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Antibacterial Activity of Guava (*Psidium guajava* L.) and Neem (*Azadirachta indica* A. Juss.) Extracts Against Foodborne Pathogens and Spoilage Bacteria

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Abstract

The antibacterial activity of guava (*Psidium guajava*) and neem (*Azadirachta indica*) extracts against 21 strains of foodborne pathogens were determined—*Listeria monocytogenes* (five strains), *Staphylococcus aureus* (four strains), *Escherichia coli* O157:H7 (six strains), *Salmonella* Enteritidis (four strains), *Vibrio parahaemolyticus*, and *Bacillus cereus*, and five food spoilage bacteria: *Pseudomonas aeroginosa*, *P. putida*, *Alcaligenes faecalis*, and *Aeromonas hydrophila* (two strains). Guava and neem extracts showed higher antimicrobial activity against Gram-positive bacteria compared to Gram-negative bacteria except for *V. parahaemolyticus*, *P. aeroginosa*, and *A. hydrophila*. None of the extracts showed antimicrobial activity against *E. coli* O157:H7 and *Salmonella* Enteritidis. The minimum inhibitory concentration (MIC) of ethanol extracts of guava showed the highest inhibition for *L. monocytogenes* JCM 7676 (0.1 mg/mL), *S. aureus* JCM 2151 (0.1 mg/mL), *S. aureus* JCM 2179 (0.1 mg/mL), and *V. parahaemolyticus* IFO 12711 (0.1 mg/mL) and the lowest inhibition for *Alcaligenes faecalis* IFO 12669, *Aeromonas hydrophila* NFRI 8282 (4.0 mg/mL), and *A. hydrophila* NFRI 8283 (4.0 mg/mL). The MIC of chloroform extracts of neem showed similar inhibition for *L. monocytogenes* ATCC 43256 (4.0 mg/mL) and *L. monocytogenes* ATCC 49594 (5.0 mg/mL). However, ethanol extracts of neem showed higher inhibition for *S. aureus* JCM 2151 (4.5 mg/mL) and *S. aureus* IFO 13276 (4.5 mg/mL) and the lower inhibition for other microorganisms (6.5 mg/mL). No significant effects of temperature and pH were found on guava and neem extracts against cocktails of *L. monocytogenes* and *S. aureus*. The results of the present study suggest that guava and neem extracts possess compounds containing antibacterial properties that can potentially be useful to control foodborne pathogens and spoilage organisms.

Introduction

FOODBORNE PATHOGENS SUCH AS diarrheagenic serotypes of *Escherichia coli*, *Salmonella* spp., *Listeria monocytogenes*, and *Aeromonas hydrophila* are widely distributed in nature, causing considerable mortality and morbidity in the population. It has been reported that there are more than 1.3 billion cases of human salmonellosis annually with 3 million deaths worldwide (Pang *et al.*, 1995). Among the various diarrheagenic serotypes of *E. coli*, enterohemorrhagic *E. coli* O157:H7 is implicated in a large number of food borne outbreaks in many parts of the world (Mead *et al.*, 1999). *L. monocytogenes* has been isolated from various environments and is reported to cause 25% of all the deaths resulting from foodborne outbreaks in the United States annually (CDC, 1995). *Aeromonas* spp. represents a group of ubiquitous microorganisms in

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aquatic environments (Monfort and Baleux, 1990). These bacteria have broad host range and have often been isolated from humans with diarrhea (Janda and Abbot, 1998).

Novel approaches to the development of new antimicrobials remain an important area of research. In recent years, multiple drug/chemical resistance in both human and plant pathogenic microorganisms has developed due to indiscriminate use of commercial antibiotics commonly applied in the treatment of infectious diseases (Loper *et al.*, 1999; Davis, 1994; Service, 1995). This situation has led scientists to search for new antimicrobials from various sources, including medicinal plants (Cordell, 2000).

Psidium guajava L. (guava), a fruit plant belonging to the family Myrtaceae, is found all over the world. Guava leaves, roots, and fruits have been used for the prevention and treatment of diarrhea (Lutterodt, 1989; Almeida *et al.*, 1995), and a high level of antibacterial activity was detected in guava leaves (Hidetoshi and Danno, 2002). In several studies, guava showed significant antibacterial activity against common foodborne diarrhea-causing bacteria such as *Staphylococcus* sp., *Shigella* sp., *Salmonella* sp., *Bacillus* sp., *E. coli*, *Clostridium* sp., and food spoilage bacteria such as *Pseudomonas* spp. (Lutterodt, 1989; Hidetoshi and Danno, 2002; Abdelrahim *et al.*, 2002; Jaiarj *et al.*, 1999).

Azadirachta indica A. Juss (neem) belonging to the family Meliaceae is an evergreen tree, cultivated in various parts of the Indian subcontinent. Neem has a long history of use in the traditional medical systems of India (Ayurvedic, Unani-Tibb). Each part of the neem plant has some medicinal property and is thus commercially exploitable. Extracts from neem leaves, seeds, and bark possess a wide spectrum of antibacterial action against Gram-negative and Gram-positive microorganisms, including *Mycobacterium tuberculosis*, *Vibrio cholerae*, and *Klebsiella pneumoniae* (Biswas *et al.*, 2002). Pant *et al.* (1986) reported antifungal activity of leaf extracts of neem. Recently, the antibacterial activity of neem seed oil was assessed *in vitro* against 14 strains of pathogenic bacteria (Biswas *et al.*, 2002). However, very limited or no work has been done evaluating the antibacterial activity of neem plant extracts against foodborne pathogens.

Therefore, this study was designed to assess the antibacterial activity of guava and neem extracts *in vitro* against selected foodborne pathogens and food spoilage bacteria.

Materials and Methods

Plants

Guava and neem leaves were tested. These plant samples were collected from the fields of Bangladesh.

Test organisms

A total of 26 strains of foodborne pathogens and food spoilage bacteria, including *Listeria monocytogenes*, *Staphylococcus aureus*, *Vibrio parahaemolyticus*, *Escherichia coli* O157:H7, *Salmonella* Enteritidis, *Bacillus cereus*, *Pseudomonas* spp., *Alcaligenes faecalis*, and *Aeromonas hydrophila* were used in the study (Table 1). The stock cultures of the test organisms in medium containing 20% glycerol were kept at −84°C. Working cultures were kept at 4°C on tryptic soy agar slants (NISSUI, Tokyo, Japan) and were periodically transferred to fresh slants. A loopful of culture from the slant was transferred to Tryptic soy broth and grown overnight at 37°C. The overnight grown culture was used for the subsequent study.

Preparation of plant extracts

The guava and neem leaves were collected and washed in distilled water and air-dried, and then cut into small pieces. The pieces of the leaves were air-dried at 37°C for 24 hours and the dried leaves were ground using a grinder (IWATANI, Tokyo, Japan) into a fine powder.

Chloroform extracts. Thirty grams of each ground plant sample was added in 120 mL of chloroform (WAKO, Osaka, Japan) in sterile bottles (800 mL) and rotated with constant agitation (130 rpm) overnight at 20°C in a temperature-controlled bioshaker (BR-40 LF, TAITEC, Tokyo, Japan). The chloroform fraction was separated using sterile cheesecloth and filter through sterile Whatman filter paper (no. 2).

Ethanol extracts. The residual plant material of each sample after chloroform extraction was dried at 40°C overnight in an oven. Then 120 mL

Table 1. Test Organisms Used in This Study

Code no. of test organisms	*Organisms*	*No. of type culture*	*Origin*
1Lm	*Listeria monocytogenes*	ATCC 43256	Mexican-style cheese
2Lm	*L. monocytogenes*	ATCC 49594	Scott A
3Lm	*L. monocytogenes*	JCM 7671	Lax ham
4Lm	*L. monocytogenes*	JCM 7672	Roast beef
5Lm	*L. monocytogenes*	JCM 7676	Salami sausage
6Sa	*Staphylococcus aureus*	JCM 2151	Unknown
7Sa	*S. aureus*	JCM 2179	Unknown
8Sa	*S. aureus*	JCM 2874	Wound
9Sa	*S. aureus*	IFO 13276	Human lesion
10Vp	*Vibrio parahaemolyticus*	IFO 12711	Shirasu food poisoning, Japan
11Ec	*Escherichia coli* O157:H7	MN 28	Bovine feces
12Ec	*E. coli* O157:H7	CR 3	Bovine feces
13Ec	*E. coli* O157:H7	DT 66	Bovine feces
14Ec	*E. coli* O157:H7	MY 29	Bovine feces
15Ec	*E. coli* O157:H7	E 615	Tomato juice
16Ec	*E. coli* O157:H7	JCM 1649	Urine
17 Sal	*Salmonella Enteritidis*	SE 1	Chicken feces
18 Sal	*S. Enteritidis*	SE 2	Bovine feces
19 Sal	*S. Enteritidis*	SE 3	Chicken feces
20 Sal	*S. Enteritidis*	IDC 7	Egg
21Bc	*Bacillus cereus*	IFO 3457	Unknown
22 Pa	*Pseudomonas aeroginos*	PA 01	Unknown
23 Pp	*P. putida*	KT 2440	Unknown
24 Af	*Alcaligenes faecalis*	IFO 12669	Unknown
25 Ah	*Aeromonas hydrophila*	NFRI 8282	Unknown
26 Ah	*A. hydrophila*	NFRI 8283	Unknown

of ethanol was added to each dried residue and agitated (130 rpm) overnight at 20°C in a temperature-controlled bioshaker (BR-40 LF, TAITEC). The ethanol fraction was separated using sterile cheesecloth and filter through sterile Whatman filter paper (no. 2).

Aqueous extracts. The residual plant material of each sample after ethanol extraction was dried at 40°C overnight in an oven. Then 120 mL of sterile distilled water was added to each dried residue and agitated (130 rpm) overnight at 20°C in a temperature-controlled bioshaker (BR-40 LF, TAITEC). The aqueous fraction was separated using sterile cheesecloth and filter through sterile Whatman filter paper (no. 2).

All the extracts were then concentrated with a rotary vacuum evaporator (EYELA, Tokyo, Japan) at 40°C and the concentrated extracts were diluted to 10 mg/mL using 10% dimethyl sulfoxide (DMSO) solvent, filter (0.45 μm) sterilized, and kept at −20°C until use.

Antimicrobial sensitivity testing

The antibacterial activity of all the plant extracts was done according to the method of Bauer *et al.* (1966). The 8-mm diameter discs (ADVANTEC; Toyo Roshi Kaisha, Ltd., Tokyo, Japan) were impregnated with 50 μL of different concentrations of each plant extract before being placed on the inoculated agar plates. The inocula of the test organisms were prepared by transferring a loopful of culture into 9 mL of sterile Mueller Hinton broth (Difco, Sparks, MD) and incubated at 37°C for 5 to 6 hours except for *Listeria monocytogenes*, which was grown overnight. The bacterial cultures were compared with McFarland (Jorgensen *et al.* 1999) turbidity standard (10^8 CFU/mL) and streaked evenly in three directions keeping at a 60° angle onto the surface of the Mueller Hinton agar plate (10 × 40 mm) with sterile cotton swab. Surplus suspension was removed from the swab by rotating the swab against the side of the tube before the plate was seeded. After the inocula dried, the impregnated discs were placed on the agar using ethanol-dipped and flamed forceps and were gently pressed down to ensure contact. Plates were kept at refrigeration temperature for 30 to 60 minutes for better absorption. During this time microorganisms will not grow but absorption of extracts would take

place. Negative controls were prepared using the same solvent without the plant extract. Reference antibiotics (streptomycin and gentamycin) were used as positive controls.

The inoculated plates containing the impregnated discs were incubated in an upright position at 37°C overnight and/or 24 to 48 hours (if necessary). The results were expressed as the diameter of inhibition zone around the paper disk (8 mm).

Determination of the minimum inhibitory concentration

The minimum inhibitory concentrations (MICs) of all the extracts were determined by microdilution techniques in Mueller Hinton broth according to Sanches *et al.* (2005). Inoculates were prepared in the same medium at a density adjusted to 0.5 McFarland turbidity standard (Jorgensen *et al.*, 1999) (10^8 colony-forming units [CFU]/mL) and diluted 1:10 for the broth microdilution procedure. Microtiter plates were incubated at 37°C and the MICs were recorded after 24 hours of incubation. Two susceptibility endpoints were recorded for each isolate. The MIC was defined as the lowest concentration of extracts at which the microorganism tested did not demonstrate visible growth. Minimum bactericidal concentration (MBC) was defined as the lowest concentration yielding negative subcultures or only one colony.

Antibacterial activity at different temperatures

The effect of temperature on antibacterial activity of plant extracts was determined by the methods as described by Lee *et al.* (2004). The plant extract solutions were incubated at 37, 50, 75, and 100°C, in a water bath for 30 minutes. Then, the plant extracts heated at different temperatures were cooled and stored at 4°C until use. The antibacterial activity was assayed by the methods described by Bauer *et al.* (1966).

Antibacterial activity at different pH values

The effect of pH on the antibacterial activities of plant extracts of guava and neem were assayed by using methods reported previously with slight modification (Shibata *et al.*, 1995; Ohno *et al.*, 2003). Briefly, the pH of the plant extracts (5 mg/mL) was adjusted to the range of 5.0 to 9.0 with either 50 mM phosphate buffer or 20 mM Tris-HCl buffer. Then the pH-adjusted mixtures were filtered through 0.45-μm membrane filters, stored at 4°C and used within 30 minutes. The antibacterial activity against the cocktails of five strains of *L. monocytogenes*, four strains of *S. aureus*, six strains of *E. coli* O157:H7, and four strains of *Salmonella* Enteritidis was done by the disc diffusion method described above.

Statistical analysis

The inhibition zones were calculated as means $\pm$ SD ($n = 3$). The significance among different data was evaluated by analysis of variance (ANOVA) using the Microsoft Excel program. Significant differences in the data were established by least significant difference at the 5% level of significance.

Results and Discussion

Ethanol extracts of guava were found to have antimicrobial activity against all *L. monocytogenes* strains except for *L. monocytogenes* JCM 7672, all *S. aureus* strains, *V. parahaemolyticus* IFO12711, *Alcaligenes faecalis* IFO 12669, and all *A. hydrophila* strains. The aqueous extract of guava was active against *L. monocytogenes* JCM 7671, all *S. aureus* strains, *V. parahaemolyticus* IFO12711, *B. cereus*, *P. aeroginosa* PA01, *Alcaligenes faecalis* IFO 12669, and all *A. hydrophila* strains (Table 2). However, *S. aureus* JCM2894 and *A. hydrophila* NFRI 8282 were least sensitive to ethanol and aqueous guava extracts, respectively. The chloroform extracts of guava did not show any antibacterial activity against the test strains. The ethanol and aqueous extracts of guava exhibited the highest antimicrobial activities and were able to inhibit 46% and 38% of the test organisms, respectively. Guava extracts exhibited antibacterial activity against both Gram-positive and Gram-negative bacteria. However, no antibacterial activity was found against *E. coli* O157:H7, *Salmonella* Enteritidis, and *Pseudomonas putida* (Table 2). The ethanol extracts of guava showed antibacterial activity against other test microorganism, with zones of inhibition very close to the zones of inhibition of reference antibiotics, gentamycin, used in this

TABLE 2. ANTIBACTERIAL ACTIVITY OF GUAVA AND NEEM EXTRACTS AGAINST FOODBORNE PATHOGENS AND SPOILAGE BACTERIA

Code no. of test organisms	Plant extracts					
	Zones of inhibition[a]					
	Guava		Neem		Antibiotics	
	EtOH	H_2O	$CHCl_3$	EtOH	SM	GM
1Lm	19.4 ± 0.21	—	16.0 ± 0.38	—	17.2 ± 0.20	18.4 ± 0.12
2Lm	21.9 ± 0.26	—	14.2 ± 0.26	20.0 ± 0.46	19.5 ± 0.30	22.9 ± 0.56
3Lm	21.4 ± 0.26	18.0 ± 0.47	—	14.5 ± 0.26	21.5 ± 0.60	23.0 ± 0.30
5Lm	21.4 ± 0.21	—	—	12.0 ± 0.35	18.0 ± 0.47	22.6 ± 0.21
6Sa	15.3 ± 0.06	12.0 ± 0.31	—	13.0 ± 0.38	18.0 ± 0.46	24.5 ± 0.49
7Sa	11.5 ± 0.10	11.5 ± 0.20	—	—	16.5 ± 0.70	22.0 ± 0.12
8Sa	10.7 ± 0.30	11.0 ± 0.56	—	9.0 ± 0.45	16.5 ± 0.80	20.4 ± 0.21
9Sa	12.0 ± 0.21	10.2 ± 0.21	—	12.0 ± 0.21	17.4 ± 0.40	18.1 ± 0.25
10Vp	14.8 ± 0.26	13.1 ± 0.25	—	12.8 ± 0.44	24.5 ± 0.50	30.0 ± 0.31
21Bc	—	13.5 ± 0.20	—	—	16.2 ± 0.20	22.0 ± 0.31
22 Pa	—	12.0 ± 0.38	—	—	15.5 ± 0.26	21.5 ± 0.66
23 Pp	—	—	—	—	19.3 ± 0.21	21.0 ± 0.31
24 Af	11.4 ± 0.21	14.5 ± 0.36	—	—	—	9.5 ± 0.50
25 Ah	12.2 ± 0.21	10.0 ± 0.38	—	—	10.7 ± 0.26	19.2 ± 0.21
26 Ah	13.0 ± 0.21	14.0 ± 0.21	—	—	11.0 ± 0.47	15.0 ± 0.47

[a]Represents mean ± S.D mm ($n = 3$); $p > 0.05$; EtOH = ethanol; H_2O = aqueous; $CHCl_3$ = chloroform; SM = streptomycin (30 μg); GM = gentamycin (10 μg).

study. This result is consistent with the previous reports by Jaiarj *et al.* (1999), Gnan and Demello (1999), Oliver-Bever (1986), and Sanches *et al.* (2005).

Hidetoshi and Danno (2002) reported four flavonoid compounds extracted by 90% (v/v) aqueous methanol from guava exhibited antibacterial activity against *B. cereus* and *Salmonella* Enteritidis. However, in our experiment the ethanol extracts of guava did not show any activity against *B. cereus* IFO 3457 and *Salmonella* Enteritidis.

The chloroform extracts of neem showed antibacterial activity against *L. monocytogenes* ATCC 43256 and *L. monocytogenes* ATCC 49594. However, ethanol extracts of neem showed activity against *L. monocytogenes* ATCC 49594, *L. monocytogenes* JCM 7671, *L. monocytogenes* JCM 7676, and *Staphlococcus aureus* JCM 2151, *S. aureus* JCM 2894, *S. aureus* IFO 13276, and *V. parahaemolyticus* IFO 12711. The aqueous extracts of neem did not show any antibacterial activity against the test strains.

The chloroform and ethanol extracts of neem showed antibacterial activity against 7% and 26% of the test organisms, respectively. In addition, ethanol extracts of neem showed antibacterial activity against *V. parahaemolyticus* IFO 12711, however the chloroform extracts of neem did not show antibacterial activity against *V. parahaemolyticus* IFO 12711. The ethanol extract of neem showed zones of inhibition against test microorganism were very close to the zones of inhibition of reference antibiotics used in this study (Table 2). Preliminary studies carried out by several investigators showed significant effects of neem extracts on several bacterial strains (Rao *et al.*, 1986; Chopra *et al.*, 1952, 1956; Chopra, 1958; Rojanapo, 1985). Mahmoodin, one of the neem's medicinal compounds, showed significant antibacterial activity against various Gram-positive and Gram-negative microorganisms (Seddiqui *et al.*, 1992). The antibacterial activity of neem extracts against *Staphylococcus aureus* (Schneider, 1986), *Streptococcus pyogenes*, Cornebacterium, and *E. coli* (Thaker and Anjaria, 1986), *Salmonella typhosa* (Patel and Trivedi, 1962; Chopra, 1958) has been reported. In our experiment, extract of neem did not show antimicrobial activity against any of the Gram-negative bacteria tested but were highly effective in controlling Gram-positive and spoilage microorganism.

The MIC results are listed in Table 3. The MIC of ethanol extract of guava showed the highest inhibition for *L. monocytogenes* JCM 7676

TABLE 3. MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM BACTERICIDAL CONCENTRATION (MBC) OF GUAVA AND NEEM EXTRACTS AGAINST FOODBORNE PATHOGENS AND SPOILAGE BACTERIA

	Plant extracts			
	MIC (MBC)			
	Guava		*Neem*	
Code no. of test organisms	*EtOH*	*H_2O*	*$CHCl_3$*[a]	*EtOH*
1Lm	0.3 (0.4)	—	5.0 (5.5)	—
2Lm	0.4 (0.5)	—	5.0 (5.5)	6.5 (7.0)
3Lm	0.1 (0.5)	7.0 (7.5)	—	6.5 (7.0)
5Lm	0.1 (0.2)	—	—	6.5 (7.0)
6Sa	0.1 (0.2)	4.5 (5.0)	—	4.5 (5.0)
7Sa	0.1 (0.2)	4.0 (4.5)	—	6.5 (7.0)
8Sa	0.4 (0.5)	3.5 (4.0)	—	6.5 (7.0)
9Sa	0.7 (0.8)	5.0 (5.5)	—	4.5 (5.0)
10Vp	0.1 (0.2)	3.0 (3.5)	—	6.5 (7.0)
21Bc	—	3.0 (3.5)	—	—
22 Pa	—	—	—	—
23 Pp	—	—	—	—
24 Af	4.0 (4.5)	0.9 (1.0)	—	—
25 Ah	1.5 (2.0)	2.0 (2.5)	—	—
26 Ah	4.0 (4.5)	3.5 (4.0)	—	—

Dashes represent no positive activity found during preliminary screening.

Parentheses value represents minimum bactericidal concentration (MBC).

[a]MIC was done by disk diffusion method.

(0.1 mg/mL), *S. aureus* JCM 2151 (0.1 mg/mL), *S. aureus* JCM 2179 (0.1 mg/mL) and *V. parahaemolyticus* IFO 12711 (0.1 mg/mL). The same extract showed the lowest inhibition for *A. faecalis* IFO 12669 (4.0 mg/mL) and *A. hydrophila* NFRI 8283 (4.0 mg/mL). The aqueous extracts of guava showed the highest inhibition for *A. faecalis* IFO 12669 (0.9 mg/mL) and the lowest inhibition for *L. monocytogenes* JCM 7671 (7.0 mg/mL). Guava leaf extracts have been reported to have MIC values ranging from 150 μg/mL to 4 mg/mL for inhibition of bacterial pathogens (Prabu *et al.*, 2006; Hidetoshi and Danno, 2002; Sanches *et al.*, 2005).

The MIC of the chloroform extract of neem showed the same inhibition concentrations for *L. monocytogenes* ATCC 43256 (5.0 mg/mL) and *L. monocytogenes* ATCC 49594 (5.0 mg/mL). However, the ethanol extract showed the highest inhibition for *V. parahaemolyticus* IFO 12711 (4.5 mg/mL) and the lower inhibition against the other pathogens (6.5 mg/mL).

The antibacterial activity of the extracts heated to 50°C for 30 minutes was found almost unchanged compared to nonheated extracts, while the inhibitory effect of boiled plant extracts was significantly decreased (Table 4). The activity of aqueous extracts of guava was lost completely when heated to 100°C against a cocktail of *S. aureus* strains. The antibacterial activity was not affected at pH 5.0, however, significant decrease of inhibitory activity was found at pH 9.0. The antibacterial activity of the ethanol extract of guava was lost completely at pH 9.0 against a cocktail of *L. monocytogenes*. The ethanol extract of neem showed no activity against cocktail of *L. monocytogenes* at pH 7.0 and 9.0 (Table 5). Lisboa *et al.* (2006) showed similar findings with bacteriocin-like substances

TABLE 4. EFFECT OF TEMPERATURE ON ETHANOL AND AQUEOUS EXTRACTS OF GUAVA AND ETHANOL EXTRACT OF NEEM AGAINST COCKTAILS OF THE FOODBORNE PATHOGENS AND SPOILAGE BACTERIA

		Zones of inhibition[a]					
		Temperature (°C)					
Plant extracts	*Cocktails of test organisms (serotype code numbers)*	*4*	*25*	*37*	*50*	*75*	*100*
Guava EtOH	*L. monocytogenes* (1Lm–3Lm, 5Lm)	12.0 ± 0.2	12.0 ± 0.1	12.0 ± 0.3	11.0 ± 0.5	10.5 ± 0.2	10.0 ± 0.4
Guava EtOH	*S. aureus* (6Sa–9Sa)	12.0 ± 0.4	12.0 ± 0.5	12.0 ± 0.6	10.5 ± 0.4	10.0 ± 0.3	9.0 ± 0.1
Guava H_2O	*S. aureus* (6Sa–9Sa)	10.0 ± 0.3	11.0 ± 0.5	10.0 ± 0.6	10.0 ± 0.5	9.0 ± 0.2	0.0 ± 0.0
Neem EtOH	*S. aureus* (6Sa–9Sa)	14.0 ± 0.3	12.0 ± 0.3	10.2 ± 0.2	10.0 ± 0.8	9.0 ± 0.3	9.0 ± 0.6
Neem EtOH	*L. monocytogenes* (2Lm, 3Lm, 5Lm)	11.5 ± 0.3	11.5 ± 0.2	10.0 ± 0.5	10.0 ± 0.4	9.0 ± 0.4	9.0 ± 0.7

[a]Represents mean ± SD mm ($n = 3$); $p > 0.05$.

Table 5. Effect of pH on Ethanol and Aqueous Extracts of Guava and Ethanol Extract of Neem Against Cocktails of the Foodborne Pathogens and Spoilage Bacteria

		Zones of inhibition[a]		
		pH		
Plant extracts	*Cocktails of test organisms (serotype code no.)*	*5.0*	*7.0*	*9.0*
Guava EtOH	*L. monocytogenes* (1Lm–3Lm, 5Lm)	12.0 ± 0.4	10.0 ± 0.2	0.0 ± 0.0
Guava EtOH	*S. aureus* (6Sa–9Sa)	11.0 ± 0.1	10.0 ± 0.1	9.8 ± 0.1
Guava H_2O	*S. aureus* (6Sa–9Sa)	12.1 ± 0.6	10.5 ± 0.1	10.0 ± 0.4
Neem EtOH	*S. aureus* (6Sa–9Sa)	14.2 ± 0.1	9.5 ± 0.2	9.0 ± 0.5
Neem EtOH	*L. monocytogenes* (2Lm, 3Lm, 5Lm)	11.5 ± 0.1	0.0 ± 0.0	0.0 ± 0.0

[a]Represents mean ± SD mm ($n = 3$); $p > 0.05$.

produced by *Bacillus amyloliquefaciens* isolated from Brazilian Atlantic forest against *L. monocytogenes*.

Conclusion

In this study, guava and neem extracts showed antibacterial activity against selected foodborne pathogens and spoilage microorganisms. The result of this study also suggests that guava and neem extracts possess compounds containing antibacterial properties that can be useful to control foodborne pathogens and spoilage organisms. Antibacterial extracts obtained in the present study will be applied to actual foods to assess the microbiological condition of the particular food or food products with extended shelf-life.

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