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Research Article

Antimicrobial Potential of Selected Plant Extracts Against *Listeria monocytogenes* in Fish and Processing Environments

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Pathogen Control

Abstract

Background: *Listeria monocytogenes* is a serious food-borne pathogen of global significance responsible for listeriosis in humans, generally associated with Ready-To-Eat foods (RTE). This study investigated the inhibitory potential of extracts from *Terminalia chebula* and *Embelia ribes* against *L. monocytogenes in-vitro*, in seafood and on food processing surfaces.

Materials and methods: Aqueous and ethanolic extracts were prepared from the fruits of *Terminalia chebula* and *Embelia ribes*. The antimicrobial activities the extracts were tested by disc diffusion and agar well diffusion assays. The Minimum Bactericidal Concentration (MBC) was determined using a microplate dilution assay and the minimum bactericidal concentration was also determined. The extracts were tested for their anti-biofilm activities on steel and plastic surfaces. Tuna meat artificially spiked with *L. monocytogenes* was dip-treated with plant extract to determine the effect on *L. monocytogenes* populations during storage at 2°C.

Results: Ethanolic extract of *E. ribes* and aqueous extract of *T. chebula* showed the highest anti-*L. monocytogenes* activity with a Minimum Bactericidal Concentration (MBC) of 12.5 mg/ml. Significant reductions in the biofilm-forming ability of *L. monocytogenes* on both plastic and stainless steel surfaces were achieved with ethanolic extract *E. ribes* and aqueous extract of *T. chebula*. This extract, applied to tuna slices as a dip treatment, could reduce *L. monocytogenes* counts by over 2.6 log CFU.

Conclusion: This study provides strong evidence for the anti- *L. monocytogenes* activities of plant extracts, which can be potentially useful in controlling *L. monocytogenes* in foods and food processing environments.

Keywords: *Listeria monocytogenes; Terminalia chebula; Embelia ribes;* Biofilm; Antimicrobial;

Introduction

L. monocytogenes is a Gram-positive, facultatively anaerobic, non-spore-forming rod-shaped bacterium, a natural inhabitant of the soil, water and decaying plant materials [1,2]. *L. monocytogenes* is also an important food-borne pathogen causing infections of varying intensities in the elderly, pregnant women, neonates and immunocompromised individuals [3]. *Listeria* infections manifest as septicemia, meningitis, encephalitis and in pregnant women, listeriosis may result in miscarriages or stillbirths [4-6]. In the United States alone, *L. monocytogenes* is responsible for about 1,600 illnesses each year, with over 94% hospitalization and 15-20% death [7]. In 2010, the global burden of listeriosis was estimated at 23,150 cases and 5,463 deaths [8]. Listeria outbreaks are most frequently linked to Ready-To-Eat (RTE) foods, including deli meats, scalded sausages, hot dogs, RTE salads, soft cheeses (particularly those made from unpasteurized milk), smoked seafood and contaminated fruits and vegetables [9,10]. RTE fishery products have been the sources of *Listeria* outbreaks, including cold-smoked and gravad fish, which are modified atmosphere packaged [11-13]. The incidence of *L. monocytogenes* in fish and fishery products has been reported from India [14-18].

Due to its ubiquitous nature, broad host range and the ability to proliferate at different environmental conditions, including refrigerated temperatures, *L. monocytogenes* is a successful foodborne pathogen and a serious threat to food safety. The bacterium is able to grow at broad ranges of temperatures (0.4°C to 45°C) and pH conditions (4.3-9.5) and survive at relatively low water activity (as low as 0.90) and high salt concentrations (up to 10%) [5,19]. The capacity of *L. monocytogenes* to withstand harsh environmental conditions, along with its ability to colonize, multiply and persist on processing equipment, poses a significant challenge to the food industry. *Listeria* has a strong ability to attach to stainless steel, glass, plastic and rubber to produce biofilms, which are resistant to commonly employed sanitizers and biocides, posing a threat to food safety [20-22]. *L. monocytogenes* has been isolated from various food processing surfaces and equipment, including saws and tables in slaughterhouses, the deck of fishing vessels, the floor of fish landing centers, plate freezers and food handlers from fish processing plants [23,24].

Considering the numerous outbreaks, product recalls and fatalities associated with *L. monocytogenes* contamination, preventing the entry, growth and proliferation of this pathogen in foods is of critical importance. Natural antimicrobials of bacterial and plant origin are potentially useful in controlling *L. monocytogenes* in foods and food contact surfaces. The interest in natural compounds as food preservatives is increasing, which has led to research efforts focused on identification, extraction and food applications of compounds from natural origins that have better bacteriostatic/bactericidal activities and offer consumer safety than their synthetic counterparts. Many compounds from plant sources have been effectively used as natural food preservatives to produce a microbiologically safe product with retained quality attributes [25,26]. Several natural compounds, like bacteriocins, organic acids, essential oils, chitosan, etc., have antimicrobial properties against *Listeria spp.* In this context, the goal of the present study was to identify new antimicrobials of plant origin for the control of *Listeria monocytogenes* in fish and processing surfaces.

Materials and Methods

Plant Materials and Preparation

Terminalia chebula and *Embelia ribes* used in this study were collected from Dakshina Kannada District, Karnataka, India. Based on their applications in traditional Indian medicine, the fruits of these plants were used for solvent extraction. The fruits were cleaned, dried under shade at room temperature for 24 hours and ground in a mixer into a fine powder.

Preparation of Aqueous Extracts of Plants

One-gram of powdered plant material was soaked in 10 ml of distilled water and incubated at room temperature (28 ± 2°C) for one week with intermittent shaking. The extracts were then centrifuged at 5000 rpm and the resulting supernatant was filtered using a 0.2 μm syringe filter. The final extract was used at a 100 mg/ml concentration for subsequent testing.

Preparation of Ethanol Extracts

Ethanol extracts were prepared by soaking powdered plant materials in 95% ethanol (1 g per 10 ml) for one week with continuous shaking. Afterwards, the crude ethanolic extracts were obtained by evaporating the solvent to dryness at 50°C under reduced pressure using a rotary evaporator (Steroglass, Germany). The resulting residues were resuspended in Dimethylsulphoxide (DMSO) to achieve a final concentration of 100 mg/ml (w/v), filtered through a 0.2 μm syringe filter and stored at room temperature until further use [27].

Antimicrobial Susceptibility Testing

Bacterial Strains and Culture Conditions

Listeria monocytogenes strains MTCC 1143, MTCC 657, ATCC 19111 were used in this study. The isolates were preserved on Brain Heart Infusion (BHI) agar slants at 4°C (HiMedia, Mumbai, India) and broth cultures were subsequently prepared in Tryptic Soy Broth (TSB).

Agar Well Diffusion Method

The agar well diffusion method was performed as described previously [28]. Briefly, *L. monocytogenes* cultures were grown in TSB (HiMedia, Mumbai, India) to 0.5 McFarland units. The liquid culture was uniformly spread on Tryptone Soya Agar (TSA) plates using sterile cotton swabs. The plates were allowed to dry for 5 min inside a laminar flow. Wells measuring 6-8 mm in diameter were cut into the agar surface and 100 μl of aqueous and ethanol plant extracts were dispensed into each well. The plates were incubated upright at 37°C for 16-18 hours. Antibacterial activity was assessed by measuring the diameter of the inhibition zones around the wells, with a gentamicin (10 μg) disc (HiMedia, Mumbai, India) serving as the positive control.

Agar Disc Diffusion Method

The inhibitory activity of plant extracts against *L. monocytogenes* was determined by agar disc diffusion method as described previously, with minor modifications [29]. TSA plates were inoculated with *L. monocytogenes* as described in the previous section. Sterile 10-mm filter paper discs were loaded with 50 µl of plant extract and placed on the inoculated plates. The final concentration of extract tested was 5 mg/disc. Each disc was pressed down to ensure complete contact with the agar surface and distributed evenly so that they were at least 24 mm apart. The agar plates were then incubated at 37°C. After 16 to 18 hours of incubation, the diameters of the zones of inhibition were measured. Gentamycin was used as a control.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC was determined by the micro broth dilution method [28]. Bacterial strains were grown to an OD_{600} of approximately 0.5. The test antimicrobial solution was prepared and diluted to a 2X concentration. A volume of 100 µL of 2X BHI broth was dispensed into all wells of a sterile microtiter plate. Subsequently, 100 µL of the 2X antimicrobial solution was added to the first well in column 1 and mixed thoroughly by pipetting up and down 5-8 times. A serial two-fold dilution was performed across columns 1 to 10 by transferring 100 µL from one column to the next, mixing in each step and discarding 100 µL from column [10]. Sterile water (100 µL) was added to columns 11 and 12 to serve as controls. A *Listeria monocytogenes* inoculum was prepared at a concentration of 10^4 to 10^5 CFU/mL by dilution with broth or saline. Each well in columns 1 to 11 was inoculated with 5 µL of the bacterial suspension, while column 12 was left uninoculated to act as a sterility control and blank for plate reading. The plates were incubated at 37°C for 12-18 hours. Bacterial growth was recorded either manually or spectrophotometrically using an ELISA reader. The microbroth dilution assay used to determine the MIC of the plant extracts was also employed to assess their Minimum Bactericidal Concentration (MBC). Aliquots from each well of the microtiter plate were transferred onto TSA and incubated at 37°C for 24 h. The lowest concentration at which no visible bacterial colony growth was observed was recorded as the MBC of the extract against *L. monocytogenes.* This procedure was performed in triplicate to ensure reproducibility of the results.

Biofilm Assay Using Polystyrene Microtiter Plates

The ability of various *Listeria monocytogenes* strains to adhere to polystyrene surfaces was assessed using a microtiter plate assay as previously described [30]. Bacterial strains were cultured overnight in tryptic soy broth with 0.6% yeast extract (TSB-YE) without agitation. Overnight cultures were diluted 1:40 in fresh TSB-YE, vortexed and 100 µl of the suspension was dispensed into six wells of a microtiter plate. Wells containing only fresh medium served as blanks. The plates were incubated at 30°C for 40 hours. After incubation, wells were washed five times with sterile distilled water and air-dried for 30 min. Biofilm cells were stained with 1% freshly prepared crystal violet for 30 min at room temperature. Wells were then washed five times with 150 µl sterile distilled water, air-dried and destained with 200 µl of 95% ethanol at 8°C for 45 min. Finally, 100 µl of the destained solution was transferred to a fresh microtiter plate and absorbance was measured at 595 nm to quantify biofilm formation [22].

Inhibition of Biofilm Formation on Stainless Steel and Plastic Surfaces

The effect of plant extracts on biofilm formation on stainless steel and plastic surfaces was evaluated using a previously described procedure with minor modifications [31]. Stainless steel and plastic surface chips measuring 10 × 10 mm were used to investigate biofilm formation by *Listeria monocytogenes* (MTCC 1143). Before use, each chip was individually cleaned and sanitized by immersion in a neutral detergent for 1 hour, followed by rinsing with sterile distilled water, drying and subsequent cleaning with 70% (v/v) alcohol. After the sanitation process, the stainless steel chips underwent an additional sterilization step by autoclaving at 121°C for 15 minutes to ensure complete sterility before biofilm assays. *Listeria monocytogenes* (MTCC 1143) culture was used as the inoculum after establishing a viable cell concentration of 10^5 CFU/mL, determined through a growth curve constructed by correlating optical density with plate counts. Stainless steel and plastic chips (three chips per tube) were immersed in 5 mL of BHI broth. Plant extracts were added to each tube at concentrations equivalent to half of their respective MBCs to evaluate their inhibitory effects on biofilm formation by *L. monocytogenes.* Control tubes, containing chips without any plant extract, were prepared separately for each surface type. The broths were inoculated with 125 µL of the bacterial suspension (corresponding to a 1:40 dilution of the overnight culture) and incubated at 37°C for 48 hours without shaking to facilitate biofilm development.

After the 2-day incubation, the chips were carefully removed from each tube using sterile forceps and rinsed with sterile saline. Biofilms were scraped from the chips using sterile swabs, which, along with the chips, were transferred into tubes containing 9 ml physiological saline and vortexed for 2 min. Serial dilutions of the suspension were plated in duplicate on PCA and incubated at 37°C for 24 h. Colony-forming units (CFUs) were then enumerated to determine biofilm cell counts.

Storage Study of Tuna Slices Dip Treated with Extracts

Yellowfin tuna (Thunnus albacares), purchased from the local fish market in Versova, Mumbai, was cut into uniform slices weighing approximately 10g each and pasteurized by dipping in sterile water maintained at 90°C for 1 min to eliminate contaminating bacteria. Two extracts, which demonstrated the highest antimicrobial activity against *Listeria monocytogenes*, were selected for preparing the dip solutions. Aqueous solutions of the *Terminalia chebula* fruit extract and ethanolic extract of *Embelia ribes* fruit were prepared to dip the tuna slices. The concentration of the dip solution was adjusted to 12.5 mg/mL for both extracts, corresponding to their MBCs. The 10 g fish slices were then immersed in 100 mL of the prepared solution. After treatment, the slices were inoculated with *L. monocytogenes* by dipping them into a 100 mL cell suspension (10^8 CFU/mL) and placed in separate sterile plastic pouches. Control slices were treated with sterile water and spiked with the same bacterial suspension. All slices were stored at 2°C and sampling was performed at regular intervals to determine the counts of *L. monocytogenes.* For this, 10 g of tuna slice was homogenized with 90 ml sterile physiological saline (0.85% NaCl w/v) and 10-fold serially diluted in physiological saline. One-hundred microliters from each dilution was spread plated in duplicates on TSA, incubated at 37°C for 24 hours and the colony counts were determined as CFU/g.

Results

Preparation of Extracts

All the extracts were naturally coloured. Ethanol extracts were brightly coloured compared to their aqueous counterparts. Ethanol extracts could be easily resuspended in 100% DMSO, but this resulted in preparations that were more turbid compared to the original ethanol preparations. Filteration using 0.2 μm filter reduced the turbidity of aqueous extracts, but extracts in DMSO remained turbid. The extracts were active even after a month when stored at ambient temperature.

Antimicrobial Activities of Extracts

The extracts were tested for their antibacterial activity against *L. monocytogenes* (MTCC 1143) using both well and disc diffusion methods (Fig. 1). Disc diffusion test revealed maximum activity by *E. ribes* Ethanolic Extract (ERE), followed by *T. chebula* Water Extract (TCW). By the well diffusion method, the maximum Inhibition was exhibited by the extract TCW, followed by ERE. The details are shown in Table 1.

Minimum Bactericidal Concentrations (MBC) of Extracts Against L. monocytogenes

Both TCW and ERE exhibited an MBC of 12.5 mg/ml, whereas TCE was bactericidal at 25 mg/ml (Table 2). All three *L. monocytogenes* strains tested were inhibited at comparable MBCs, indicating no significant variation in strain sensitivity. Due to the color and turbidity of the extracts, visual determination of MIC was unreliable; therefore, subsequent experiments were conducted using MBC or ½ MBC concentrations of the extracts.

Effect on Biofilm-Forming Ability of L. monoctyogenes

Three strains of *L. monocytogenes*, viz., MTCC 1143, MTCC 657 and MTCC 1143, were tested for their ability to form biofilms on the polystyrene surface. MTCC 1143 exhibited better biofilm-forming ability compared to the other two strains (data not shown). Based on this, MTCC 1143 was selected to study further the effect of TCW and ERE on the biofilm-forming ability of this strain on steel and plastic surfaces.

Biofilm Formation on Plastic and Steel Surfaces and Its Inhibition

L. monocytogenes (MTCC 1143) formed biofilms on steel and plastic chips after 40 hours of incubation. Treatment with extracts at their ½ MBC significantly reduced the biofilm cell counts on both surfaces compared to the control. Both the extracts were effective against biofilm formation, with more than one log reduction in the biofilm cell counts (Table 3). A higher reduction of biofilm quantity was observed on the plastic surface than on steel (Fig. 2).

Dip Treatment of Tuna Slices with Plant Extracts

The extracts ERE and TCW, which showed maximum bactericidal activity, were chosen to give a dip treatment on Yellowfin tuna slices spiked with *L. monocytogenes.* The dip solutions were prepared with a final 12.5 mg/ml concentration for both extracts. A one-minute dip significantly (p=0.05) reduced the *L. monocytogenes* counts in both the treatments (Table 4). The decreasing trend in counts continued for an additional day (day 3) of storage at 2°C (Fig. 3). From day 3 onwards, the bacterial counts increased steadily in both treatments. Both the extracts were effective in reducing the count significantly compared to the control. Tuna slices treated with extract ERE showed fewer survivors than TCW, while the control slices did not show much difference in count throughout the storage period (Fig. 3).

Extracts (100 mg/ml)	Zones of Inhibition (mm)	
	Disc diffusion assay	Well diffusion assay
TCW	27.3 ± 0.57	27.0 ± 1.0
TCE	17.3 ± 0.57	18 ± 2.0
ERE	28.3 ± 0.57	25.6 ± 1.52
Gentamycin (10 µg)	22.6 ± 0.6	22.6 ± 0.6
TCW=*Terminalia chebula* Water extract; TCE=*Terminalia chebula* Ethanolic extract; ERE=*Embelia ribes* Ethanololic extract. Data represent means of three replicates (n=3) ± standard deviation.		

Table 1: Antimicrobial screening of plant extracts against *L. monocytogenes*.

Extracts	**MBC (mg/ml)**		
	L. monocytogenes MTCC 1143	*L. monocytogenes* MTCC 657	*L. monocytogenes* ATCC 19111
TCW	12.5	12.5	12.5
TCE	25	25	25
ERE	12.5	12.5	12.5

Table 2: Minimum Bactericidal Concentrations (MBCs) of plant extracts against L. monocytogenes.

Extract*	**Plastic Surface**		**Steel Surface**	
	Biofilm cell count/cm²	**Log reduction in cell number**	**Biofilm cell count/cm²**	**Log reduction in cell number**
Control**	2.19×10^5	-	1.90×10^5	-
TCW	1.95×10^4	1.05	1.82×10^4	1.02
TCE	4.06×10^4	0.73	4.92×10^4	0.59
ERW	1.68×10^4	1.12	1.62×10^4	1.07
ERE	9.16×10^3	1.38	1.17×10^4	1.21
* All extracts were used at their ½ MBCs ** Control without any extract.				

Table 3: Plant extract-mediated reduction in biofilm formation by *L. monocytogenes* (MTCC 1143) on plastic and steel surfaces.

Treatments	**Day 0**	**Day 1**	**Day 3**	**Day 5**	**Day 7**
ERE	$4.93 \pm 0.005^{aA*}$	4.57 ± 0.04^{bA}	4.79 ± 0.02^{cA}	5.47 ± 0.015^{dA}	5.68 ± 0.04^{eA}
TCW	5.02 ± 0.01^{aB}	4.65 ± 0.06^{bA}	4.93 ± 0.01^{aA}	5.92 ± 0.02^{cB}	6.32 ± 0.06^{dB}
Control	7.60 ± 0.01^{aC}	7.33 ± 0.04^{bB}	7.66 ± 0.06^{aB}	7.67 ± 0.01^{aC}	7.63 ± 0.02^{aC}
*Data represent means of three replicates (n=3) ± standard error. The statistical differences between bacterial counts across different sampling days with respect to ERC and TCW were determined using Duncan's Multiple Range Test. Values within a row with different superscripts (lower case letters) and values within a column with different superscripts (upper case letters) are significantly different (p=0.05).					

Table 4: Changes in *L. monocytogenes* counts of tuna slices dip-treated with plant extracts and stored at 2°C.

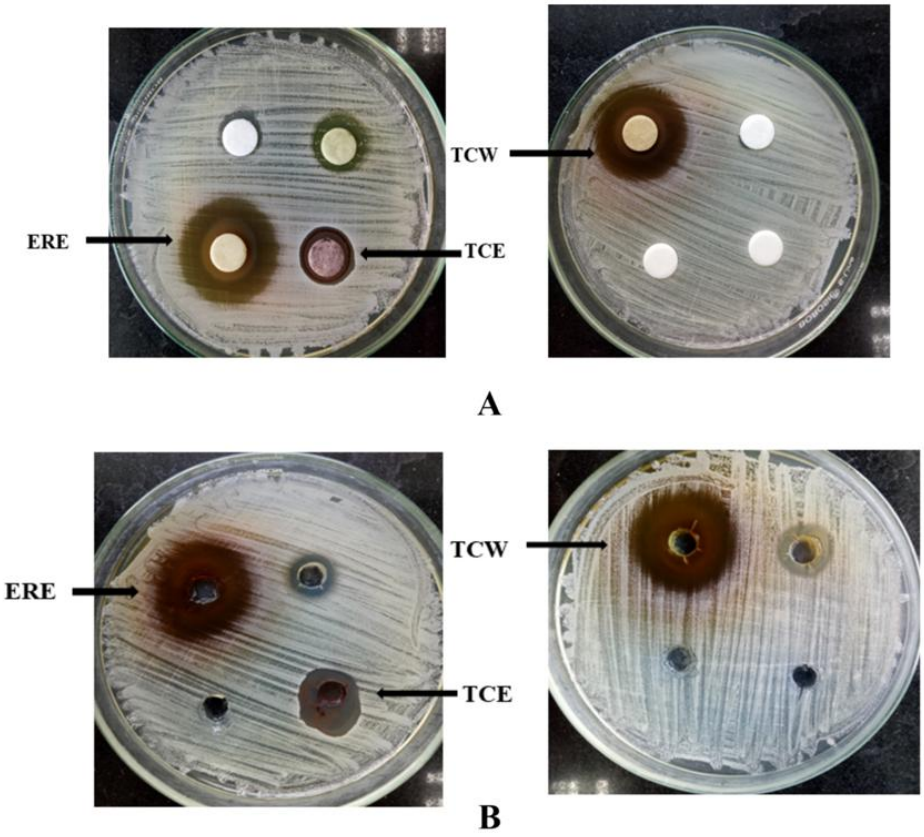

Figure 1: Anti-*Listeria monocytogenes* activities of *Terminalia chebula* Water (TCW) and Ethanol extracts (TCE) and *Embelia ribes* Ethanol (ERE) extract demonstrated by disc diffusion and agar well diffusion methods.

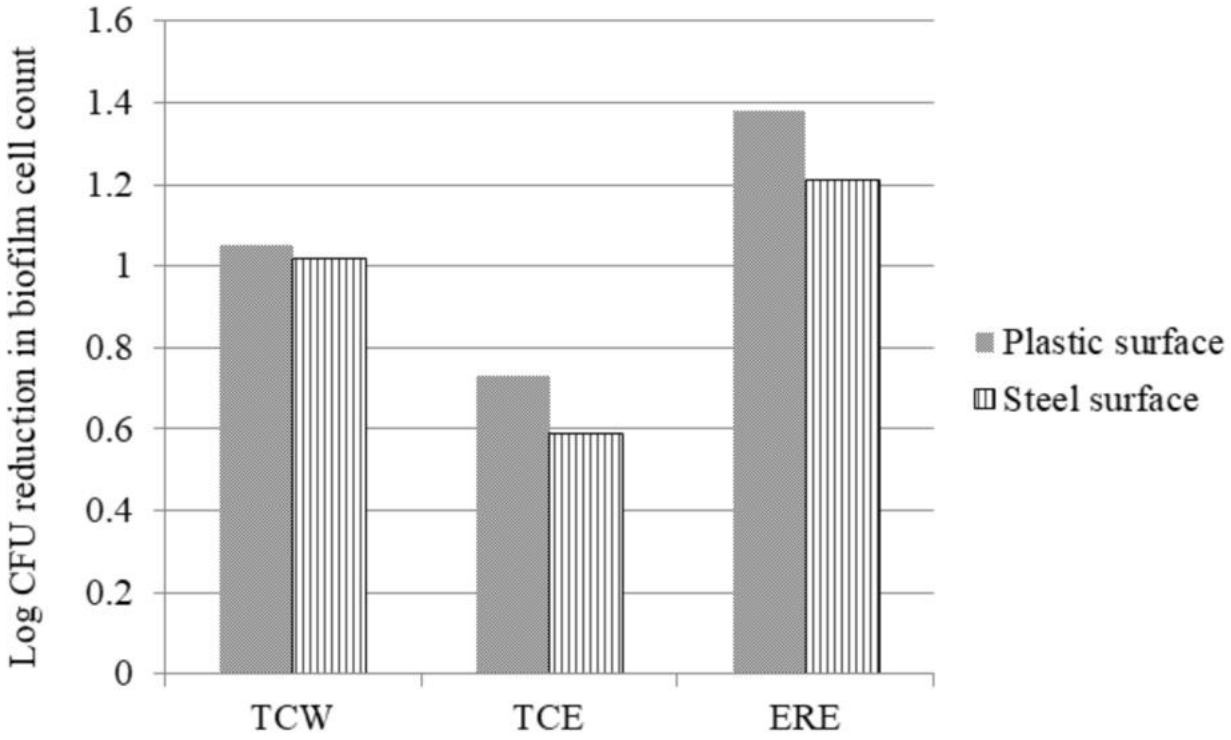

Figure 2: Reduction in biofilm cells of *L. monoctyogenes* on plastic and stainless-steel surfaces following treatment with *Terminalia chebula* Water (TCW) and Ethanol extracts (TCE) and *Embelia ribes* Ethanol (ERE) extract.

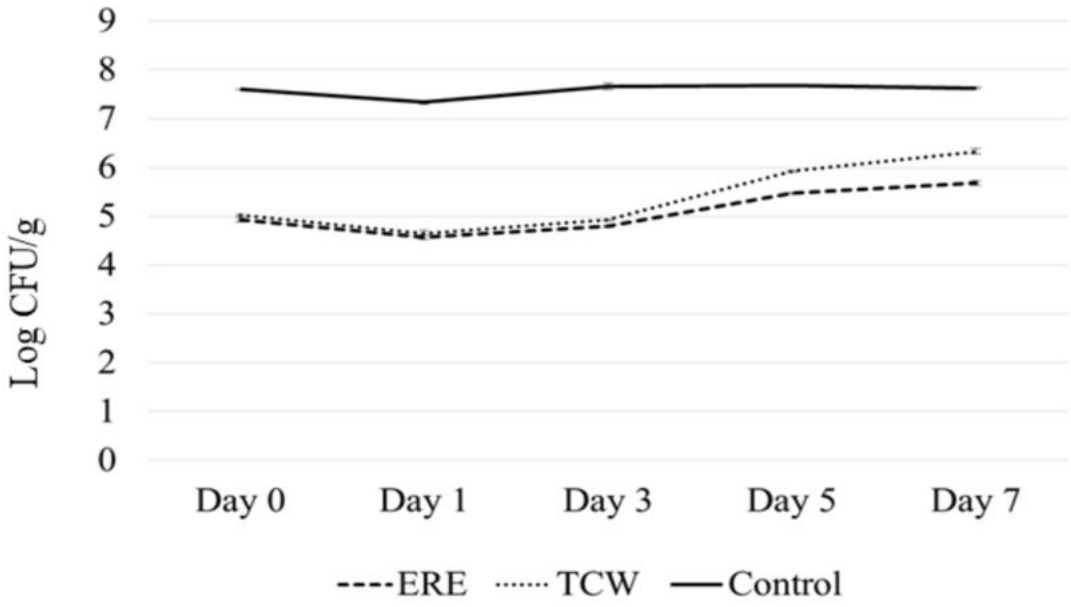

Figure 3: Effect of dip treatment with *Terminalia chebula* Water (TCW) and *Embelia ribes* Ethanol (ERE) extract on yellowfin tuna (*Thunnus albacares*) slices spiked with *Listeria monocytogenes* MTCC 1143.

Discussion

Antimicrobial Activity of Plant Extracts Against L. monocytogenes

A number of natural compounds found in plants, such as extracts of herbs and fruits, have been shown to possess antimicrobial activities against *L. monocytogenes* [32]. In the present study, both aqueous and ethanol extracts of *Terminalia chebula* fruits and ethanol extracts obtained from the *Embelia ribes* fruits could effectively inhibit the growth of *L. monocytogenes* on food processing surfaces and in food systems. Although previous studies have documented the antibacterial activity of extracts from *Terminalia chebula* fruit against clinically important bacterial strains, their bactericidal and anti-biofilm activities against *L. monocytogenes* have not been investigated [29,33]. The aqueous extract of this fruit demonstrated significant antibacterial activity against both Gram-positive and Gram-negative bacteria, including *Staphylococcus epidermidis, Staphylococcus aureus, Bacillus subtilis, Salmonella* Typhi and *Pseudomonas aeruginosa* [28]. The water extracts of *Terminalia chebula* fruits showed a better antibacterial activity than their ether and alcoholic counterparts and had a Minimum Inhibitory Concentration (MIC) and Minimum Bacteriocidal Concentration (MBC) of 125 and 150 mg/l, respectively, against *Helicobacter pylori* even after autoclaving [34]. The findings of the present study were consistent with these earlier reports. Both aqueous (TCW) and ethanolic (TCE) extracts exhibited antimicrobial activity against the Gram-positive pathogen *L. monocytogenes*, with MBCs of 12.5 mg/ml and 25 mg/ml, respectively. In a previous study, the crude methanolic extract of *Terminalia chebula* demonstrated the highest activity against *Bacillus subtilis* (23 mm inhibition zone), followed by *Listeria innocua* (16 mm) and the lowest activity against *Enterococcus faecalis* (12 mm) [35]. Zones of inhibition against *L. monocytogenes* for the aqueous and ethanol extracts in the present study were 27.3 mm and 17.3 mm, respectively. Many researchers have reported the presence of different active agents in *Terminalia chebula*, which may be responsible for its antibacterial effects against *L. monocytogenes.* Miyasaki and colleagues identified active compounds like chebulagic acid, chebulinic acid, corilagin and terchebulin in *Terminalia chebula* that were effective in killing *Acinetobacter baumannii* [36]. It is also important to note that in the present study, the susceptibility of the pathogen varied between the solvent extract and the aqueous extract. This indicates the involvement of more than one active principle of biological significance in the activity against microorganisms.

The ethanolic extract made from dried berries of *Embelia ribes* showed significant antibacterial activities against *L. monocytogenes.* At a concentration of 12.5 mg/ml, this extract completely inhibited the growth of *L. monocytogenes in-vitro*. Although no previous reports are available on its efficacy against *Listeria* spp., several studies have demonstrated its broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria [37,38]. In the present study, the aqueous extract showed no antibacterial activity, whereas the ethanolic extract effectively eliminated *L. monocytogenes* at 12.5 mg/ml. This may be attributed to the limited ability of water to extract bioactive compounds that are more readily soluble in organic solvents. Embelin, a natural

benzoquinone derived from *Embelia ribes* berries, has been reported to exhibit stronger activity against Gram-positive bacteria. *Bacillus cereus, Bacillus subtilis* and *Staphylococcus aureus* were more susceptible to embelin with a MIC index less than 4. In contrast, against Gram-negative organisms like *Escherichia coli*, *Shigella flexneri*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, it showed bacteriostatic activities with MIC index values greater than 4 [37]. The remarkable sensitivity of *L. monocytogenes* to the ethanolic extract of *Embelia ribes* observed in this study may be attributed to embelin, a compound with well-documented antibacterial activity against Gram-positive bacteria.

Anti-Biofilm-Forming Activity of Plant Extracts

Both ERE and TCW extracts showed more than one log reduction in the biofilm cell counts when compared to that of the control. A study revealed the anti-quorum sensing potential of the methanol extract of *Terminalia chebula* fruit [39]. A bioactive Fraction (F7) obtained from methanol extract showed a significant reduction in QS-regulated production of extracellular virulence factors in *P. aeruginosa* and a significant reduction in biofilm formation of the organism on polystyrene plates. The F7 could bring about a reduction of 65% in biofilm formation in 24 h at a concentration of 1 mg/ml, that was increased to 85% at 5 mg/ml. In the present study, aqueous extract made from the fruits of *T. chebula* showed a significant reduction in the biofilm cells when applied at a concentration of 6.25 mg/ml on both plastic and steel surfaces.

A biofilm-inhibition study using embelin at a concentration significantly lower than the MBC of *Streptococcus* mutans demonstrated a significant antibiofilm effect of the natural compound embelin, which reduced the biofilm-forming ability of the bacteria on polystyrene plates [40]. In the present study, ethanol extract of *Embelia ribes* successfully caused more than one log reduction in the biofilm cell counts on both plastic and steel surfaces.

Antibacterial Activity of Plant Extracts Against Listeria monocytogenes in the Food System

Natural compounds are in demand as food preservatives due to their health benefits and safety. The most common application method is the direct addition of natural compounds to food. Dipping, spraying and coating treatment of food with active solutions are currently applied to the product before packaging as valid options. Even though many plant products show bactericidal activity against pathogenic organisms in microbiological media, only a few of those antimicrobials have been tested in foods. In the present study, tuna slices treated with the ethanolic extract of *Embelia ribes* fruits (12.5 mg/ml) exhibited a significant 2.67-log reduction in *L. monocytogenes* counts. Similarly, treatment with the aqueous extract of *Terminalia chebula* fruits (12.5 mg/ml) for one minute resulted in a 2.61-log reduction. Following storage at 2°C, both extracts achieved an overall reduction of approximately 3 log units. These findings suggest that aqueous and ethanolic extracts of *Terminalia chebula* fruits, as well as ethanolic extracts of *Embelia ribes* fruits, possess promising antimicrobial potential and can be applied in the food industry to control *L. monocytogenes* in seafood and processing environments.

Conclusion

Our study has shown *T. chebula* and *E. ribes* extracts as promising anti-*Listeria* compounds for application in fishery products. Further studies are necessary to determine the stability of the extracts under different processing conditions. The mechanism of antimicrobial activity of the extracts requires further studies to determine the active compound(s) responsible for the antimicrobial activity and their application in combination with other natural antimicrobials in controlling *L. monoctyogenes* in fresh and ready-to-eat fishery products.

Conflict of Interest

The authors declare no conflict of interest for this paper.

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