. arizonae*, which is yet to be explained. For example, the study of Harizon *et al.* (2015) and Musa *et al.* (2019) identified new lupane-type triterpenoids from *S. alba* bark and leaf extract and has antibacterial activities against human pathogens.

The maximum allowable concentration of *S. alba* on goldfish was 300 ppm, this concentration was used for subsequent *In vivo* antibacterial assay. In the study of Avenido and Serrano Jr. (2012), the use of *S. caseolaris* as prophylaxis has no adverse effects on the histology of the *Penaeus monodon*. This present study and related literature suggest that *S. alba* is a promising source of phyto-medicine and a favorable source of antimicrobials for aquaculture use. Moreover, the treatment of *S. alba* ethanolic leaf extract to *S. arizonae* infected goldfish, has reduced the mortality and delayed the onset of mortality, further increasing the promise of *S. alba* ethanolic extract as a treatment against *S. arizonae*.

This study, as the first study to test the efficacy of mangrove leaf extract against the uncommon ornamental fish pathogen, would be a great help to the hobbyist and eventually, the ornamental fish industry should *S. arizonae* infection would occur. Furthermore, the use of organic and natural resources may reduce the threat of multiple drug resistance. However, it should be noted that this study has limitations. First, only presumptive identification of *S. arizonae* was conducted, second, preliminary phytochemical analyses were performed, and lastly, the toxicity of *S. alba* in goldfish was conducted using a small sample size and inadequate replications. Therefore we recommend further PCR techniques be conducted to verify the isolation of *S. arizonae* and robust toxicity assay with adequate sampling size. With the promising antibacterial activity of *S. alba*, toxicity may

also be evaluated on tissue and cellular levels.

## 5 | CONCLUSIONS

The present study revealed three primary results, (1) mangrove ethanolic leaf extracts, specifically *S. alba* extract, have antimicrobial activity against *S. arizonae* comparable to the commercial and standard antibiotics, (2) presence of flavonoids, saponin, sterols, tannin, and terpenoids were detected in *S. alba* ethanolic leaf extracts, and (3) *S. alba* ethanolic extract treatment in *S. arizonae* infected goldfish reduced and delayed the onset of mortality. Therefore, *S. alba* is a potential source of antibacterial phytomedicine against *S. arizonae*.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## AUTHORS' CONTRIBUTION

**JSL & JS** research design, field and laboratory experiments, manuscript preparation and revision; **AAG, PM, MNR** field and laboratory experiments; **DKG** research design and supervision, and review of the manuscript.

## DATA AVAILABILITY STATEMENT

All data that support the findings of the study are available within the manuscript. Should any clarifications be needed, it could be requested from the corresponding author.

## REFERENCES

- Abeysinghe PD (2010) Antibacterial activity of some medicinal mangroves against antibiotic resistant pathogenic bacteria. *Indian Journal of Pharmaceutical Sciences* 72(2): 167–172.
- Ahmed BS, Mostafa AA, Darwesd, OM, Abdel-Rahim EA (2020) Development of specific nano-antibody for application in selective and rapid environmental diagnoses of *Salmonella arizonae*. *Biointerface Research in Applied Chemistry* 10(6): 7198–7208.
- Audah KA, Amsyir J, Almasyhur F, Hapsari AM, Sutanto H (2018) Development of extract library from Indonesian biodiversity: exploration of antibacterial activity of mangrove *Bruguiera cylindrica* leaf extracts. *IOP Conference Series: Earth and Environmental Science* 130(1): 012025.
- Avenido P, Serrano AE Jr (2012) Effects of the apple mangrove (*Sonneratia caseolaris*) on antimicrobial, immunostimulatory and histological responses in black tiger shrimp postlarvae fed at varying feeding frequency. *Aquaculture, Aquarium, Conservation & Legislation International Journal of the Biofluc Society* 5(3): 112–123.
- Bandaranayake WM (2002) Bioactivities, bioactive compounds and chemical constituents of mangrove plants. *Wetlands Ecology and Management* 10(6): 421–452.

- Behbahani BA, Yazdi FT, Shahidi F, Noorbakhsh H, Vasiee A, Alghooneh A (2018) Phytochemical analysis and antibacterial activities extracts of mangrove leaf against the growth of some pathogenic bacteria. *Microbial Pathogenesis* 114(1): 225–232.
- Caldwell ME, Ryerson DL (1939) Salmonellosis in certain reptiles. *The Journal of Infectious Diseases* 65(3): 242–245.
- Cameron-Veas K, Fraile L, Napp S, Garrido V, Grilló MJ, Migura-Garcia L (2018) Multidrug resistant *Salmonella enterica* isolated from conventional pig farms using antimicrobial agents in preventative medicine programmes. *Veterinary Journal* 234(1): 36–42.
- Carvalho J Jr, McMillan VM, Ellis RB, Betancourt A (1990) Endogenous endophthalmitis due to *Salmonella arizonae* and *Hafnia alvei*. *Southern Medical Journal* 83(3): 325–327.
- Casner PR, Zuckerman MJ (1990) *Salmonella arizonae* in patients with AIDS along the U.S.-Mexican border. *The New England Journal of Medicine* 323(3): 198–199.
- Chandrasekaran M, Kannathasan K, Venkatesalu V, Prabhakar K (2009) Antibacterial activity of some salt marsh halophytes and mangrove plants against methicillin resistant *Staphylococcus aureus*. *World Journal of Microbiology and Biotechnology* 25(1): 155–160.
- Chapin KC and Lauderdale TL (2007) Reagents, stains, and media: bacteriology. In: Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA (Eds) *Manual of clinical microbiology*. ASM Press, USA.
- Cortes E, Zuckerman MJ and Ho H (1992) [Recurrent \*Salmonella arizonae\* infection after treatment for metastatic carcinoma](#). *Journal of Clinical Gastroenterology* 14(2): 157–159.
- Di Bella S, Capone A, Bordi E, Johnson E, Musso M, ... Petrosillo N (2011) [Salmonella enterica ssp. arizonae infection in a 43-year-old Italian man with hypoglycemia: a case report and review of the literature](#). *Journal of Medical Case Reports* 5: 323.
- Djouossi MG, Tamokou JD, Ngnokam D, Kuate JR, Tapondjou LA, ... Voutquenne-Nazabadioko L (2015) Antimicrobial and antioxidant flavonoids from the leaves of *Oncoba spinosa* Forssk (Salicaceae). *BMC Complementary and Alternative Medicine* 15: 134.
- dos Santos RR, Xavier RGC, de Oliveira TF, Leite RC, Figueiredo HCP, Leal CAG (2019) [Occurrence, genetic diversity, and control of \*Salmonella enterica\* in native Brazilian farmed fish](#). *Aquaculture* 501: 304–312.
- Eswaraiah G, Peele KA, Krupanidhi S, Kumar RB, Venkateswarulua TC (2019) Studies on phytochemical, antioxidant, antimicrobial analysis and separation of bioactive leads of leaf extract from the selected mangroves. *Journal of King Saud University - Science* 32(1): 842–847.
- Galib SM, Mohsin ABM (2010) Exotic ornamental fishes of Bangladesh. *Bangladesh Journal of Progressive Science and Technology* 8(2): 255–258.
- Harizon, Pujiastuti B, Kurnia D, Sumiarsa D, Shiono Y, Supratman U (2015) Antibacterial triterpenoids from the bark of *Sonneratia alba* (Lythraceae). *Natural Products Communications* 10(2): 277–280.
- Hoag JB, Sessler CN (2005) A comprehensive review of disseminated *Salmonella arizonae* infection with an illustrative case presentation. *Southern Medical Journal* 98(11): 1123–1129.
- Jortner BS, Larsen C (1984) [Granulomatous ventriculitis of the brain in arizonosis of turkeys](#). *Veterinary Pathology* 21(1): 114–115.
- Kathiresan K, Bingham BL (2001) Biology of mangroves and mangrove ecosystems. *Advances in Marine Biology* 40: 81–251.
- Khlif I, Jellali K, Michel T, Halabalaki M, Skaltsounis LA, Allouche N (2015) Characteristics, phytochemical analysis and biological activities of extracts from Tunisian *Chetoui olea europaea* variety. *Journal of Chemistry* 2015: 418731.
- Kodama H, Nakanishi Y, Yamamoto F, Mikama T, Izawa H, ... Kudo N (1987). *Salmonella arizonae* isolated from a pirarucu, *Arapaima gigas* Cuvier, with septicemia. *Journal of Fish Diseases* 10(6): 509–512.
- Kolker S, Itsekzon T, Yinnon AM, Lachish T (2012) Osteomyelitis due to *Salmonella enterica* subsp. *arizonae*: the price of exotic pets. *Clinical Microbiology and Infection* 18(2): 167–170.
- Kumar S, Pandey AK (2013) Chemistry and biological activities of flavonoids: an overview. *The Scientific World Journal* 2013: 162750.
- Lee YC, Hung MC, Hung SC, Wang HP, Cho HL, ... Wang JT (2016) [Salmonella enterica subspecies arizonae infection of adult patients in Southern Taiwan: a case series in a non-endemic area and literature review](#). *BMC Infectious Diseases* 16: 746.
- Lopez D, Cherigo L, de Sedas A, Spadafora C, Martinez-Luis S (2018) [Evaluation of antiparasitic, anticancer, antimicrobial and hypoglycemic properties of organic extracts from Panamanian mangrove plants](#). *Asian Pacific Journal of Tropical Medicine* 11(1): 32–39.
- Mahajan RK, Khan SA, Chandel DS, Kumar N, Hans C, Chaudhry R (2003) [Fatal case of Salmonella enterica subsp. arizonae gastroenteritis in an infant with microcephaly](#). *Journal of Clinical Microbiology* 41(12): 5830–5832.
- Mierziak J, Kostyn K, Kulma A (2014) [Flavonoids as important molecules of plant interactions with the environment](#). *Molecules* 19(10): 16240–16265.
- Miranda CD, Godoy FA, Lee MR (2018) Current status of the use of antibiotics and the antimicrobial resistance in the Chilean Salmon farms. *Frontiers in*

- Microbiology 9: 1284.
- Mishra MP, Rath S, Swain SS, Ghosh G, Das D, Padhy RN (2017) [In vitro antibacterial activity of crude extracts of 9 selected medicinal plants against UTI causing MDR bacteria](#). Journal of King Saud University-Science 29(1): 84–95.
- Musa WJA, Bialangi N, Situmeang B, Silaban S (2019) Triterpenoid compound from metanol extract of mangrove leaves (*Sonneratia alba*) and anti-cholesterol activity test. Jurnal Pendidikan Kimia 11(1): 18–23.
- Muyot FB, Mutia MTM, Manejar AJA, Guihem GL, Muñoz MJ (2019) Status of ornamental fish industry in the Philippines: prospects for development. The Philippine Journal of Fisheries 26(2): 12–32.
- Nabeelah BS, Fawzi MM, Gokhan Z, Rajesh J, Nadeem N, ... Pandian SK (2019) [Ethnopharmacology, phytochemistry, and global distribution of mangroves- a comprehensive review](#). Marine Drugs 17(4): 231.
- Nishioka H, Doi A, Takegawa H (2017) [Pyelonephritis in Japan caused by \*Salmonella enterica\* subspecies \*arizonae\*](#). Journal of Infection and Chemotherapy 23(12): 841–843.
- Nowinski RJ, Albert MC (2000) [Salmonella osteomyelitis secondary to iguana exposure](#). Clinical Orthopaedics and Related Research 372(1): 250–253.
- Nuhu T, Olayinka BO, Bolaju RO, Adabara N (2017) [Salmonella arizonae: an uncommon uropathogen?](#) Gulf Medical Journal 6(1): 22–26.
- Okla MK, Alamri SA, Alatar AA, Hegazy AK, Al-Ghamdi AA, ... Abdel-Maksoud MA (2019) Antioxidant, hypoglycemic, and neurobehavioral effects of a leaf extract of *Avicennia marina* on autoimmune diabetic mice. Evidence-Based Complementary and Alternative Medicine 19: 1263260.
- Primavera JH, Sadaba RS, Lebara MJHL, Altamirano JP (2004) Handbook of mangroves in the Philippines-Panay. SEAFDEC Aquaculture Department, Iloilo.
- Ravikumar S, Inbaneson SJ, Suganthi P, Gnanadesigan M (2011) [In vitro antiplasmodial activity of ethanolic extracts of mangrove plants from South East coast of India against chloroquine-sensitive \*Plasmodium falciparum\*](#). Parasitology Research 108(4): 873–878.
- Ruangpan L, Tendencia EA (2004) Laboratory manual of standardized methods for antimicrobial sensitivity tests for bacteria isolated from aquaculture. SEAFDEC Aquaculture Department, Iloilo, Philippines.
- Saad S, Taher M, Susanti D, Qaralleh H, Awang AF (2012) [In vitro antimicrobial activity of mangrove plant \*Sonneratia alba\*](#). Asian Pacific Journal of Tropical Biomedicine 2(6): 427–429.
- Sachithanandam V, Lalitha P, Parthiban A, Mageswaran T, Manmadhan K, Sridhar R (2019) [A Review on antidiabetic properties of Indian mangrove plants with reference to island ecosystem](#). Evidence-Based Complementary Alternative Medicine 19: 4305148.
- Sahoo G, Mulla NS, Ansari ZA, Mohandass C (2012) [Antibacterial activity of mangrove leaf extracts against human pathogens](#). Indian Journal of Pharmaceutical Sciences 74(4): 348–351.
- Santos L, Ramos F (2018) Antimicrobial resistance in aquaculture: current knowledge and alternatives to tackle the problem. International Journal of Antimicrobial Agents 52: 135–143.
- Seligmann E, Saphra L, Wassermann M (1944) Occurrence of some unusual *Salmonella* types in man including a new type, *Salmonella georgia*. American Journal of Hygiene 40(3): 227–231.
- Smilack JD, Goldberg MA (1975) [Bone and joint infection with \*Arizona hinshawii\*: report of a case and a review of the literature](#). The American Journal of Medical Sciences 270(3): 503–507.
- Smith KF, Schmidt V, Rosen GE, Amaral-Zettler L (2012) [Microbial diversity and potential pathogens in ornamental fish aquarium water](#). PloS one 7(9): e39971.
- Thatoi H, Samantaray D, Das SK (2016) [The genus \*Avicennia\*, a pioneer group of dominant mangrove plant species with potential medicinal values: a review](#). Frontiers in Life Science 9(4): 267–291.
- Tonguthai K, Chinabut S, Somsiri T, Chanratchakol P, Kanchanakhan S (1999) Diagnostic procedures for finfish diseases. Aquatic Animal Health Research Institute, Bangkok, Thailand.
- Walczak N, Puk K, Guz L (2017) [Bacterial flora associated with diseased freshwater ornamental fish](#). Journal of Veterinary Research 61(4): 445–449.
- Wang J, Li Y, Xu X, Liang B, Wu F, ... Song H (2017) [Antimicrobial resistance of \*Salmonella enterica\* serovar \*typhimurium\* in Shanghai, China](#). Frontiers in Microbiology 8(1): 510.
- Watts JEM, Schreier HJ, Lanska L, Hale MS (2017) The rising tide of antimicrobial resistance in aquaculture: sources, sinks and solutions. Marine Drugs 15(6): 158.
- Xie Y, Yang W, Tang F, Chen X, Ren L (2015) Antibacterial activities of flavonoids: structure-activity relationship and mechanism. Current Medicinal Chemistry 22(1): 132–149.



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