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Prebiotic Potential and Chemical Composition of Seven Culinary Spice Extracts

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Abstract: The objective of this study was to investigate prebiotic potential, chemical composition, and antioxidant capacity of spice extracts. Seven culinary spices including black pepper, cayenne pepper, cinnamon, ginger, Mediterranean oregano, rosemary, and turmeric were extracted with boiling water. Major chemical constituents were characterized by RP-HPLC-DAD method and antioxidant capacity was determined by measuring colorimetrically the extent to scavenge ABTS radical cations. Effects of spice extracts on the viability of 88 anaerobic and facultative isolates from intestinal microbiota were determined by using Brucella agar plates containing serial dilutions of extracts. A total of 14 phenolic compounds, a piperine, cinnamic acid, and cinnamaldehyde were identified and quantitated. Spice extracts exhibited high antioxidant capacity that correlated with the total amount of major chemicals. All spice extracts, with the exception of turmeric, enhanced the growth of *Bifidobacterium* spp. and *Lactobacillus* spp. All spices exhibited inhibitory activity against selected *Ruminococcus* species. Cinnamon, oregano, and rosemary were active against selected *Fusobacterium* strains and cinnamon, rosemary, and turmeric were active against selected *Clostridium* spp. Some spices displayed prebiotic-like activity by promoting the growth of beneficial bacteria and suppressing the growth of pathogenic bacteria, suggesting their potential role in the regulation of intestinal microbiota and the enhancement of gastrointestinal health. The identification and quantification of spice-specific phytochemicals provided insight into the potential influence of these chemicals on the gut microbial communities and activities. Future research on the connections between spice-induced changes in gut microbiota and host metabolism and disease preventive effect in animal models and humans is needed.

Keywords: *Bifidobacterium*, intestinal bacteria, *Lactobacillus*, minimum inhibitory concentration, spice

Practical Application: This research shows that aqueous extract of culinary spice exhibited high antioxidant capacity that correlated with the total amount of major chemicals. Some spices displayed prebiotic-like activity by promoting the growth of beneficial bacteria and suppressing the growth of pathogenic bacteria. These results provide a basis for further study on the connections between spice-induced changes in gut microbiota and host metabolism and disease preventive effect in animal models and humans.

Introduction

The gastrointestinal tract, especially the large intestine, houses the most abundant and complex microbiota in humans. Most of intestinal bacteria belong to the phylum *Firmicutes* (including *Clostridium*, *Enterococcus*, *Lactobacillus*, and *Ruminococcus* genera) and *Bacteroidetes* (including *Prevotella* and *Bacteroides* genera), which make up more than 90% of known phylogenetic categories and dominate the distal gut microbiota. Other lower abundance bacteria include *Actinobacteria*, *Fusobacteria*, *Proteobacteria*, and *Verrucomicrobia*. Diet is one of the important factors contributing to the gut microbial composition that ultimately affects human health. Obesity and associated metabolic diseases, including type 2 diabetes, are intimately linked to diet (Sonnenburg and Backhed 2016). A number of recent *in vitro*, *in vivo*, and human studies showed that polyphenols or polyphenol–rich dietary sources, particularly tea, wine, cocoa, fruits, and fruit juices, influence the relative abundance of different bacterial groups within the gut microbiota by reducing the numbers of potential pathogens and certain gram-negative *Bacteroides* spp. and enhance beneficial bifidobacteria and lactobacilli (Duenas and others 2015).

Spices are derived from bark, fruit, seeds, or leaves of plants and often contain spice-specific phytochemicals. Spices have been used not only for seasoning of foods but also for medicinal purposes, and have a number of demonstrated disease preventive functions such as antimicrobial, antiinflammatory, antimutagenic activities, and are known to reduce the risk of cancer, heart disease, and diabetes (Surh 2003; Bi and others 2017; Kocaadam and Sanlier 2017). They are best known for their strong antioxidant properties that exceed most foods. It was reported that of the 50 food products highest in antioxidant concentrations among 1113 U.S. food samples, 13 were spices. Among them, oregano, ginger, cinnamon, and turmeric ranked #2, 3, 4, and 5, respectively (Halvorsen and others 2006). Previous research from our group reported that consumption of hamburger meat with spice mix added prior to cooking resulted in a reduction in the concentration of malondialdehyde, a lipid peroxidation marker, in the meat and in plasma and urine of healthy volunteers, and improved postprandial endothelial dysfunction in men with Type 2 diabetes (Li and others 2010a, b). Subsequent study reported that commercial spices in dry or fresh form exhibited significant antioxidant capacity that correlated with total phenolic content but

not with the concentration of chemical biomarker (Henning and others 2011).

There is limited amount of information regarding the activity of culinary spice extracts against clinical isolated intestinal bacteria, and a limited number of bacterial strains have been assessed for their susceptibility or antimicrobial activity against spices. Gunes and colleagues reported that minimum inhibitory concentration (MIC) of curcumin against 7 standard bacterial strains (*Staphylococcus aureus*, methicillin-resistant *S. aureus*, *Enterococcus faecalis*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*) is in the range of 129 to 293 μg/mL (Gunes and others 2016a). Cinnamaldehyde, a bioactive component of cinnamon, was shown to exhibit more potent in vitro antibacterial properties against 5 common foodborne pathogenic bacteria (*Bacillus cereus*, *Listeria monocytogenes*, *S. aureus*, *E. coli*, and *Salmonella anatum*) with MIC being 125 to 500 μg/mL as compared to crude cinnamon stick extract (625 to >2500 μg/mL; Shan and others 2007), but cinnamaldehyde did not modulate the population of selected *Lactobacillus* and *Bifidobacterium* counts in mouse cecal content (Khare and others 2016). Supplementation of rosemary extract was reported to increase *Bacteroides/Prevotella* groups and reduce the *Lactobacillus/Leuconostoc/Pediococcus* group in the caecum of both obese and lean rats (Romo-Vaquero and others 2014). Based on potential health benefits demonstrated from our group, this study investigated major chemical constituents, antioxidant activity, and *in vitro* effect of 7 spice extracts on the growth of 33 beneficial *Bifidobacterium* spp. and *Lactobacillus* spp., and established their antimicrobial activity against 88 intestinal, pathogenic, and toxigenic bacterial strains.

Materials and Methods

Reagent and chemicals

All organic solvents were High-performance liquid chromatography (HPLC) grade (Thermo Fisher Scientific, Fairlawn, N.J., U.S.A.). Deionized water was purified by the Milli-Q system (Millipore, Bedford, Mass., U.S.A.). Reference standards apigenin, capsaicin, dihydrocapsaicin, cinnamic acid, cinnamaldehyde, *p*-coumaric acid, bisdemethoxycurcumin, demethoxycurcumin, curcumin, luteolin, luteolin-3-glucuronide, piperine, rosmarinic acid, and vanillin were purchased from Sigma-Aldrich (St. Louis, Mo., U.S.A.). Ferulic acid was purchased from USP (Rockville, Md., U.S.A.) and 6-gingerol from Chromadex (Irvine, Calif., U.S.A.). They were used for the identification and quantification of spice chemicals.

Spice samples and extract preparation

Seven culinary spice samples, black pepper (BLP), cayenne pepper (CAP), cinnamon (CIN), ginger (GIN), Mediterranean oregano (ORE), rosemary (ROS), and turmeric (TUR; McCormick Company, Inc., Sparks, Md., U.S.A.) were extracted by refluxing 7 g of dry spice powder in 70 mL (or 100 mg/mL) of Milli-Q water for 30 min. Aqueous food extracts were reported to be more effective on the cultured bacteria (*Lactobacillus reuteri*, *Lactobacillus rhamnosus*, *Bifidobacterium lactis*, *E. coli* 0157:H7, and *E. coli* LF82) than the organic extracts (Sutherland and others 2009). After cooling down, approximately 38.5 mL of supernatants were collected after centrifuging and filtration, and then stored at −20 °C. The concentration of each aqueous extract was 182 mg dry spice per milliliter after adjusting for final volume of 38.5 mL, which was used directly for chemical analysis and the measurement of antioxidant activity.

Identification and quantification of phenolic compounds

After mixing well, an aliquot of 1.0 mL of aqueous extract was mixed with 1.0 mL of dimethyl sulfoxide. The resulting mixture was then vortexed for 5 min and centrifuged at 20600 $\times$ g for 10 min. An aliquot of 25 μL of the supernatant was injected into HPLC. The quantification of phenolic compounds was performed in a 2690 Waters HPLC system equipped with a diode array detector and a Zorbax SB C18 column (4.6 $\times$ 150 mm, 3.5 μm; Agilent, Santa Clare, Calif., U.S.A.) connected to a disposable pre-column (C18, 5 μm, 4.6 $\times$ 20 mm, Phenomenex, Torrance, Calif., U.S.A.) with temperature held at 30 °C. The mobile phase consisted of 0.1% phosphoric acid in water (A) and 100% acetonitrile (B). A linear gradient was performed with initial 2% B increasing to 25% B in 25 min, and then to 40% B from 25 to 32 min, to 50% B from 32 to 40 min and 70% B from 40 to 50 min. The flow rate was 0.75 mL/min and the chromatograms were recorded at 280 nm for CAP, BLP, CIN, and GIN, 310 nm for GIN, 330 nm for ORE and ROS, and 425 nm for TUR. Chemicals from ROS extract were analyzed using Phenomenex Prodigy (250 $\times$ 4.6 mm) column with same mobile phase, flow rate, and column temperature but slightly modified gradient. Chemical identification for each spice was determined by comparing peak retention time and UV absorption spectrum to those from reference standard. The concentrations of major chemicals from each spice extract were determined using external calibration curves. Stock solutions of reference standards were prepared in the concentration of 1.00 mg/mL in methanol and calibration solutions were prepared by diluting stock solutions with 50% aqueous methanol. For all calibration curves, there were linear relationships between peak area and concentration in the range of 0.7825 to 100 μg/mL. Lithospermic acid in ORE was characterized based on the published UV absorption maxima (Grevsen and others 2009) and its concentration was determined by using the calibration curve of rosmarinic acid.

Trolox equivalent antioxidant capacity

Trolox equivalent antioxidant capacity (TEAC) was determined by measuring the ability to scavenge 2′,2′-azinobis (3-ethylbenzothiazline-6-sulfonic acid (ABTS) radical cation produced by the oxidation of ABTS with manganese dioxide as previously described (Lu and others 2015). Briefly, ABTS radical cations were prepared by adding solid manganese dioxide (80 mg) to a 5 mM aqueous stock solution of ABTS diammonium salt (20 mL using a 75 mM sodium/potassium [Na/K] buffer of pH 7). Trolox (6-hydroxy-2, 5, 7, 8-tetramethyl-chroman-2-carboxylic acid) was used as an antioxidant standard. Extract was 1st diluted in Na/K buffer, and the resulting solution was mixed with 200 μL of ABTS$^{·+}$ solution in 96-well plates. Absorbance was read at 750 nm after 30 min in a VersaMax microplate reader (Molecular Devices, Sunnyvale, Calif., U.S.A.). Final value is expressed as trolox equivalent (TE).

Bacterial strains, growth stimulation, and inhibition tests

Eighty-eight strains of bacteria included in this study were clinical isolates from patients in the Greater Los Angeles VA Healthcare Center as published (Li and others 2015a), or purchased from American Type Culture Collection (ATCC). Altogether 17 strains of *Bifidobacterium* species consisting of 11 strains recovered from human intestinal contents and 6 ATCC *Bifidobacterium* type strains were studied. Similarly, 16 strains of *Lactobacillus* species consisting of 12 strains recovered from human intestinal contents and 4 ATCC *Lactobacillus* type strains were studied. Other bacterial

strains, including 1 *Akkermansia*, 7 *Bacteroides* spp., 21 *Clostridium* spp., including 12 toxigenic *Clostridium difficile*, 2 *E. coli*, 3 *Finegoldia magna*, 11 *Fusobacterium* spp., 1 *Peptoniphilus asaccharolyticus*, 1 *Peptostreptococcus anaerobius*, 4 *Ruminococcus* spp., 2 *Salmonella typhi*, and 2 *S. aureus* strains, were also tested. These bacteria have been identified by 16S rRNA sequence analysis as published (Li and others 2015a).

Brucella agar (BD BBL, Sparks, Md., U.S.A.) plates containing serial dilutions of each spice extract and control plates without the tested spice were prepared (Hecht and others 2012; Jousimies-Somer and others 2012). Spice extracts (182 mg dry spice per milliliter) were 1st diluted by 20 times to give 9.0 mg/mL concentration which was further diluted to give 4.50, 2.25, 1.13, 0.56, 0.28, 0.14, and 0.07 mg/mL of extracts. The bacterial strains were cultured on Brucella agar plates for 48 h before testing. A suspension of colonies was used to prepare Brucella broth tubes with a density equal to that of a 0.5 McFarland standard corresponding to density of 10^8 bacterial colony forming units (CFU)/mL. A volume of 10 μL of these suspensions was inoculated on the spice extract-containing plates, achieving a final inoculum of 10^5 CFU/spot. Brucella agar plates were used as a positive growth control. The plates were incubated in an anaerobic chamber for 48 h at 37 °C. Anaerobic conditions consisted of a gas mixture of 5% CO_2, 5% H_2, and 90% N_2; the residual oxygen was removed by palladium catalysts (Jousimies-Somer and others 2012). After incubation, the plates were examined.

The MICs were determined. MIC is defined as the lowest concentration of each spice resulting in no growth or a marked change in the appearance of growth as compared to the control plate, as described in the Clinical and Laboratory Standards Institute-approved protocol (Hecht and others 2012). Further, the concentrations resulting in the change of the appearance of growth as compared to the control plate were recorded to establish for each test strain the concentration where the tested spice had no effect on the growth, where the spice stimulated the growth, and where the growth stimulation by the test spice reached a plateau (Li and others 2015a).

Statistical analysis

Chemical analysis and antioxidant assay were carried out in triplicate and data were presented as mean ± standard deviation (SD). Microbiology assays were performed in duplicate independent assays and averaged data were reported.

Results and Discussion

Major chemical constituents and antioxidant capacity

Spices often contain a variety of chemicals that include phenolic compounds, alkaloids, and terpenoids that are often characteristic for each particular spice. In this study, we identified and quantitated 14 major phenolic ingredients from the aqueous extract of CAP, GIN, ORE, ROS, and TUR, an alkaloid piperine from BLP, and cinnamic acid and cinnamaldehyde from CIN. A small number of phenolic compounds such as ferulic acid, vanillin, and rosmarinic acid were present in more than 1 spice (Table 1). Because our extraction method was designed to simulate cooking and not to optimize to extract all constituents, the number of chemicals reported was limited. However, they consisted of marker compounds and major bioactive constituents present in aqueous extracts. The total concentration of major chemicals quantified was the highest in ROS (2475 ± 87.0) and lowest in CAP (30.7 ± 3.8) μg/mL.

The order was ROS ≥ ORE > CIN > GIN > TUR > BLP > CAP.

Average antioxidant capacity of each spice extract determined as TE is also listed in Table 1. The antioxidant capacity ranged from 140.4 to 13.1 mM TE with ROS and ORE being the highest and GIN the lowest. The order of TE was ROS $\sim$ ORE > TUR > CIN > BLP $\sim$ CAP > GIN. The antioxidant capacity of spice extracts correlated well with the total chemical concentration ($r = 0.86$, $r^2 = 0.74$).

Results from our chemical investigations indicated that most spice extracts contain either moderate or high content of spice-specific phytochemicals. The identification and quantitation of these phytochemicals provide insight into the potential influence of these chemicals on the gut microbial communities and activities. Among the chemicals characterized, majority belongs to polyphenols and phenolic acids. Convincing evidences suggest that the beneficial effects attributable to dietary (poly)phenols depend on their biotransformation by the gut microbiota. Most polyphenols present in the diet are in the form of esters, glycosides, or polymers that cannot be absorbed in their native form. It is estimated that about 5% to 10% of dietary polyphenols are absorbed and reach plasma. Over 95% of the intake passes to the colon and is fermented by gut microbiota to produce small aromatic or phenolic acids (Clifford 2004). For example, rosmarinic acid, found abundantly in ORE, ROS, and in many *Lamiaceae* plants, is an ester of caffeic acid and 3-(3′,4′-dihydroxyphenyl)lactic acid. A recent study reported a complete degradation of rosmarinic acid, and the generation of microbial product caffeic acid after *in vitro* fermentation using human feces (Mosele and others 2014). The caffeic acid undergoes subsequent microbial transformation to yield hydroxyphenylpropionic acids as major metabolites, a process mediated by microbial chlorogenate esterases or by *Lactobacillus johnsonii* (Bel-Rhlid and others 2009). Results of human intervention study confirmed these *in vitro* findings (Mosele and others 2014). These and other microbial metabolites were found to selectively inhibit the growth of pathogenic bacteria and stimulate the growth of beneficial microorganisms (Madureira and others 2016).

Growth stimulatory effect

Table 2 illustrates the growth stimulatory effect of spice extracts on 17 strains of *Bifidobacterium* and 16 strains of *Lactobacillus*. Values represent the lowest concentrations of spice extract that stimulated growth. Of the 17 *Bifidobacterium* strains tested, the growth of 1 strain was stimulated by ORE at 0.56 mg/mL, the lowest concentration showing the stimulating effect. At 1.13 mg/mL, both ORE and BLP enhanced the growth of another strain. At 2.25 mg/mL, 1 strain was stimulated by BLP, 4 by GIN, and 6 by ORE. At 4.5 mg/mL, 7 strains were stimulated by BLP, 10 by CAP, 6 by GIN, 4 by ORE, and 1 by TUR.

Of the 16 *Lactobacillus* strains tested, the growth of 1 strain was enhanced by BLP and another one by CAP at the concentration of 0.56 mg/mL. At 1.13 mg/mL, 4 strains were stimulated by BLP, 5 by CAP, 7 by GIN, and 10 by ORE. At 2.25 mg/mL, 2 strains were stimulated by BLP, 7 by CAP, 4 by GIN, and 2 by TUR. Overall, all spice extracts showed better growth stimulatory effect on the *Lactobacillus* spp. as compared to *Bifidobacterium* spp. Some foods can exert growth stimulatory effect because their ingredients can be used as substrates by these bacteria, or they can enhance nutrients consumption by affecting bacteria metabolism (Hervert-Hernandez and others 2009; Madureira and others 2016).

Prebiotics are defined as substances that induce the growth or activity of microorganisms that contribute to the well-being of their

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Table 1–Concentrations (µg/mL) of major ingredients and antioxidant capacity (mM) in black pepper (BLP), cayenne pepper (CAP), cinnamon (CIN), ginger (GIN), Mediterranean oregano (ORE), rosemary (ROS), and turmeric (TUR) extracts.

	BLP	CAP	CIN	GIN	ORE	ROS	TUR
Piperine	87.6 ± 4.2						
Capsaicin		12.9 ± 1.3					
Dihydrocapsaicin		4.1 ± 0.3					
Ferulic acid		1.4 ± 0.3		2.0 ± 0.1			13.2 ± 1.7
Luteolin		1.1 ± 0.3					
Apigenin		11.2 ± 1.6					
Cinnamic acid			66.8 ± 5.5				
Cinnamaldehyde			926 ± 56.0				
6-Gingerol				170 ± 8.5			
Vanillin				3.30 ± 0.3			8.2 ± 1.0
P-coumaric acid				1.80 ± 0.2			11.8 ± 1.3
Rosmarinic acid					1297 ± 11.0	1830 ± 72.0	
Lithospermic acid					706 ± 19.0		
Luteolin–3-glucuronide						645 ± 15.0	
Bisdemethoxycurcumin							27.6 ± 3.0
Demethoxycurcumin							20.4 ± 1.9
Curcumin							22.8 ± 1.5
Total concentration	87.6 ± 4.2	30.7 ± 3.8	992.8 ± 61.5	177.1 ± 9.1	2003 ± 30.0	2475 ± 87.0	104 ± 10.4
Antioxidant capacity	21.8 ± 0.8	21.2 ± 1.1	58.8 ± 0.6	13.1 ± 0.2	139.6 ± 4.1	140.4 ± 6.0	91.9 ± 2.1

Table 2–Lowest concentrations[a] (mg/mL) of black pepper (BLP), cayenne pepper (CAP), cinnamon (CIN), ginger (GIN), Mediterranean oregano (ORE), rosemary (ROS), and turmeric (TUR) extracts showing the growth stimulation of *Bifidobacterium* and *Lactobacillus* species.

Source	Strain no.	Genus	Species	BLP	CAP	CIN	GIN	ORE	ROS	TUR
ATCC	15703	*Bifidobacterium*	*Adolescentis*	nd[b]	9	9	4.5	4.5	9	nd
Stool	19896	*Bifidobacterium*	*Adolescentis*	2.25	4.5	9	4.5	1.13	nd	nd
Stool	19814	*Bifidobacterium*	*Animalis*	9	4.5	9	4.5	2.25	9	nd
Stool	19909	*Bifidobacterium*	*Animalis*	nd	nd	nd	nd	9	9	nd
Stool	19886	*Bifidobacterium*	*Bifidum*	4.5	4.5	9	4.5	2.25	9	nd
Stool	19893	*Bifidobacterium*	*Bifidum*	4.5	4.5	9	4.5	2.25	9	nd
ATCC	15696	*Bifidobacterium*	*Bifidum*	4.5	2.25	9	2.25	2.25	9	nd
ATCC	15700	*Bifidobacterium*	*Breve*	9	4.5	nd	nd	nd	nd	nd
Stool	19855	*Bifidobacterium*	*Catenaforme*	4.5	4.5	9	2.25	0.56	9	nd
Stool	19815	*Bifidobacterium*	*Infantis*	4.5	4.5	9	nd	2.25	9	nd
ATCC	15697	*Bifidobacterium*	*infantis*	4.5	9	nd	2.25	nd	nd	nd
Stool	19907	*Bifidobacterium*	*Longum*	1.13	4.5	9	4.5	2.25	9	nd
ATCC	15707	*Bifidobacterium*	*Longum*	nd	4.5	9	2.25	nd	nd	nd
Stool	19860	*Bifidobacterium*	*Longum*	nd	nd	nd	nd	9	9	nd
Stool	19891	*Bifidobacterium*	*Longum*	9	9	nd	nd	4.5	9	nd
Stool	19892	*Bifidobacterium*	*Longum*	9	nd	nd	nd	4.5	9	nd
ATCC	27919	*Bifidobacterium*	*Pseudocatenulatum*	4.5	4.5	nd	9	4.5	9	4.5
ATCC	4356	*Lactobacillus*	*Acidophilus*	nd	9	nd	nd	nd	nd	nd
Stool	19925	*Lactobacillus*	*Breve*	4.5	4.5	9	2.25	9	9	4.5
ATCC	9595	*Lactobacillus*	*Casei*	1.13	1.13	9	1.13	1.13	9	nd
Stool	19882	*Lactobacillus*	*Casei*	1.13	2.25	9	1.13	1.13	9	nd
Stool	19908	*Lactobacillus*	*Casei*	4.5	1.13	9	2.25	1.13	9	4.5
Stool	19983	*Lactobacillus*	*Crispatus*	nd	2.25	nd	nd	nd	nd	nd
Stool	19935	*Lactobacillus*	*Fermentum*	2.25	2.25	9	1.13	1.13	9	nd
ATCC	14931	*Lactobacillus*	*Fermentum*	0.56	0.625	9	1.13	1.13	9	nd
Stool	19897	*Lactobacillus*	*Gasseri*	nd	1.25	nd	nd	4.5	nd	nd
Stool	19879	*Lactobacillus*	*Gasseri*	nd	4.5	nd	nd	nd	nd	nd
ATCC	53103	*Lactobacillus*	Gg	4.5	0.56	4.5	2.25	1.13	4.5	2.25
Stool	19911	*Lactobacillus*	*Johnsonii*	nd	2.25	nd	nd	4.5	nd	nd
Stool	19883	*Lactobacillus*	*Lactis*	1.13	2.25	9	1.13	1.13	9	nd
Stool	19884	*Lactobacillus*	*Plantarum*	4.5	1.13	9	2.25	1.13	9	2.25
Stool	19888	*Lactobacillus*	*Reuteri*	1.13	1.13	9	1.13	1.13	9	nd
Stool	19920	*Lactobacillus*	*Rhamnosus*	2.25	2.25	9	1.13	1.13	9	nd

[a]Values are average of 2 tests.
[b]nd: growth stimulation was not detected.

host (Hutkins and others 2016). *Lactobacillus* and *Bifidobacterium* are widely established bacterial genera as prebiotic target organisms. These 2 genera produce acetic acid and lactic acid as the major end-metabolites and do not contain any known pathogens. Previous studies by our group and others have identified dietary sources functioning as prebiotics by stimulating the growth of *Bifidobacterium* spp. and *Lactobacillus* spp. in both *in vitro* and human studies (Li and others 2015a, b; Molan and others 2009; Mandalari and others 2010; Vendrame and others 2011). Evidence is growing in support of the prebiotic effect of foods high in polyphenols

Table 3–Minimum inhibitory concentrations[a] (mg/mL) of black pepper (BLP), cayenne pepper (CAP), cinnamon (CIN), ginger (GIN), Mediterranean oregano (ORE), rosemary (ROS), and turmeric (TUR) extracts against intestinal bacterial species.

Strain	Source	Genus	Species	BLP	CAP	CIN	GIN	ORE	ROS	TUR
19982	ATCC	*Akkermansia*	*Muciniphila*	>9	>9	9	>9	>9	>9	>9
25285	ATCC	*Bacteroides*	*Fragilis*	>9	>9	>9	>9	>9	>9	>9
18241	Stool	*Bacteroides*	*Fragilis*	>9	>9	>9	>9	>9	>9	>9
18257	Stool	*Bacteroides*	*Fragilis*	>9	>9	4.5	>9	>9	9	>9
18286	Stool	*Bacteroides*	*Fragilis*	>9	>9	>9	>9	>9	>9	>9
18249	Stool	*Bacteroides*	*Ovatus*	>9	>9	>9	>9	>9	>9	>9
18271	Stool	*Bacteroides*	*Thetaiotaomicron*	>9	>9	>9	>9	>9	>9	>9
29742	ATCC	*Bacteroides*	*Thetaiotaomicron*	>9	>9	>9	>9	>9	>9	>9
	Table 2	*Bifidobacterium*	spp.; 17 strains	>9	>9	>9	>9	>9	>9	>9
16469	Stool	*Clostridium*	*Bifermentans*	>9	>9	>9	>9	>9	>9	>9
16351	Stool	*Clostridium*	*Bolteae*	>9	>9	9	>9	>9	>9	>9
17059	Stool	*Clostridium*	*Butyricum*	>9	>9	>9	>9	>9	>9	>9
17162	Stool	*Clostridium*	*Butyricum*	>9	>9	3.42	>9	>9	4.5	5.65
	Stool	*Clostridium*	*Difficile*; 12 strains	>9	>9	4.5–9[b]	>9	>9	>9	>9
17490	Stool	*Clostridium*	*Orbiscindens*	>9	>9	4.5	>9	>9	6.82	>9
14572	Stool	*Clostridium*	*Perfringens*	>9	>9	>9	>9	>9	>9	>9
14824	Stool	*Clostridium*	*Perfringens*	>9	>9	>9	>9	>9	>9	>9
16448	Stool	*Clostridium*	*Perfringens*	>9	>9	9	>9	>9	>9	>9
16523	Stool	*Clostridium*	*Sordellii*	>9	>9	9	>9	>9	>9	>9
1	Stool	*Escherichia*	*Coli* toxin +	>9	>9	>9	>9	>9	>9	>9
2	Stool	*Escherichia*	*Coli* toxin +	>9	>9	>9	>9	>9	>9	>9
29328	ATCC	*Finegoldia*	*Magna*	>9	>9	2.25	9	9	9	9
18421	Stool	*Finegoldia*	*Magna*	3.42	>9	9	9	9	9	9
29328	ATCC	*Finegoldia*	*Magna*	>9	>9	6.82	>9	>9	>9	>9
9817	ATCC	*Fusobacterium*	*Mortiferum*	>9	>9	>9	>9	>9	>9	>9
25286	ATCC	*Fusobacterium*	*Necrophorum*	>9	>9	6.82	>9	6.82	4.5	>9
15529	Intraabdominal abscess	*Fusobacterium*	*Necrophorum*	>9	>9	6.82	>9	4.5	4.5	>9
16534	Intraabdominal abscess	*Fusobacterium*	*Necrophorum*	>9	>9	4.5	>9	4.5	4.5	9
14060	Stool	*Fusobacterium*	*Nucleatum*	>9	>9	9	>9	>9	>9	>9
14131	Stool	*Fusobacterium*	*Nucleatum*	>9	>9	9	>9	>9	>9	>9
16981	Stool	*Fusobacterium*	*Nucleatum*	>9	>9	9	>9	>9	>9	>9
16996	Blood	*Fusobacterium*	*Nucleatum*	>9	>9	4.5	>9	>9	>9	>9
16961	Stool	*Fusobacterium*	*Nucleatum*	>9	>9	6.82	>9	>9	>9	>9
25586	ATCC	*Fusobacterium*	*Nucleatum*	>9	>9	6.82	>9	9	>9	>9
8501	ATCC	*Fusobacterium*	*Varium*	>9	>9	>9	>9	>9	>9	>9
	Table 2	*Lactobacillus*	spp.; 16 strains	6.82→9[c]	>9	9→9[d]	>9	>9	>9	>9
18410	Stool	*Peptoniphilus*	*Asaccharolyticus*	>9	>9	9	>9	9	9	>9
18406	Stool	*Peptostreptococcus*	*Anaerobius*	>9	>9	2.25	>9	9	9	>9
17492	Stool	*Ruminococcus*	*Gnavus*	6.82	6.82	1.71	4.5	0.94	0.36	2.25
17457	Stool	*Ruminococcus*	*Obeum*	9	4.5	1.71	4.5	1.25	0.42	1.71
17493	Stool	*Ruminococcus*	*Productus*	>9	9	4.5	>9	2.5	2.25	9
27756	ATCC	*Ruminococcus*	*Torques*	4.5	4.5	1.71	3.42	1.71	0.64	2.84
3	Stool	*Salmonella*	*Typhi*	>9	>9	>9	>9	>9	>9	>9
4	Stool	*Salmonella*	*Typhi*	>9	>9	>9	>9	>9	>9	>9
3110	Foot ulcer, DM	*Staphylococcus*	*Aureus*	>9	>9	>9	>9	>9	>9	>9
3144	Foot ulcer, DM	*Staphylococcus*	*Aureus*	>9	>9	>9	>9	>9	>9	>9

[a]Values are average of 2 tests.
[b]Range; 1 *C. difficile* strain had MIC 4.5 mg/mL, 11 strains 9 mg/mL.
[c]Range; 1 *Lactobacillus acidophilus* and 1 *Lactobacillus gasseri* strain with MIC of 6.82 mg/mL and 1 *L. gasseri* and 1 *L. johnsonii* strain with MIC of 9 mg/mL.
[d]Range; 1 L. acidophilus strain had MIC 9 mg/mL, 15 *Lactobacillus* spp. strains >9 mg/mL.

(Duenas and others 2015). Our results suggest that some of the tested spices exhibit prebiotic-like effect by stimulating the growth of a number of *Bifidobacterium* and *Lactobacillus* species.

Bifidobacteria are normal inhabitants of the gastrointestinal tract. The composition, diversity, or relative abundance of *Bifidobacterium* species has been implicated in several intestinal disease conditions (Arboleya and others 2016). Bifidobacteria have been shown to alleviate infectious diarrhea through their effects on the immune system (Picard and others 2005). Lower levels of bifidobacteria were linked to higher prevalence of *E. coli* in obese children (Gao and others 2015); similarly, lower levels of bifidobacteria have been demonstrated in overweight subjects (Schwiertz and others 2010) and in patients with long-term asthma (Hevia

and others 2016), suggesting a role of bifidobacteria in human health. Our study reported new findings in that among 7 spice extracts ORE was most active in promoting the growth of *Bifidobacterium* whereas GIN, BLP, and CAP produced more modest stimulatory activity.

Studies of *Lactobacillus rhamnosus* GG (LGG, ATCC 53103) have shown promising results in treating diarrhea caused by viruses and bacteria (Vanderhoof and others 1999; Guandalini and others 2000), atopic disease (Kalliomäki and others 2001), and in prevention of gastrointestinal and respiratory tract infections (Hojsak and others 2010). In our study, all spice extracts were found to promote the growth of LGG strain at concentration ranging from 0.56 to 4.5 mg/mL. *L. reuteri* and *L. rhamnosus* are often added to dairy

products, or formulated as dietary supplements for controlling dysbiotic bacterial overgrowth during an active infection. BLP, CAP, GIN, and ORE enhanced the growth of these species at either 1.13 or 2.25 mg/mL. Our data are consistent with a study by Sutherland who reported that aqueous extracts of ginger and red chili enhanced the growth of *L. reuteri* and *L. rhamnosus* (Sutherland and others 2009).

Antimicrobial effect

Table 3 lists the MICs of 88 bacterial strains representing the major genera and species found in the human intestinal microbiota. Data revealed that all spice extracts were inactive against all *Bifidobacterium* and *Lactobacillus* isolates with MIC of >9 mg/mL, except that BLP was active against 1 *Lactobacillus acidophilus* and 1 *Lactobacillus gasseri* strain with MIC of 6.82 mg/mL and 1 *L. gasseri* and 1 *L. johnsonii* strain with MIC of 9 mg/mL. All spice extracts were inactive against all *Bacteroides* isolates, except that CIN was active against 1 strain of *Bacteroides fragilis* with MIC of 4.5 mg/mL. Extract of CIN, GIN, and ORE showed moderate activity against another group of gram-negative anaerobes, *Fusobacterium* spp., with MIC ranging from 4.5 to 9.0 mg/mL. None of the tested spices were bactericidal against the tested *E. coli* and *S. typhi* strains. CIN inhibited the growth of 11 toxigenic *C. difficile* at 9 mg/mL and 1 strain at 4.5 mg/mL. All the other spices were inactive against *C. difficile*. Data from other *Clostridium* showed that minimal growth inhibitions were observed in 2 strains by CIN, in 2 strains by ROS, and 1 strain by TUR with MICs ranging from 3.42 to 6.82 mg/mL. Both BLP and CIN showed higher activity against *F. magna* with MIC of 3.42 and 2.25 mg/mL, respectively. All 4 *Ruminococcus* strains tested were highly susceptible to all spices, with MIC ranging from 0.36 to 4.50 mg/mL being observed for CIN, ORE, and ROS. All spices were inactive against 2 strains of *S. aureus*.

Previous research on the diet and intestinal bacteria interactions has been centered mainly on the antimicrobial properties of foods or their constituents against pathogenic microorganisms. To date, there is a range of foods and their phenolic constituents that have been shown to have antimicrobial properties (Puupponen-Pimiä and others 2005; Vaquero and others 2007; Duda-Chodak 2012; Cueva and others 2015). Although all of the spices tested in this study were known to have activity against limited strains of bacteria (Al-Turki 2007; Bozin and others 2007; Shan and others 2007; Saeed and Tariq 2009; Sutherland and others 2009; Gunes and others 2016b; Shareef and others 2016); however, a comprehensive study comprising of 7 culinary spices on the viability of 88 intestinal, including toxigenic and pathogenic, bacterial strains has not previously been reported.

Conclusions

Gut microbiota is a mixed population, where the interactions among different species are crucial to establish the balance. Despite the inherent limitation of *in vitro* culture model, this work provided new findings of the effect of spices on bacteria isolates from normal intestinal microbiota. We showed that all tested spices, with the exception of TUR, promoted the growth of *Bifidobacterium* spp. and *Lactobacillus* spp. with varying degree. All spices exhibited high inhibitory activity against *Ruminococcus* species, but minimal or no activity against selected strains of *Bacteroides*, *Finegoldia*, *E. coli*, *Salmonella*, and *Staphylococcus*. *Fusobacterium* spp. were susceptible to CIN, and *F. necrophorum* also to ORE and ROS. CIN exhibited modest activity against toxigenic *C. difficile* and CIN, ROS, or TUR against a few strains of other *Clostridium*. It is worthwhile

to point out that among the spices investigated, ORE, BLP, CAP, and GIN possessed prebiotic-like effects by promoting the growth of beneficial bacteria in one hand and suppressing pathogenic bacteria on the other, suggesting their potential role in the regulation of intestinal microbiota and the enhancement of gastrointestinal health. Further research on the connections between spice-induced changes in gut microbiota and host metabolism and/or disease preventive effect in animal models and humans is needed.

Authors' Contributions

Q. Lu, Z. Li, S. Henning, D. Heber, and S. Finegold designed the study, assisted interpretations, and revised the manuscript. Q. Lu wrote and revised the manuscript. P. Summanen, R. Lee, and J. Huang carried out the experimental work and supported data interpretations.

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