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Full Length Research Paper

Antimicrobial activity of seed, pomace and leaf extracts of sea buckthorn (*Hippophae rhamnoides* L.) against foodborne and food spoilage pathogens

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The present study was conducted to evaluate the total phenolic content (TPC) and antibacterial properties of crude extracts of sea buckthorn (*Hippophae rhamnoides* L.) pomace, seeds and leaves against 17 foodborne pathogens. The methanolic extract of leaves exhibited high total phenolic content (278.80 mg GAE/g extract) and had low minimum inhibitory concentration (MIC) value of 125 µg/ml against *Listeria monocytogenes. Salmonella typhimurium* strain was found to be resistant against all tested extracts. The antilisterial activity of the methanolic extract of leaves was tested on carrots. Bacterial enumeration was significantly reduced by 0.15 to 0.31, 0.26 to 1.72 and 0.59 to 4.10 log cfu/g after 0 to 60 min exposure when treated with 125, 2500 and 5000 µg/ml extract, respectively. Thus, in addition to its use as a functional food ingredient, leaves extract from sea buckthorn (SBT) can possibly be used as a biosanitizer in food industries.

Key words: Antimicrobial activity, *Hippophae*, *Listeria monocytogenes*, natural sanitizer, seabuckthorn.

INTRODUCTION

Foodborne diseases resulting from consumption of contaminated food with pathogenic bacteria and/or their toxins is a priority concern to public health. The committee on food safety (FAO/WHO) concluded that illness due to contaminated food is the most prevalent health problem in the world and an important cause of reduced economic productivity (Kaferstein et al., 1997). In this context, the identification and evaluation of antimicrobial agents for the control of these pathogens, to assure consumers a safe, wholesome and nutritious food supply, is a matter of global concern. Many different chemical and synthetic compounds have been used as antimicrobials to inhibit bacteria in foods. With the increase of bacterial resistance to antibiotics, there is considerable interest in investigating the antimicrobial effects of naturally-derived compounds, such as essential oils and plant extracts, against a range of bacteria, to develop other classes of natural antimicrobials useful for infection control or for the preservation of food, which have a natural or 'green' image (Negi et al., 2005). The versatile composition of plant essential oils and extracts and the large antimicrobial spectrum, associated with their low toxicity, make them potential natural agents for food preservation (Conner, 1993).

The antimicrobial activity of plant extracts may reside in a variety of different components, including aldehyde and phenolic compounds (Lai and Roy, 2004). Naturally occurring combinations of these compounds can be synergistic and often result in extracts having greater antimicrobial activity than the purified individual constituents (Delaquis et al., 2002). *Hippophae rhamnoides* L., Elaeagnaceae, commonly known as sea buckthorn (SBT) is a temperate, 3 to 15 feet tall, branched and thorny nitrogen fixing deciduous shrub (Rousi, 1971). The non-toxicological effect of the extracts of this plant is known from their use in traditional *Amchi* system of medicine. All parts of the plant are considered to be a good source of bioactive substances. The medicinal effects of SBT have been suggested to be due

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to the presence of high antioxidant contents (Eccleston et al., 2002).

Previous studies demonstrated that extracts of whole fruit, fruit pulp, pulp oil and seed oil possess immuno-modulatory and anti-oxidant activity (Geetha et al., 2002) and reported to be useful in treating gastric ulcers (Xing et al., 2002). Antimicrobial activity of SBT seed extract against *Bacillus cereus, Bacillus coagulans, Bacillus subtilis, Listeria monocytogenes* and *Yersinia enterocolitica* have been previously reported (Negi et al., 2005; Chauhan et al., 2007). However, to the best of our knowledge, the antimicrobial activity of SBT leaves and pomace have not been investigated. The antilisterial activities of SBT extracts in a real food system have also not been reported. Therefore, this study was undertaken to determine the potential of SBT extract as a natural sanitizing agent.

MATERIALS AND METHODS

SBT specimens were collected from trans-Himalayan Ladakh region, India in August, 2009 and initially identified by morpho-logical features. A voucher specimen has been deposited in the institute herbarium.

Preparation of methanolic and aqueous extracts

Briefly, 10 g of air-dried and finely ground samples (pomace, seeds and leaves) were extracted with methanol and distilled water in a soxhlet apparatus for 6 h each to obtain the extracts in respective solvents. The solvents were evaporated using a rotary vacuum evaporator and stored at 4°C.

Determination of total phenols

Total phenolic content (TPC) in the extracts were determined by the modified Folin-Ciocalteu method (Wolfe et al., 2003). An aliquot of the extracts was mixed with 5 ml Folin-Ciocalteu reagent (previously diluted with water 1:10, v/v) and 4 ml (75 g/L) of sodium carbonate. The tubes were vortexed for 15 s and allowed to stand for 30 min at 40°C for color development. Absorbance was measured at 765 nm in a UV-VIS spectrophotometer. Samples of extract were evaluated at a final concentration of 0.1 mg/ml. TPC was expressed as mg/g gallic acid equivalent (GAE).

Microbial strains

17 foodborne pathogens were selected, which included six Gram positive and 11 Gram-negative species (Table 2). Strains were obtained from international repository at MTCC, India. Active cultures for experimental use were prepared by transferring a loopful of cells from stock cultures to flasks and inoculated in Luria-Bertani (LB) broth medium at 37°C for 24 h, except for *Pseudomonas fluorescens*, which was grown at 25°C for 24 h. Cultures of each bacterial strains were maintained on LB agar medium at 4°C and subculture every 15 days interval.

Agar well-diffusion method

The methanolic and aqueous extracts were dissolved in 10% dimethylsulfoxide (DMSO) (v/v) to a final concentration of 50 mg/ml and filter sterilized. The antibacterial test was carried out by agar well diffusion method using 100 µl of standardized inoculum suspension containing 10^7 cfu/ml of bacteria (NCCLS, 2000a). To prepare standardized inoculums, bacteria were grown in LB broth at 37°C with constant agitation until the density matched the turbidity of a 0.5 McFarland standard. The wells (6 mm in diameter) were punched into the agar medium using a sterile cork borer and 100 µl of the extract solution (5000 µg/ml per well) was delivered into them. DMSO was used as negative control. Standard reference antibiotics, gentamicin (10 µg) and rifampicin (30 µg), were used as positive controls. Before incubation, all Petri dishes were kept at 4°C for 4 h. The plates were incubated at 37°C for 24 h. Antibacterial activity was evaluated by measuring the diameter of the zones of inhibition (ZOI) against the tested bacteria.

Determination of MIC

The minimum inhibitory concentration (MIC) values were studied for the bacterial strains sensitive to the extracts in the well diffusion assay. A broth micro dilution susceptibility assay was performed for determination of MIC (NCCLS, 2000b). All tests were performed in Mueller Hinton Broth (MHB). The inocula of the bacterial strains were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The extracts were first dissolved in 10% DMSO and then diluted to the highest concentration (4000 µg/ml) to be tested, and then serially two-fold dilutions were made in a concentration range from 4000 to 31.25 µg/ml with MHB in 96-well microtitre plate, volume being 100 µl. The plates were dispensed with 95 µl of MHB and 5 µl of the inocula. The volume in each well was 200 µl. The plate was covered with a sterile plate sealer. Contents of each well were mixed on a plate shaker at 300 rpm for 20 s and then incubated at 37°C for 24 h. Similar tests were performed simultaneously for growth control (MHB + inocula) and sterility control (MHB + test sample). Microbial growth was determined by absorbance at 600 nm. MIC values were determined as the lowest concentration of the extract where absence of growth was recorded.

Preparation of treatment solution

Treatment solution of methanolic extract of SBT leaves [125 (MIC), 2500 (20 MIC) and 5000 (40 MIC) µg/ml] against *Listeria monocytogenes* ATCC 19111, with lowest MIC value of 125 µg/ml as compared to all other pathogens evaluated, was tested in the real food system comprising fresh carrots.

Antilisterial activity in real food system

Carrots (*Daucus carota*) were purchased on the day of processing from a local retailer and stored at 4°C until use within 4 h. After washing with tap water, the carrots were peeled, shredded into 2 to 5 mm wide strips and exposed to the UV lamp in a laminar flow cabinet for 30 min on both sides to minimize interference of the natural bacterial flora. Inoculum cell suspension of *L. monocytogenes* was prepared by transferring 10 ml of a 24 h LB culture containing 10^8 cfu/ml into 1 L of 0.1% sterile buffered peptone water (BPW) to 10^6 cfu/ml. Shredded carrots were immersed into the inoculum solution (sample: inoculum ratio 1:5 w/v) and shaken for 1 min to distribute the inoculum homogenously and placed on sterile cheese cloth for removing excess liquid and inoculated samples were transferred into sterile jars. To allow attachment of *L. monocytogenes* cells, jars partly closed with covers were stored at 20°C for 24 h before they were treated. 100 g of inoculated samples was transferred into 500 ml of different

Table 2. Antimicrobial activity of SBT extracts against foodborne bacteria.

Test microorganism	Zone of inhibition (mm)							
	Aqueous extract			Methanolic extract			Antibiotic[x]	
	Seed	Leaf	Pomace	Seed	Leaf	Pomace	G (10 µg)	R (30 µg)
Gram-positive organisms								
B. cereus ATCC 11778	$13.0^{d} \pm 1.0$	$10.7^{a,b} \pm 1.2$	-	$17.7^{i} \pm 1.5$	$17.0^{c} \pm 1.0$	-	$17.0^{b,c} \pm 3.0$	$20.0^{e} \pm 0.0$
E. faecalis ATCC 10100	-	$11.7^{b} \pm 1.5$	$11.3^{b} \pm 1.2$	$13.0^{d,e} \pm 1.0$	$14.3^{b} \pm 0.6$	$11.3^{b} \pm 1.2$	$20.0^{c,d,e} \pm 0.0$	$9.0^{a} \pm 1.0$
L. monocytogenes ATCC 15313	-	-	-	-	$9.7^{a} \pm 0.6$	-	$22.0^{d,e,f} \pm 0.0$	$12.0^{b,c} \pm 0.0$
L. monocytogenes ATCC 19111	-	-	-	$16.3^{h} \pm 0.6$	$22.3^{g} \pm 1.5$	-	$18.0^{b,c,d} \pm 2.0$	$16.0^{d} \pm 0.0$
S. aureus ATCC 12598	-	-	-	-	$10.3^{a} \pm 0.6$	$9.0^{a} \pm 1.0$	$15.7^{a,b} \pm 4.0$	$24.3^{f} \pm 1.5$
S. aureus ATCC 12600	$11.0^{c} \pm 1.0$	$11.0^{a,b} \pm 1.0$	$13.3^{c} \pm 1.5$	$14.3^{e,f,g} \pm 0.6$	$20.7^{efg} \pm 1.2$	-	$22.0^{e,f} \pm 2.5$	$19.3^{e} \pm 2.5$
Gram-negative organisms								
A. hydrophila ATCC 7966	-	$10.3^{a,b} \pm 1.5$	-	$12.7^{b,c,d} \pm 0.6$	$25.3^{h} \pm 1.5$	-	$13.0^{a} \pm 3.0$	$13.0^{c} \pm 1.0$
E. coli ATCC 8739	-	-	-	-	$14.0^{b} \pm 1.0$	-	$20.0^{c,d,e} \pm 0.0$	$10.0^{a,b} \pm 0.0$
E. coli ATCC 25922	-	-	$10.0^{a} \pm 1.0$	$11.7^{b,c} \pm 1.5$	$14.7^{b} \pm 0.6$	-	$23.0^{e,f} \pm 1.0$	$20.7^{e} \pm 1.5$
K. pneumoniae ATCC 33495	$12.7^{d} \pm 0.6$	-	-	$11.3^{b} \pm 0.6$	$14.3^{b} \pm 1.5$	-	$25.3^{f} \pm 1.5$	$17.0^{d} \pm 1.0$
P. vulgaris ATCC 6380	-	-	-	-	$18.0^{c,d} \pm 1.0$	-	$17.3^{b,c} \pm 3.0$	$13.0^{c} \pm 1.0$
P. aeruginosa ATCC 25619	$9.0^{a,b} \pm 1.0$	$9.7^{a} \pm 1.2$	-	$15.3^{f,g,h} \pm 0.6$	$9.3^{a} \pm 0.6$	$9.3^{a} \pm 0.6$	$20.0^{c,d,e} \pm 2.0$	$9.0^{a} \pm 0.0$
P. fluorescens ATCC 13525	$14.0^{e} \pm 0.0$	$13.0^{c} \pm 0.0$	-	$14.0^{d,e,f} \pm 0.0$	$19.0^{d,e} \pm 1.0$	$8.7^{a} \pm 1.2$	$23.7^{e,f} \pm 1.5$	$16.0^{d} \pm 1.0$
S. enterica ATCC 10749	$14.0^{e} \pm 0.0$	$17.0^{d} \pm 1.0$	$14.7^{d} \pm 1.2$	$14.3^{e,f,g} \pm 1.5$	$20.3^{e,f} \pm 1.5$	$11.3^{b} \pm 1.1$	$23.7^{ef} \pm 1.5$	$12.7^{c} \pm 1.5$
S. typhimurium ATCC 23564	-	-	-	-	-	-	$20.0^{c,d,e} \pm 0.0$	$12.0^{b,c} \pm 0.0$
S. flexneri ATCC 29508	$9.7^{b} \pm 0.6$	-	-	$15.7^{g,h} \pm 0.6$	$21.7^{f,g} \pm 1.5$	-	$19.3^{b,c,d,e} \pm 3.0$	$12.0^{b,c} \pm 2.0$
Y. enterocolitica ATCC 23715	$8.7^{a} \pm 0.6$	$11.0^{a,b} \pm 1.0$	-	$8.7^{a} \pm 0.6$	$10.3^{a} \pm 0.6$	-	$23.0^{e,f} \pm 3.0$	$12.0^{b,c} \pm 2.0$

[x] Standard antibiotics: G, gentamycin (10 µg/disc); R, rifampicin (30 µg/disc). [a, b, c, d, e, g, h, i] Different superscripts in a column differ significantly ($p<0.05$) by Duncan's test.

treatment solutions, sufficient to cover the entire vegetable sample in the jar and left for 0, 15, 30, 60 min at room temperature with gentle agitation. The ratio of product to treatment solution was 1:5 w/v. After decanting the treatment solution, samples were serially diluted by a factor of ten in phosphate buffer saline and enumerated.

Detection and enumeration of *L. monocytogenes*

To determine the possible presence of *Listeria* spp. on uninoculated carrot samples, 25 g of carrot was transferred to 225 ml of BPW in a stomacher bag and homogenized for 60 s at medium speed. Homogenates of carrots in BPW were incubated at 37°C for 24 h and then streaked on HiCrome Listeria Agar Base with supplement FD061 (HiMedia, India). Plates were incubated at 37°C for 48 h and then examined for presumptive *Listeria* colonies (AOAC, 1990). To enumerate *L. monocytogenes* in the inoculated carrot samples, 10 g of treated sample was suspended into 90 ml of BPW and spread plated on HiCrome Listeria Agar. Inoculated plates were incubated for 48 h at 37°C. Results were expressed as log cfu/ml. Randomly selected presumptive *L. monocytogenes* colonies were confirmed using standard biochemical tests (AOAC, 1990).

Statistical analysis

Three replicate trials were done for each experiment. Data generated was analyzed by SPSS 11.5 statistical computer package and subjected to analysis of variance and

Table 1. Total phenolic content (mg GAE/g extract) of SBT extracts.

Extract	TPC (mg GAE/g extracts)
Leaves MeOH	$278.80^{e} \pm 8.24$
Leaves Aq	$148.89^{c} \pm 5.34$
Seed MeOH	$162.56^{d} \pm 9.25$
Seed Aq	$109.57^{c} \pm 9.00$
Pomace MeOH	$107.01^{b} \pm 3.92$
Pomace Aq	$87.35^{a} \pm 6.45$

[a, b, c, d, e] Different superscripts in a column differ significantly ($p < 0.05$) by Duncan's test.

Duncan's multiple range tests to determine if significant differences ($p = 0.05$) in populations of *L. monocytogenes* existed between mean values.

RESULTS

Assay for total phenolics

The TPC ranged from 87.35 to 278.80 mg GAE, depending on the plant part and the extracting solvent (Table 1). The methanolic extracts contained the higher amounts of phenolic compounds as compared to respective water extracts. On the basis of total mean of two solvent extracts, TPC was found in the following order from high to low: leaves > seeds > pomace. The results denote the presence of antioxidant principles in the extractives.

In vitro antimicrobial activity

The *in vitro* antimicrobial activity of SBT plant extracts, against the employed bacteria, was qualitatively assessed by the presence or absence of inhibition zones and zone diameters (Table 2). The methanolic extracts of SBT pomace, seeds and leaves showed a broad spectrum of antimicrobial activity against the tested ATCC strains (64.71%); the susceptibility of the test microorganisms was less pronounced in the cases of respective aqueous extracts (39.21%). However, among the three parts of SBT, leaves had significant anti-bacterial activity against 16 tested reference strains out of 17 followed by seeds (12) and pomace (7). All the extracts of SBT used in this study possessed good antibacterial property except aqueous and methanolic extracts of pomace, which were active only against few microorganisms with lesser inhibition zones ranging from 10 to 14.7 mm and 8.7 to 1.3 mm, respectively.

The seeds exerted moderate antibacterial activity with the zones of inhibition ranging from 8.7 to 14.0 mm in aqueous extract and 8.7 to 17.7 mm in methanolic extract. The methanolic extract of seeds strongly inhibited the growth of *Bacillus cereus* (17.7 mm), whereas aqueous extract exhibited maximum inhibitory effect against *Salmonella enterica* (14.0 mm). Methanolic extract of leaves produced potent inhibitory effect against *Aeromonas hydrophila*, *Listeria monocytogenes* ATCC 19111, *Shigella flexneri, Staphylococcus aureus* ATCC 12600 and *Salmonella enterica* with the diameters of zones of inhibition of 25.3, 22.3, 21.7, 20.7 and 20.3 mm, respectively. In contrast, aqueous extract was found to be less effective against the aforementioned pathogens but *Shigella flexneri* and *Listeria monocytogenes* ATCC 19111 displayed strong resistance. *Salmonella typhimurium* was found to be resistant against all the extracts tested. All tested microorganisms were completely non-susceptible to control wells loaded with 10% DMSO.

Minimum inhibitory concentrations

The MIC of SBT extracts against the bacteria tested is shown in Table 3. Extracts which showed a clear ZOI at the maximum tested concentration (50 mg/ml) were selected for MIC determination. MIC values for methanolic extracts of different parts of SBT against the tested bacteria were lower (125 to 4000 µg/ml) than that of aqueous extracts (2000 > 4000 µg/ml). The high antimicrobial activity of methanolic extract of leaves was confirmed by the micro dilution broth assay, exhibiting MIC values of 125 µg/ml against *Listeria monocytogenes* ATCC 19111, 250 µg/ml against *Staphylococcus aureus* ATCC 12600 and *Aeromonas hydrophila* and 500 µg/ml against *Salmonella enterica*. Strains of *Escherichia coli* ATCC 8739, *Listeria monocytogenes* ATCC 15313, *Enterococcus faecalis* and *Klebsiella pneumoniae* were inhibited by 2000 to 4000 µg/ml concentration of both aqueous and methanolic extracts. However, MIC values of more than 4000 µg/ml were obtained with the aqueous extracts against *Escherichia coli* ATCC 25922, *Aeromonas hydrophila* and *Yersinia enterocolitica.*

In vivo antimicrobial activity

The sterility of the experimental procedure to prepare carrot strips, omitting the step of *Listeria* inoculation, was confirmed by negative viable counts on HiCrome Listeria Agar plates, which also involved the absence of *Listeria* on non inoculated carrots. Moreover, any subsequent contamination was limited by the high *Listeria* inoculum, which was corroborated by similar numbers of recovered cells on plate count agar (PCA) and on HiCrome Listeria Agar, a selective medium for *Listeria*. Preliminary studies were conducted to determine the level of inoculums that could be retained on the surface of shredded carrots. The level of inoculums used was approximately 7 log cfu/ml; however, the amount of pathogens attached to the surface was 5.79 log cfu/ml.

Table 3. MIC (µg/ml) of SBT extracts against foodborne bacteria.

Test microorganism	MIC (µg/ml)					
	Aqueous extracts			Methanolic extracts		
	Seed	Leaf	Pomace	Seed	Leaf	Pomace
Gram-positive organisms						
B. cereus ATCC 11778	4000	>4000	n.d.	1000	1000	n.d.
E. faecalis ATCC 10100	n.d[a]	4000	4000	2000	4000	4000
L. monocytogenes ATCC 15313	n.d.	n.d.	n.d.	n.d.	4000	n.d.
L. monocytogenes ATCC 19111	n.d.	n.d.	n.d.	2000	125	n.d.
S. aureus ATCC 12598	n.d.	n.d.	n.d.	n.d.	2000	4000
S. aureus ATCC 12600	2000	4000	4000	4000	250	n.d.
Gram-negative organisms						
A. hydrophila ATCC 7966	n.d.	>4000	n.d.	4000	250	n.d.
E. coli ATCC 8739	n.d.	n.d.	n.d.	n.d.	4000	n.d.
E. coli ATCC 25922	n.d.	n.d.	>4000	2000	4000	n.d.
K. pneumoniae ATCC 33495	2000	n.d.	n.d.	4000	4000	n.d.
P. vulgaris ATCC 6380	n.d.	n.d.	n.d.	n.d.	1000	n.d.
P. aeruginosa ATCC 25619	2000	>4000	n.d.	2000	4000	4000
P. fluorescens ATCC 13525	2000	4000	n.d.	2000	1000	4000
S. enterica ATCC 10749	2000	2000	4000	2000	500	4000
S. typhimurium ATCC 23564	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
S. flexneri ATCC 29508	4000	n.d.	n.d.	2000	500	n.d.
Y. enterocolitica ATCC 23715	>4000	>4000	n.d.	4000	2000	n.d.

[a] n.d, Not done.

Table 4. Effectiveness of SBT MeOH extract in inactivating *L. monocytogenes* ATCC 1911* (log cfu/g [x]) inoculated on carrots.

Time (min)	Growth control	M** (125 µg/ml)	20 M** (2500 µg/ml)	40 M** (5000 µg/ml)
0	$5.71^{a} \pm 0.03$	$5.64^{c} \pm 0.02$	$5.53^{d} \pm 0.01$	$5.20^{d} \pm 0.05$
15	$5.77^{a} \pm 0.06$	$5.62^{c} \pm 0.03$	$5.44^{c} \pm 0.03$	$4.63^{c} \pm 0.04$
30	$5.84^{b} \pm 0.03$	$5.55^{b} \pm 0.03$	$4.32^{b} \pm 0.03$	$3.46^{b} \pm 0.04$
60	$5.85^{b} \pm 0.02$	$5.48^{a} \pm 0.03$	$4.07^{a} \pm 0.06$	$1.69^{a} \pm 0.07$

[a, b, c, d] Different superscripts in a column differ significantly ($p<0.05$) by Duncan's test. [x] Populations of *L. monocytogenes* detected in carrot samples after treatment. * Populations of *L. monocytogenes* on carrot samples were 5.76 log cfu/g before treatment followed by 24 h incubation at 20°C for the attachment of the cells. **M, minimum inhibitory concentration.

The fate of *L. monocytogenes* on sliced carrots subjected to different concentrations of SBT methanolic leaves extract is shown in Table 4. Inoculated carrots were dipped in sterile distilled water as a control treatment and the maximum *L. monocytogenes* reduction obtained with sterile water was approximately 0.08 log cfu/g after 0 min. Treatment with SBT leaf extracts significantly reduced the number of *L. monocytogenes* on fresh-cut carrots compared with the populations on the produce treated with sterile distilled water. After 0 min, populations of *L. monocytogenes* on carrots were reduced by 0.15, 0.26 and 0.59 log cfu/g, respectively by 125, 2500 and 5000 µg/ml concentrations of the treatment solution (Figure 1).

Reductions of the pathogen counts increased with increasing exposure times and concentrations of the treatment solutions. However, *Listeria* population on carrots treated with *in vitro* MIC value (125 µg/ml), did not vary significantly for the first 15 min Treatment of carrots with 20 MIC (2500 µg/ml) treatment solution caused a 0.35, 1.47 and 1.72 log cfu/g reduction in the number of *L. monocytogenes* at 15, 30 and 60 min, respectively. Application of 5000 µg/ml concentration of the treatment solution for 60 min resulted in the lowest pathogen number (1.69 log cfu/g) surviving on sliced carrots. On the contrary, the populations in the control increased non-significantly to 5.84 and 5.85 log cfu/g after 30 and 60 min.

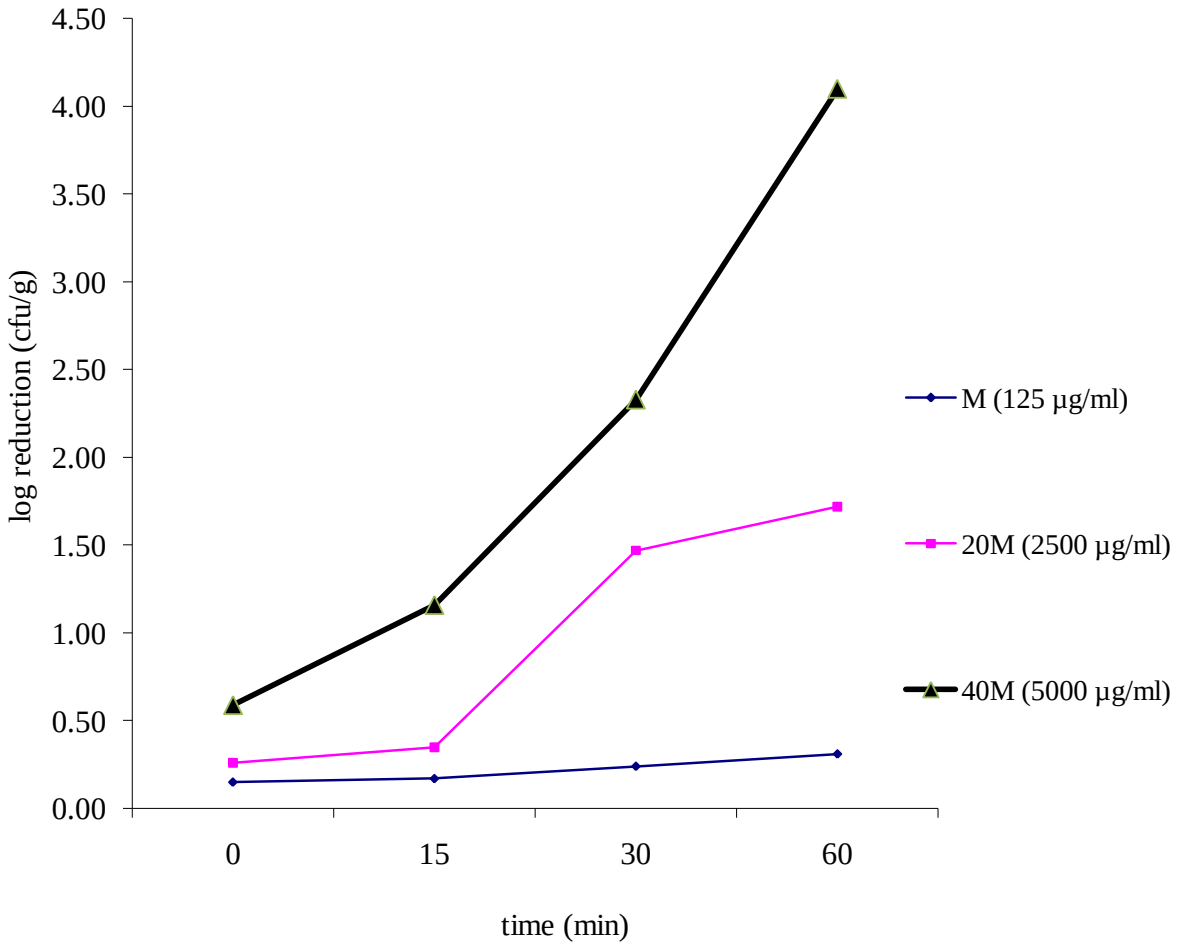

Figure 1. Log-reduction of *L. monocytogenes* ATCC 19111 in artificially contaminated fresh-cut carrots.

DISCUSSION

In the last years, the consumer's habits were focused on the use of fresh fruits and vegetables. For this reason, probably, the diseases caused by psychrotrophic bacteria such as *L. monocytogenes* increased. So, all possible strategies to prevent the proliferation of the pathogen in food, especially those using natural bioactive compounds, may contribute to the maintenance of human health. The antimicrobial activity of plant extracts depends on the type and amount of phenolics present in the plant tissue and the pathogen's inherent resistance (Martini et al., 2004). Methanolic extract of SBT leaves were found to have better amounts of TPC (278.80 mg/g extract) that might be responsible for their comparatively higher antimicrobial activity. This is in agreement with the reports of Yen et al. (1996) that methanol is a widely used and effective solvent for the extraction of antioxidants.

Carrots are a nutrient source able to support a significant growth of *Listeria* when the antilisterial compound, which is present in the juice and not on the sterile strips (Nguyen-the and Lund, 1991), and the indigenous microflora (Hu et al., 2008; Liao, 2007) are inactive or absent, achieving final levels close to those in a nutritive broth (Noriega et al., 2010). In the present study, antilisterial activity of the carrot itself was discounted firstly by the antagonistic action of the background microflora which was destroyed by UV sterilization of the carrot strips. Secondly, falcarinol and falcarindiol, polyacetylenic constituents of carrots with antimicrobial properties (Hansen et al., 2003), have been discounted due to their location in the carrot peel, which was removed during the preparation of shredded carrots that are responsible for the antilisterial activity (Nguyen-the and Lund, 1991).

The immediate reduction in *Listeria* population at 0 min by the methanolic extracts at different concentrations showed a shock antimicrobial effect on the cells attached to carrot samples. In control, reduction of *L. monocytogenes* population was 0.08 and 0.02 log cfu/ml after dipping for 0 and 15 min, respectively. Other researchers also showed that treatment of fruits and vegetables with water results in a microbial reduction of less than 1 logarithmic unit (Singh et al., 2002). These findings show small variations which may be due to

differences in the types and quantities of test micro-organisms, concentrations of treatment solutions and the types of produce used (Francis and Beirne, 2002). These studies demonstrated that the use of water is insufficient to eliminate the pathogen on fresh fruits and vegetables. Therefore, the use of effective sanitizers is necessary to inactivate pathogens.

Treatment solutions did not exert any lethal effect on *L. monocytogenes* but inhibited its proliferation with different exposure times. However, the most effective treatment was immersion in treatment solution of 40 MIC (5000 µg/ml) for 60 min which reduced the pathogen load by 4.10 log cfu/g in contrast to control where the population increased. Similar results were obtained by Sengun and Karapinar (2005) who reported that *Salmonella typhimurium* inoculated on carrots was reduced by 3.95 log cfu/g after 60 min of treatment. It is interesting to note that most of the published work points out the need to use a high concentration of natural extracts in food systems, typically 100 times the determined *in vitro* MIC value, depending on the food characteristics (Burt, 2004). In this regard, results obtained in this study indicate the technical viability of using the SBT extract as natural sanitizer at a relatively low concentration (40 times MIC) to reduce *L. monocytogenes*.

Conclusion

This study shows that the different extracts of SBT have high TPC and antibacterial properties against foodborne *L. monocytogenes*. Owing to their strong antibacterial and high total phenolic content, the leaves extract from SBT have scope for the possible use as biosanitizer in food industries. However, further works are warranted for the evaluation of toxicity and safety of the extract, even though SBT have been used in traditional medicine. It can be concluded that the results obtained with SBT leaf extract are promising from the standpoint of fresh produce safety.

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