Research in
AGRICULTURE, LIVESTOCK and FISHERIES
ISSN : P-2409-0603, E-2409-9325
An Open Access Peer-Reviewed International Journal

Article Code: 456/2024/RALF
Article Type: Research Article

Res. Agric. Livest. Fish.
Vol. 11, No. 2, August 2024: 255-269.

Efficacy of *Terminalia arjuna* (Roxb.) Bark Extracts Against *Salmonella* sp. Isolated From Aquaculture Ponds

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ARTICLE INFO

Received
10 August, 2024

Revised
27 August, 2024

Accepted
31 August, 2024

Online
September, 2024

Key words:
Terminalia arjuna
Salmonella sp.
Sensitivity
Drug resistance
Aquaculture

ABSTRACT

The present study was conducted to determine the efficacy of *Terminalia arjuna* bark extracts against *Salmonella* sp. isolated from 15 aquaculture ponds located adjacent to Bangladesh Agricultural University, Mymensingh. *T. arjuna* barks was dried, powdered and extracted using cold water (polar) and hexane (non-polar) and stored at 10ºC while *Salmonella* sp. were isolated using SS (*Salmonella Shigella*) Agar and selected 2 isolates were confirmed by Hi Assorted biochemical test kit. Antimicrobial activities of the bark extract of *T. arjuna* against fifteen selected isolates of *Salmonella* were then determined. Two of those selected isolates (Sa-2 and Sa-5) shown zone of inhibition against crude bark extract (hexane extract) of *T. arjuna*. The isolates were also screened for antibiotic resistance to eleven antibiotics. *Salmonella* isolates were serotyped and the antimicrobial resistance was determined by a disk diffusion method. About 100% of the isolates were resistant to antibiotic discs among which ampicillin (10 µg), and chloramphenicol (30 µg) showed 15%, azithromycin (10 µg) showed 45%, erythromycin (15 µg) showed 60%, tetracycline (10 µg) showed 40%, ampicillin (10 µg) showed 100%, doxycycline (30 µg) showed 46.67%, kanamycin (30 µg) and gentamycin (10 µg) showed 6%, streptomycin (25 µg) and ciprofloxacin (10 µg) showed 20%, and cefotaxime (30 µg) showed 30% resistant to isolated *Salmonella* sp. and all *Salmonella* sp. isolates were found to be 100% resistant to cold water extract but sensitive to hexane extract of *T. arjuna*. The study showed hexane extract of *T. arjuna* would be potential, ecofriendly and biodegradable herbal medicine to control multidrug resistant *Salmonella* sp. as well as aquaculture health management.

To cite this article: Islam S., M. K. C. Saha, N. Sultana, M. R. Rahman, J. Raihan, K. Farjana, R. B. Raihan, T. Tabassum, S. Acharjee, S. S. Shovon, S. S. Nibir, K. T. Kubra and T. Rahman, 2024. Efficacy of *Terminalia arjuna* (Roxb.) bark extracts against *Salmonella* sp. isolated from aquaculture ponds. Res. Agric. Livest. Fish. 11(2): 255-269.

DOI: https://doi.org/10.3329/ralf.v11i2.76076

INTRODUCTION

The contribution of fisheries and aquaculture sector to the livelihoods and food security is significant, providing millions of people with food, nutrition, income and employment, (FAO, 2024). To meet the growing demands for fish, safety is an important factor, since fish may be vehicles for the transmission of certain pathogens, such as *Salmonella* (Fernandes et al., 2018). The natural habitat of *Salmonella* is the gastrointestinal tract of animals including birds and man (Pelzer, 1989) which may find its way into the river water, coastal and estuarine sediments through fecal contamination. Aquatic environment is the major reservoirs of *Salmonella* and aids its transmission between the hosts (Cherry et al., 1972). The genus *Salmonella* has a wide variety of species, which are pathogenic and cause different types of food poisoning. The occurrence of *Salmonella* in fish and shellfish, either in fresh or marine waters has normally been associated with fecal contamination of the area from which they were harvested (Buttaiux, 1962). *Salmonella* is not a biological contaminant originally reported in fish, being introduced through contaminated water or improper handling (Santana, 2012). Some serogroups *viz., S. enterica* serovar Typhi and *S. enterica* serovar Paratyphi are restricted to humans, causing typhoid and paratyphoid fever, respectively (Holt et al., 2009). Salmonellosis causes gastroenteritis, bacteremia and more serious systemic diseases, such as typhoid and typhoid fever (Bibi et al., 2015). Presence of this bacterium was also detected in the fish feces. Setti et al., (2009) reported that 28 (49.1%) *Salmonella* isolates showed resistance to ampicillin (22 isolates), nalidixic acid (9 isolates), sulfonamide compounds (2 isolates) and tetracycline (1 isolates) and six isolates showed resistance to two antimicrobial substances. The limited effective life span of current antibiotics, the lack of compliance of patients, the uncontrolled use in agriculture, and the slow rate in releasing new antimicrobial agents have led to an alarming increase in antimicrobial resistance. The search for remedy in plants is not new (Othman et al., 2019). The plants which possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally recommended as medicinal plants.

Among hundreds of medicinal plants, *Terminalia arjuna* (Family: Combertaceae), commonly known as “Arjuna” 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). *Terminalia* sp. has many effective medicinal uses especially for heart and circulatory system. In particular, *T. arjuna* bark is well known for its antipyretic, antiatherogenic, antiastringic, hypotensive, inotropic, anti-inflammatory and antioxidant activities (Biswas et al., 2011 and Kapoor et al., 2014), due to containing a good number of phytoconstituents including tannins, saponin, flavonoids, phenolic compounds, alkaloids, triterpenoids, sterols, calcium salts, arjunine, arjunglyciside and glycosides (Ali et al., 2003; Sultana et al., 2007; Patil and Gaikwad, 2011 and Ghani, 2003). Different parts of this plant, mostly stem bark, have been popularly used in this region not only as traditional medicines but also as fish poison to catch fishes by tribal people since ancient time (Murthy et al., 2010). The routine practice of giving antimicrobial agents to domestic livestock as a means of preventing and treating diseases, as well as promoting growth is an important factor in the emergence of antibiotic resistant bacteria that are subsequently transferred to human through the food chain (Salehi et al., 2005). Increasing global demand for the preservation of eco-friendly environments, the 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 and Uchida et al., 2016). Since plant-based drugs cause much lower incidence of adverse reactions compared to synthetic pharmaceutical (Sharif et al., 2006), scientist felt the urgency to develop an alternative approach of herbal medication towards management of disease. The aim of this preliminary research was to isolate *Salmonella* sp. from aquaculture ponds and to determine the herbal efficacy of *T. arjuna* against the drug-resistant *Salmonella* sp. under *in-vitro* condition.

MATERIALS AND METHODS

Study area and duration

The experiment was conducted at Fish Disease Laboratory, Faculty of Fisheries and Agricultural Chemistry Laboratory, Faculty of Agriculture, Bangladesh Agricultural University (BAU), Mymensingh-2202 from June, 2018 to November, 2018 following a pre-determined schedule.

Collection of Arjun bark

Bulk volume of Arjun *(T. arjuna)* bark was collected from the adjacent area of Bangladesh Agricultural University, Mymensingh campus, cut into small pieces, sun dried, finely powdered using an automated grinder and sieved the powder to increase the surface area for better extraction.

Extraction of *T. arjuna* bark powder

Cold water extraction

Two hundred and fifty grams of finely powdered arjun bark was submerged in 3 L distilled water in an aspirator bottle at room temperature. The extract was taken out after 72 hours. The crude part deposited at the bottom of the flask. The deposited part was then removed and filtered by a muslin cloth. The extraction was then concentrated over water bath to one third of its volume (1 L). The extracted sample was stored in a 1 L plastic bottle and allowed to stand in a refrigerator for a week. The water extract was examined by thin layer chromatography in solvent system in a ratio of distilled water: silica gel (2:1) and it's R_f values (measure of the position of a component in a chromatographic separation) was measured. Finally, the extract was applied for the efficacy test against *Salmonella* sp. under *in-vitro* condition.

Hot extraction with hexane

One hundred gram of the experimental arjun bark powder was taken in a thimble and set in Soxhlet apparatus with addition of 500 ml hexane. The extraction procedure was continued for 48 hrs. After that, the crude material was taken out and stored in plastic bottle and the volume of the extract was concentrated under reduced pressure using a rotary evaporator. The compound present in the crude extract was examined by thin layer chromatography (TLC) solvent system in a ratio of distilled water: silica gel (2:1). The R_f value of the crude extract was measured and applied for the efficacy test against *Salmonella* sp. under *in-vitro* condition.

Collection of bacteria

Water samples for bacteriological examinations were collected from 15 aquaculture ponds located adjacent to BAU (Figure 1 (A) and (B)). About 200 mL water was collected from the mid-level of ponds in sterile plastic bottles, transported to Fish Disease Laboratory, BAU, serially diluted using 0.85% sterile physiological saline, plated on *Salmonella Shigella* Agar (SS Agar, Hi-media) and incubated at 25°C for 48 h. Single colonies were selected and streaked on the same medium; all colonies on the same plates were phenotypically identical and considered for pure culture. After performing routine biochemical identification methods, pure culture was stored using Tryptone Soya Broth (TSB, Hi-media) and 25% glycerol (v/v) (Merck) in 1.8 mL cryo-tubes (Thermo) at -20ºC until required for further use.

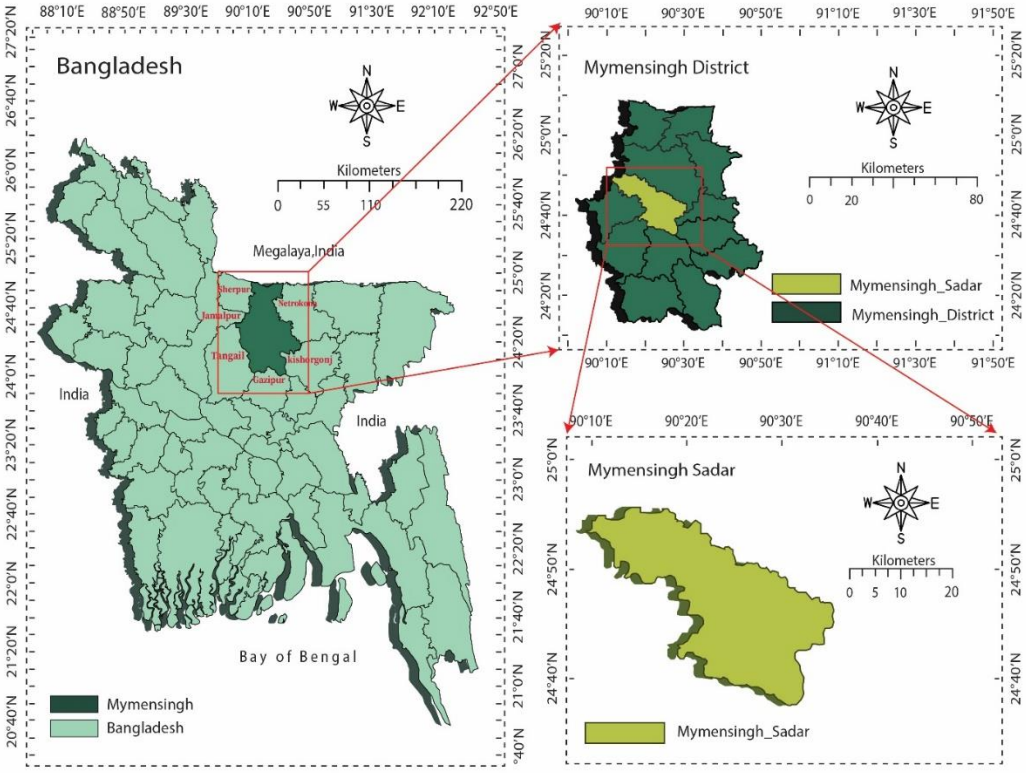


Figure 1(A). Map of study area from where the water sample were collected, Sadar Upazila, Mymensingh.

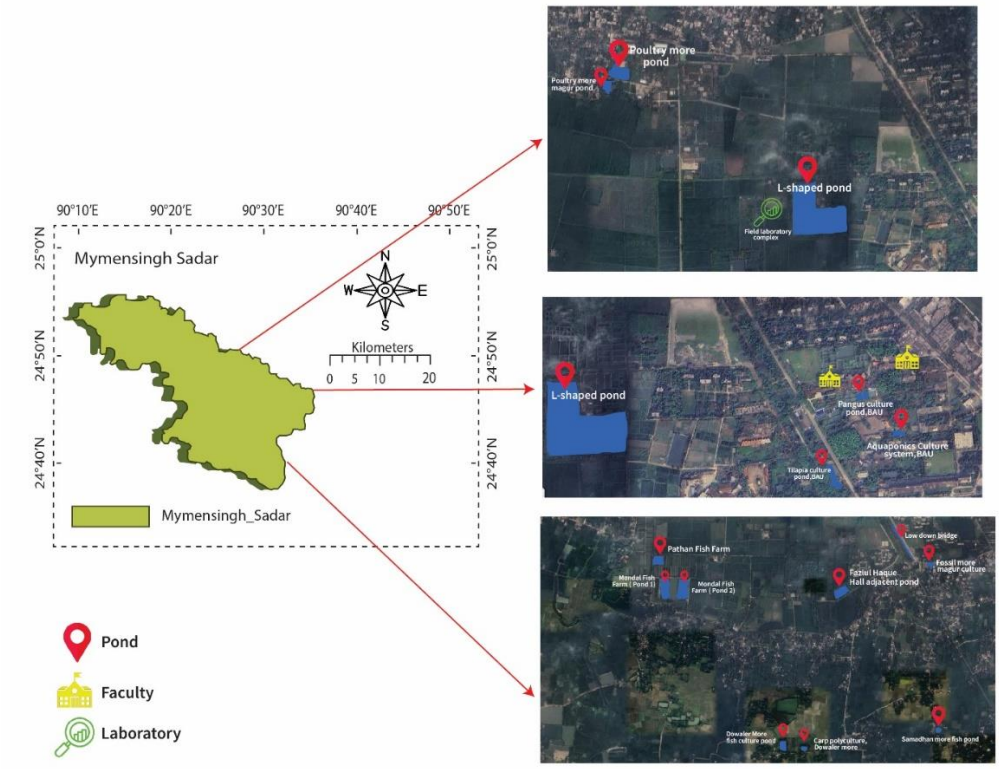


Figure 1 (B). Geographical location of the fish farms of the selected area.

Biochemical identification of *Salmonella* sp.

Identification of bacteria was carried out based on the morphological and biochemical tests. All the isolated bacterial cultures were subjected for Gram's staining using Grams stain kit (Hi-media). Biochemical identification of two isolates, marked as Sa-2 and Sa-5 were performed using Hi Assorted™ Biochemical Test kit (Hi-media), a standardized colorimetric identification system utilizing seven conventional biochemical tests and five carbohydrate utilization tests. The tests were based on the principle of pH change and substrate utilization.

Pure culture of Sa-2 and Sa-5 were done on the Blue green Agar (Hi-media) at 37°C for 24 h. Freshly cultured bacterial cells were suspended in 0.85% sterile physiological saline and the suspension was adjusted to slightly more than 0.1 OD at 620 nm. Hi Assorted™ Biochemical Test kits (Hi-media) (http://www.himedialabs.com/intl/en/products/Microbiology/Ready prepared-Media-Biochemical-Identification-Kit/HiAssorted%E2%84%A2-Biochemical-Test-Kit-KB002) were opened aseptically and 50 µl of the above inoculum was inoculate each well by surface inoculation method. The kit, containing bacterial sample was incubated at 37°C for 24 h. On incubation, organisms underwent metabolic changes which were indicated by a color changes in the media that could be either interpreted visually or after addition of the prescribed reagents. Identification was confirmed by comparing the biochemical reaction results of Sa-2 and Sa-5 and the identification index (http://himedialabs.com /TD/KB002.pdf) provided with kit.

Thin layer chromatography

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 ratios. This was manually spread very thinly on clear glass plates. A number of solvent chambers were prepared by solvents from low to high polarity and by mixing solvents of expected polarities. To obtain a chromatogram, a minute drop of *T. arjuna* bark extract (cold water extract and hexene extract) was applied with a capillary tube on a bare line along the bottom of the plate. This plate was applied in a TLC Tank containing a selected carrier solvent and mixed solvents. The samples exhibited spots on the TLC plate in ordinary conditions. After the run plates are dried and sprayed freshly prepared iodine reagents were used to detect the bands on the TLC plates. The movement of the active compound was expressed by its retention factor (R_f).

Antibiogram study

Antibiotic sensitivity test

Sa-2 and Sa-5 were tested for its antibiotic-resistance by disc diffusion method (Bauer et al., 1966) on TSA. The antibiotics (Hi-media) were 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) and resistant (R) based on the size of the zone of bacterial growth inhibition.

Determination of antibacterial activity of bark extracts

Bacterial inoculum was spread on TSA plates using sterile glass rods. About 5 mm diameter wells were prepared in the TSA plates (4 wells in a TSA plate). Bark extracts were dropped using micropipette, dried and incubated by upside down for 24 h at 37°C. The inhibition zone around the wells indicated absence of bacterial growth and was reported as positive. Absence of zone indicated as negative.

Data analysis

Collected data was analyzed using Microsoft Excel 2010 software.

RESULTS

Collection of bacterial sample

The total heterotrophic bacteria and *Salmonella* sp. in selected aquaculture pond water are presented here in figure 2. The selected ponds Poultry more pond, Poultry more magur culture pond, L-Shaped pond field fisheries complex, Low down bridge pond, Fossil more magur culture pond, Fazlul Haque Hall adjacent pond, Pathan fish farm, Mondal fish farm (pond-1), Mondal fish farm (pond-2), Pangus culture pond, BAU, Tilapia culture pond, BAU, Aquaponic culture system, Carp polyculture pond, Dowaler more, Samadhan more fish pond and Dowaler more fish culture pond were numbered as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 and 15 respectively. Among the selected ponds the highest load of both total heterotrophic bacteria and *Salmonella* sp. were recorded in poultry more *Clarias batrachus* culture pond (pond no 2) while the lowest load (< 10) of *Salmonella* sp. were observed in pond no. 3, 6, 7, 8, 9 and 13 (Figure 2).

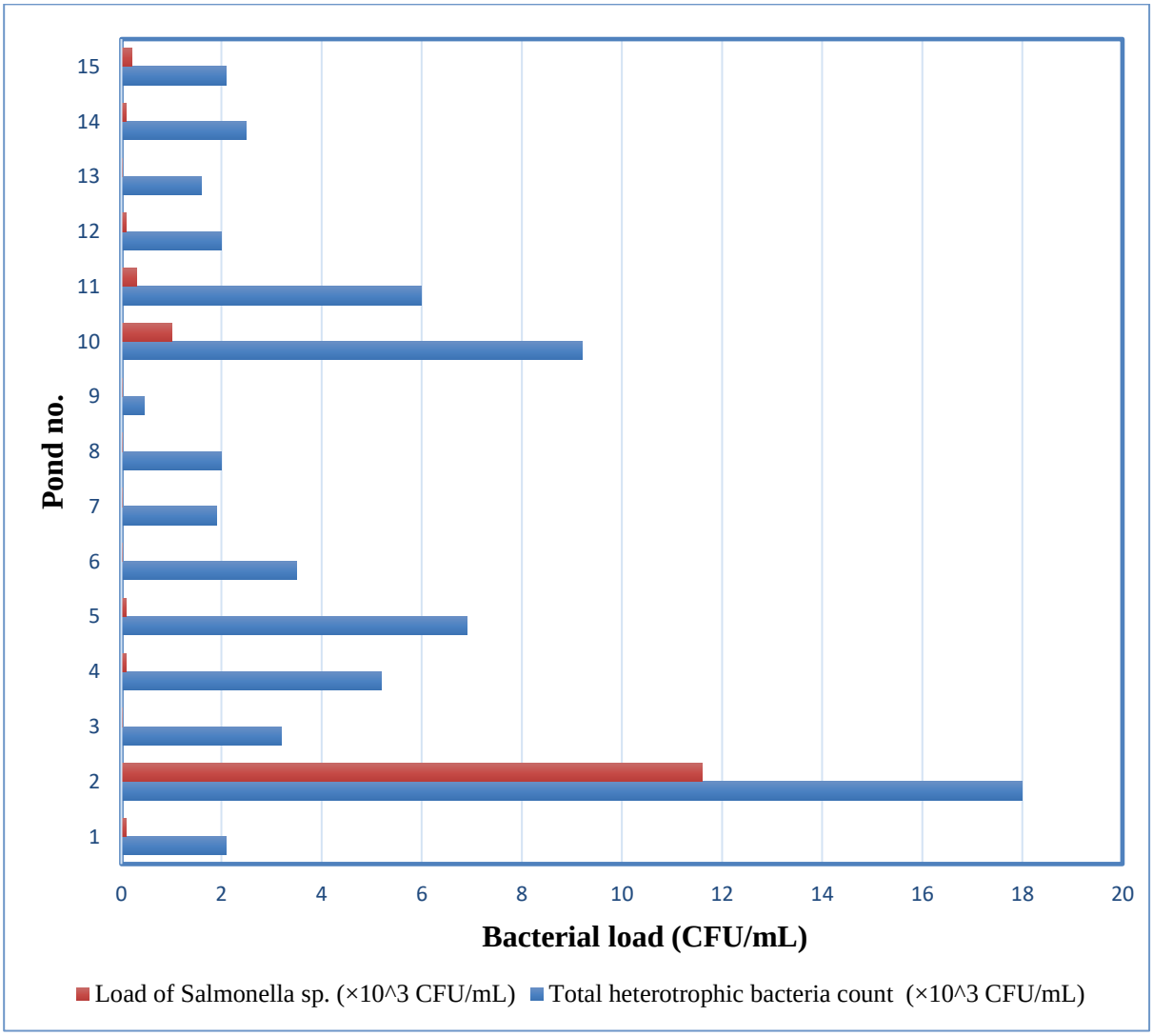


Figure 2. Summary of averages load of total heterotrophic bacterial and *Salmonella* sp from collected aquaculture pond water

Morphological and biochemical analysis of bacterial isolates

Among the isolated bacteria, fifteen isolates (Sa-1 to Sa-15) were selected for further analysis and subjected for Gram's staining using Grams stain kit (Hi-media). Two (Sa-2 and Sa-5) out of fifteen isolates were approved as *Salmonella* which optimum growth temperature was found 37˚C, positive for catalyse test, citrate utilization, nitrate reduction, H_2S production, arabinose and production of acid form glucose and sorbitol (Table 1). Negative reactions were observed in the cases of indole production, Voges-Proskauer reaction, Ornithine decarboxylase and fermentation of lactose, (Table 1). Further, 3 isolated bacterial cultures were subjected using Hi Assorted™ Biochemical Test kit (Hi-media) to confirm these isolates as *Salmonella* sp. Identification was confirmed by comparing the biochemical reaction results of Sa-2 and Sa-5 and the identification index (http://himedialabs.com /TD/KB002.pdf) provided with kit (Figure 3 (A), (B), (C) and (D)) and results indicated the isolates Sa-2 and Sa-5 are *Salmonella* sp. (Table 1).

Figure 3 (A). Hi Assorted™ Biochemical Test kit (Hi-media) before inoculation of experimental bacterium.

1	2	3	4	5	6	7	8	9	10	11	12
+	+	+	+	-	+	+	-	-	-	+	+

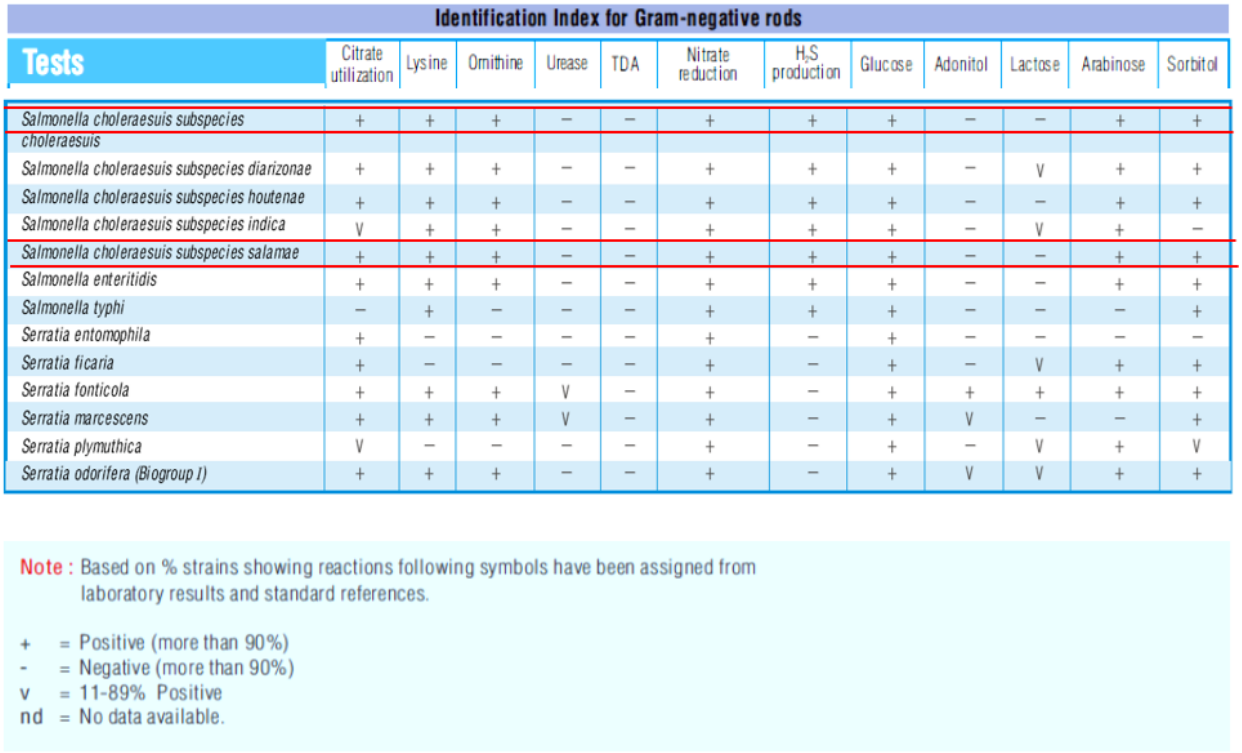
Figure 3 (B). Hi Assorted™ Biochemical Test kit (Hi-media) after inoculation of experimental bacterial suspension followed by incubation at 37ºC for 24 h. *Salmonella* sp. Sa-2 was identified by comparing the visible colour change (due to biochemical reaction) and the identification index provided with kit. (+) and (-) indicating positive and negative reactions.

1	2	3	4	5	6	7	8	9	10	11	12
+	-	+	+	-	+	+	+	-	+	+	+

Figure 3 (C). Hi Assorted™ Biochemical Test kit (Hi-media) after inoculation of experimental bacterial suspension followed by incubation at 37ºC for 24 h. *Salmonella* sp. Sa-5 was identified by comparing the visible colour change (due to biochemical reaction result) and the identification index provided with kit. (+) and (-) indicating positive and negative reactions.

Note:

Well 1: Medium for Citrate utilization Test; Well 2: Medium for Lysine utilization Test
Well 3: Medium for Ornithine utilization Test; Well 4: Medium for Urease detection Test
Well 5: Medium for Phenylalanine deamination Test; Well 6: Medium for Nitrate reduction Test
Well 7: Medium for H_2S Production Test; Well 8-12: Medium for Carbohydrate Utilization Test (with five different sugars in respective wells as Glucose, Adonitol, Lactose, Arabinose, Sorbitol).



Identification Index for Gram-negative rods

Tests	Citrate utilization	Lysine	Ornithine	Urease	TDA	Nitrate reduction	H_2S production	Glucose	Adonitol	Lactose	Arabinose	Sorbitol
Salmonella choleraesuis subspecies choleraesuis	+	+	+	−	−	+	+	+	−	−	+	+
Salmonella choleraesuis subspecies diarizonae	+	+	+	−	−	+	+	+	−	V	+	+
Salmonella choleraesuis subspecies houtenae	+	+	+	−	−	+	+	+	−	−	+	+
Salmonella choleraesuis subspecies indica	V	+	+	−	−	+	+	+	−	V	+	−
Salmonella choleraesuis subspecies salamae	+	+	+	−	−	+	+	+	−	−	+	+
Salmonella enteritidis	+	+	+	−	−	+	+	+	−	−	+	+
Salmonella typhi	−	+	−	−	−	+	+	+	−	−	−	+
Serratia entomophila	+	−	−	−	−	+	−	+	−	−	−	−
Serratia ficaria	+	−	−	−	−	+	−	+	−	V	+	+
Serratia fonticola	+	+	+	V	−	+	−	+	+	+	+	+
Serratia marcescens	+	+	+	V	−	+	−	+	V	−	−	+
Serratia plymuthica	V	−	−	−	−	+	−	+	−	V	+	V
Serratia odorifera (Biogroup I)	+	+	+	−	−	+	−	+	V	V	+	+

Note : Based on % strains showing reactions following symbols have been assigned from laboratory results and standard references.

\+ = Positive (more than 90%)
\- = Negative (more than 90%)
v = 11-89% Positive
nd = No data available.

Figure 3 (D). Identification index (http://himedialabs.com/TD/KB002.pdf) provided with the Hi Assorted™ Biochemical Test kit (Hi-media) exhibited that *Salmonella* sp. (Sa-2 and Sa-5,) are closely related to *Salmonella choleraesuis* subspecies

Thin layer chromatography

In order to determine the retention factor (R_f), the plate was applied in a TLC Tank containing a selected carrier solvent and mixed solvents. The samples exhibited spots on the TLC plate in ordinary conditions. After the run plates were dried and sprayed freshly prepared iodine reagents were used to detect the bands on the TLC plates. The retention factor (R_f), values were calculated for cold water extract and hexane extract by using the following formula: The retention factor (R_f), values were calculated for cold water extract and hexane extract by using the following formula:

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

Here the R_f value indicate that Arjun bark extract has compound to inhibit microbial growth (Table 2).

Antibiotic sensitivity

Among the fifteen isolated three bacterial cultures were subjected to antibiotic sensitivity test. The applied antibiotic and herbal extracts were exposed their inhibitory effect on pathogenic bacteria *Salmonella* sp. on TSA agar plate at 24 hrs. It is observed that azithromycin (10 µg), streptomycin (25 µg) ciprofloxacin (10 µg) and cefotaxime (30 µg) showed 33.33%, erythromycin (15 µg), tetracycline (10µg) and doxycycline (30 µg) showed 66.67%, ampicillin (10 µg) showed 100%, and kanamycin (30 µg) and gentamycin (10 µg) showed 0% (Figure 5 and 6). Hexane extracts of *T. arjuna* bark powder showed sensitivity against isolated bacterial cultures whereas cold-water extract showed resistant against isolated bacterial cultures (Figure 5 and 6).

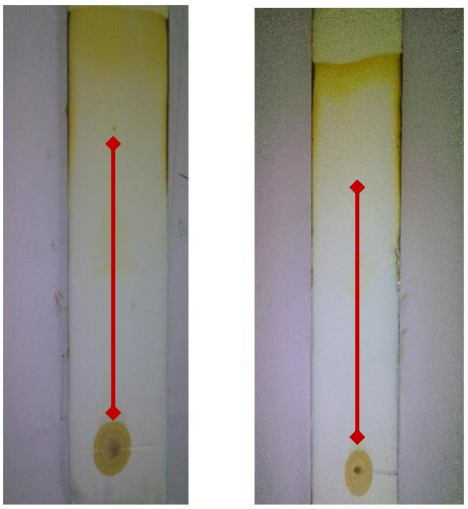


Figure 4. (A) TLC in Cold water extract, (B) TLC in hexane extract.

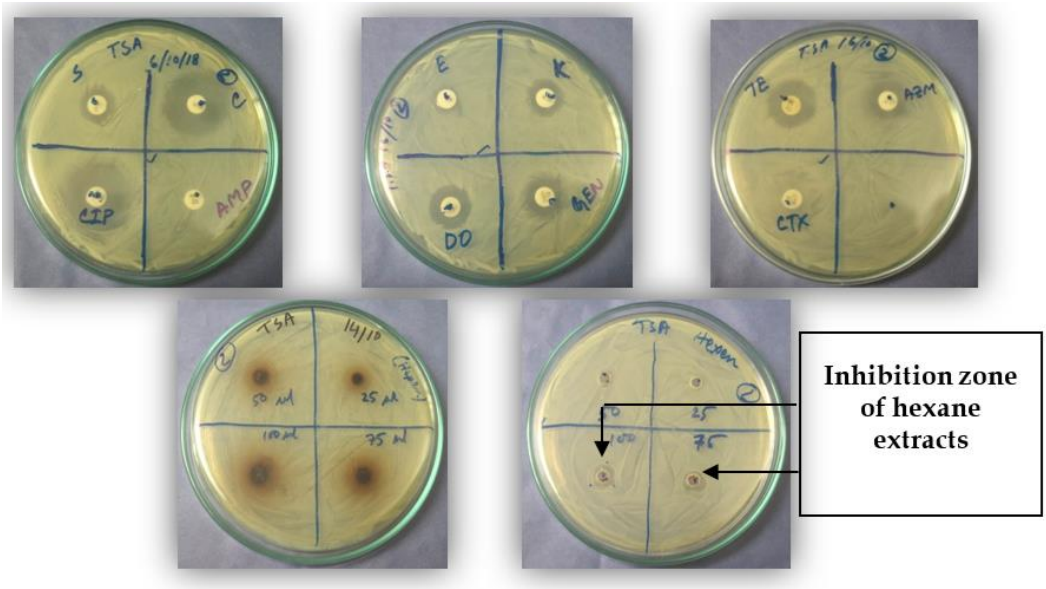


Figure 5. Antibiogram study of *Salmonella* sp. (Sa-2) using drug discs and *T. arjuna* bark extracts.

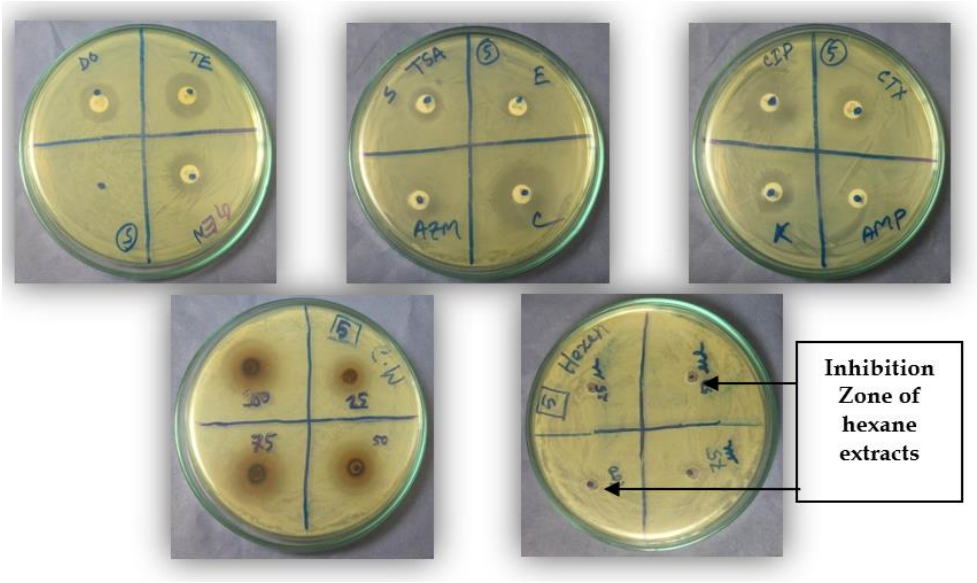


Figure 6. Antibiogram study of *Salmonella* sp. (Sa-5) using drug discs and T. *arjuna* bark extracts.

Table 1. Biochemical test of *Salmonella* sp. using Grams staining (Hi-media)

Characteristics	***Salmonella sp.* (Sa-2)**	***Salmonella sp.* (Sa-5)**
Pigment	Colorless with black center (in SS Agar)	Colorless with black center (in SS Agar)
Gram stain	–	–
Cell morphology	Cocci	Cocci
Growth on TSA	+	+
Growth on MRS Agar	+	+
Growth on cetrimide agar	–	–
Growth on TSA at:		
4˚C	–	–
37˚C	+	+
42˚C	+	+
Indole production	–	+
Methyl red	ND	ND
Voges-Proskauer	–	+
Catalase production	+	+
Coagulase test	ND	ND
Citrate utilization	+	+

Table 1. Biochemical test of *Salmonella* sp. using Grams staining (Contd.)		
Lysine decarboxylase	+	–
Ornithine decarboxylase	V	V
Urease	+	+
Nitrate production	+	+
H_2S production	+	+
Glucose	–	+
Adonitol	–	–
Lactose	–	+
Arabinose	+	+
Sorbitol	+	+

(+) = positive reaction, (-) = negative reaction, V = variable (11% to 89% positive), ND = Data not available

Table 2. R_f value of the extracted sample

Extracting solvent	TLC solvent system	R_f value	Probable compound present in the crude extract
Cold water extract	Distilled water: Sillica (2:1)	4/12.4 = 0.322	**1**
Hexane extract	Distilled water: Sillica (2:1)	3.8/11.1 = 0.28	**1**

Table 3. Sensitivity of *Salmonella* sp. (Sa-2) and (Sa-5) to various antibiotics and experimental herbal agent with radius of inhibition zone

Name of organisms	Chloramphenicol (30 µg)	Streptomycin (10 µg)	Azithromycin (15 µg)	Ampicillin (25 µg)	Erythromycin (15 µg)	Cefotaxime (30 µg)	Kanamycin (30 µg)	Doxycycline (30 µg)	Gentamycin (10 µg)	Ciprofloxacin (5 µg)	Tetracycline (30 µg)	Cold water extract	Hexane extract
Sa-2	S	S	S	R	R	R	S	S	S	S	S	R	S
Radius of inhibition zone(mm)	13	5	7	NA	NA	NA	6	10	10	11	10	NA	7
Sa-5	S	S	S	R	R	S	S	R	S	R	R	R	S
Radius of inhibition zone(mm)	11	6	9	NA	NA	10	8	NA	8	NA	NA	NA	6

S = Sensitivity, R = Resistant, NA = Not Applicable

DISCUSSION

Contribution of aquaculture to the global supply of fish, crustaceans, mollusks, and other aquatic animals has increased dramatically in recent few years. People are now following various strategies to maintain good health of fish and farm. Among with the increased aquaculture production, it's also increased the risk factor for consumers due to the inappropriate use of drugs and antibiotics in aquaculture. Various antibiotics and growth promoting agents are widely used in aquaculture, livestock and poultry rearing facilities in Bangladesh without proper awareness about their application. Multi drug resistance is now a great threat for aquaculture system. Frequent use of inappropriate doses of drug increased the drug resistant capacity of bacteria which affect total production as well as causes to great loss in aquaculture sector (Gastalho et al*.,* 2014). In this study, the highest load of *Salmonella* sp. was recorded as 1.16×10^4 CFU/mL in pond number 2. Olugbojo et al., (2015) recorded 5×10^3 CFU/mL *Salmonella* sp*.* in pond water which was in within optimum range according to ICMSF (1986). Thus, *Salmonella* sp. content of every pond water, except pond number 2, were within safe limit.

In the present study, efficacy of Arjun bark powder was observed against multi drug resistance *Salmonella* sp. and drug-resistant capacity of this species was identified against various commercial drug. Similar type of study was carried out by Setti et al., (2009*)*, a total of isolates of 28 *Salmonella* sp. showed resistance to ampicillin (22 isolates), nalidixic acid (9 isolates), sulfonamide compounds (2 isolates) and tetracycline. And six isolates showed resistances to two antimicrobial substances. Similar study was carried out by Elhadi et al., (2014) where a total of 140 *salmonella* sp. were isolated and the isolates were tested for susceptibility to 18 selected antimicrobial agents. Overall antimicrobial resistance pattern indicated that most isolates were resistant to streptomycin 10 (43.5%), sulfamethoxazole 8 (34.8%) and trimethoprim 5 (21.7%), which constitutes a serious health risk for humans.

All the experimental *Salmonella* sp. were found resistant to several antibiotics but susceptible to the crude extracts of medicinal plants *T. arjuna* in the present study. Resistance of *Salmonella* sp. to various antibiotics such as chloramphenicol, azithromycin, erythromycin, tetracycline, ampicillin, doxycycline, gentamycin, ciprofloxacin, streptomycin, cefotaxime, and kanamycin has been reported (Fig. 5 and 6) where azithromycin (10µg), streptomycin (25µg), ciprofloxacin (10µg) and cefotaxime (30µg) showed 33.33%, erythromycin (15µg), tetracycline (10µg) and doxycycline (30µg) showed 66.67%, ampicillin (10µg) showed 100%, and kanamycin (30µg) and gentamycin (10µg) showed 0% resistant to isolated *Salmonella* sp. (Figure 5 and 6). Besides, all the *Salmonella* sp. isolates were found to be 100% resistant to cold water extract but 100% sensitive to hexane extract of *T. arjuna*, which is very rare for this pathogen. Hexane extract of *T. arjuna* exhibited inhibition zone of 7 mm, 6 mm and 6 mm respectively and revealed maximum inhibition zone against the Sa-2 isolate (Figure 5). So, it can be said that hexane extract of *T. arjuna* is more effective than some antibiotics against *Salmonella* sp. isolates.

One of the interesting findings of this study is that the multiple antibiotic-resistant strains of *Salmonella* sp. showed susceptibility to organic extracts of herbal medicines of *T. arjuna*. Thus, it can be opined that some compounds are present in the hexane extract of the bark of *T. arjuna* that shows the antimicrobial activity. Sadika et al., (2012) showed that antimicrobial screening of water extracts of *T. arjuna* did not show any antimicrobial activity at 30 µg/mL, 100 µg/mL, 300 µg/mL, 500 µg/mL, 1000 µg/mL and 1500 µg/mL concentrations. But methanol and ethanol extracts showed antimicrobial activity at 1000 µg/mL and 1500 µg/mL concentrations against all test microorganisms. This may be one of the probable reasons of cold-water extract of *T. arjuna* not to respond against the isolates in this study. On the other hand, result of hexane extracts of these medicinal plants displayed higher inhibitory activity against *Salmonella* sp. compared to aqueous cold-water extracts, indicating that the active compounds are relatively polar secondary metabolites. This hypothesis is supported by the inhibitory effects of herbal extracts of the medicinal plants against the *Salmonella* sp. *in vitro*. Methanol and ethanol extracts of bark of *T. arjuna* contain phenols, flavonoids, tannin, saponin, alkaloids, glycosides, phytosterols and carbohydrate. Hexane extract contains alkaloids and

phytosterols while chloroform extract contains flavonoids and alkaloids (Sadika et al., 2012). Alkaloids are a big and structurally diverse group of secondary metabolites that can be found in around 300 plant families. According to the chemical structure there are two broad divisions in the classification. The first division contains the non-heterocyclic or atypical alkaloids, also called protoalkaloids or biological amines, such as hordenine or N-methyltyramine and colchicine (an antibiotic). The second division includes the heterocyclic or typical alkaloids such as hygrines belonging to the pyrrole and pyrrolidine group and quinine belonging to the quinolone group and quinolone group (Evans, 2009). Alkaloids are known in both traditional and modern medicine to have several pharmacological activities (Cushnie et al., 2014). The antibacterial activity of the alkaloids found in the ethanolic extract of *Datura stramonium* was tested using the agar well diffusion method against *E. coli*, *P. aeruginosa*, *S. aureus*, *Proteus mirabilis*, and *K. pneumonia* (Altameme, 2015). Quinones also have antimicrobial activity that comes from their ability to donate free radicals. They can also form irreversible complexes with amino acids in proteins, thus inactivating them. These properties of quinones make it possible for them to attack surface adhesions, polypeptides in the cell wall, and membrane enzymes of microorganisms (Cowan, 1999). The hexane extract of *T. arjuna* provided significant recovery of animal as well as human against the infection by the most virulent strains of *Salmonella* sp., which exhibited equivalent efficacy to azithromycin, chloramphenicol, ciprofloxacin, tetracycline and also other commercial bacterial drug (Sudhir et al., 2016).

These findings indicate that medicinal plant extracts could be used as a natural alternative to the synthetic antibiotics to control *Salmonella* sp. infection in fish and human. As crude extracts contain multiple secondary metabolites, chances of the development of resistance against crude plant extracts are likely to be lesser than those of pure antibiotics. Its potential application in the treatment of bacterial infection in aquaculture of Bangladesh would therefore be promising. This work will lead to develop herbal drugs from the plant *T. arjuna* for the antibacterial activity against *Salmonella.* Taken together, results suggest that medicinal plant extracts could be used as a potential alternative to the synthetic antibiotics to control diseases cause by *Salmonella* sp. in aquaculture as well as human. Further detailed investigation is required to elucidate the mode of action of *T*. *arjuna* crude extracts in preventing and/or recovering fish disease caused by *Salmonella* sp.

CONCLUSION

This initial work will lead to develop herbal drugs from the plant *T. arjuna* for the antibacterial activity against *Salmonella* sp. As crude extracts contain multiple secondary metabolites, chances of the development of resistance against crude plant extracts are likely to be lesser than those of pure antibiotics. Taken together, results suggest that medicinal plant extracts could be used as a potential alternative to the synthetic antibiotics in controlling infection cause by *Salmonella* sp. in aquaculture as well as human. Further detailed investigation is required to elucidate the mode of action of *T*. *arjuna* crude extracts in preventing and/or recovering fish disease caused by *Salmonella* sp.

ACKNOWLEDGEMENTS

Authors are thankful to the respective authority of the Department of Agricultural Chemistry and Department of Aquaculture, Bangladesh Agricultural University, Mymensingh 2202.

CONFLICT OF INTEREST

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.

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.

Author's contribution

Sohidul Islam: Implementation of the study, sample collection and preparation, antibiotic sensitivity test and initial manuscript drafting; **Mr. Kartik Chandra Saha:** Conceptualization, experimental designing, manuscript review and editing; **Naima Sultana:** Literature review, Methodology, investigation and data recording; **Md. Raimur Rahman:** Investigation and data recording; **Johir Raihan:** Investigation and data recording; **Kaniz Farjana:** Investigation, data collection and recording; **Rakiba Binta Raihan:** Data collection and recording; **Tamanna Tabassum:** Data correction, formal analysis and organizing the manuscript; **Sourav Acharjee:** Reviewing the manuscript; **Shadman Shakib Shovon:** Data analysis and preparing the manuscript; **Salman Shahriar Nibir:** Reviewing and organizing the manuscript**; Khadiza Tul Kubra:** Reviewing and editing the manuscript; **Tanvir Rahman:** Conceptualization, experimental designing, supervision, funding acquisition, project administration, resource management, manuscript review and editing.

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