JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Study of the Chemical Composition and Antimicrobial Activities of Ethanolic Extracts from Roots of *Scutellaria baicalensis* Georgi

Yingjian Lu, Rolf Joerger, and Changqing Wu*

Department of Animal and Food Science, 044 Townsend Hall, University of Delaware, 531 South College Avenue, Newark, Delaware 19716, United States

ABSTRACT: *Scutellaria baicalensis* Georgi (SBG), commonly named Huangqin, showed strong in vitro antimicrobial effects. However, limited research is available to systematically evaluate the effects of extraction methods on the phytochemical composition of SBG and its associated antimicrobial effects. In addition, limited studies have tested SBG as a natural antimicrobial agent on fresh produce such as tomatoes. In the current study, powered roots of SBG were extracted with 60, 80, and 100% ethanol, and their antiviral and antibacterial activities were evaluated. SBG ethanol extracts (SBGEEs) at 6.25 mg/mL showed limited antiviral activities against coliphage MS2 and hepatitis A virus. The SBG 80% ethanol extract (SBG80%EE) exhibited the lowest minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) against six foodborne pathogens. SBG80%EE had the highest contents of flavonoids and phenolic acids determined by high-performance liquid chromatography (HPLC). Among these bioactive compounds, ferulic acid had the lowest MIC and MBC values, 0.4 and 1.0 mg/mL, respectively, followed by baicalein and baicalin. Washing with SBG80%EE (12.5 mg/mL) resulted in >1 log reduction of *Salmonella enterica* serovars Typhimurium, Kentucky, Senftenberg, and Enteritidis on surface-inoculated grape tomatoes. None of SBGEE solutions changed the total phenolic content, color, or pH values of grape tomatoes. The quantification of these antimicrobial flavonoids and phenolic acids is important to maintain the quality and antimicrobial efficacy of SBG extracts. In addition, the application of SBG on tomatoes has provided valuable insights on the potential usage of this antimicrobial extract.

KEYWORDS: antibacterial activities, antiviral activities, *Scutellaria baicalensis* Georgi, phenolic acids

INTRODUCTION

Scutellaria baicalensis Georgi (SBG), commonly named Huangqin, is not only widely used in traditional Chinese herbal medicine but also used as a food additive. SBG contains numerous flavonoids, including baicalin, baicalein, wogonin, and chrysin.[1] Some of these flavonoids such as baicalin and baicalein from SBG have been demonstrated to have antioxidant,[2] antibacterial,[3] antiviral,[4] and anti-inflammatory activities.[5] Limited publications are available on the effects of extraction methods on the phenolic acid contents and their antibacterial activities from SBG. To our best knowledge, ours is the first study to investigate the antibacterial activities of phenolic acids from SBG and to compare their effects with those of extensively studied flavonoids such as baicalin and baicalein.

In previous research using an agar-well diffusion method, SBG extracts showed strong antibacterial effects against *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella anatum* among 46 tested herb and spice extracts.[6] Although SBG has been used as a natural antibacterial agent against foodborne pathogens such as *Staphylococcus* spp. and *Salmonella* spp. in homemade mayonnaise,[7] limited research is available about its application in wash water for fresh produce such as tomatoes. In addition, the impact of SBG extract on food quality has not been addressed.

Fresh produce has become one of the leading causes of foodborne illnesses in the United States.[8,9] High-risk fresh produce such as raw tomatoes have caused several large outbreaks of human salmonellosis over the past few years. In 2010, the Dutch health and food safety authorities issued an update relating to an outbreak of hepatitis A infection associated with semidried tomatoes in oil, which affected 13 people in The Netherlands.[10] Due to the limited efficacy of widely used chlorinated wash water on inactivation of food pathogens for fresh produce, alternatives to chlorine such as natural plant extracts have received much attention in the past few years. However, most of the previous research mainly focused on the in vitro antibacterial effects of crude extracts; limited research is available to systematically evaluate the impacts of extraction methods on the phytochemical composition and their associated antimicrobial effects. The quantification of the antimicrobial phytochemicals is important to maintain the quality and antimicrobial efficacy of plant extracts, which has not been adequately addressed in previous research. In addition, limited studies have been done to apply the extracts in wash water for fresh produce and investigate the impacts of food matrix on the antimicrobial efficacy of these plant extracts.

The objective of this research was to evaluate the impacts of extraction methods on the phytochemical compositions of three SBG ethanol extracts and to measure the antimicrobial effects of SBG ethanol extracts and their compounds against foodborne *L. monocytogenes*, *S. aureus*, *S. enterica* serovars Typhimurium, Kentucky, Senftenberg, and Enteritidis, coliphage MS2 (MS2), and hepatitis A virus (HAV). The antibacterial effects of SBG ethanol extracts against *S. enterica* on the surface of grape tomatoes were also studied, and the impacts of SBG ethanol extracts on color,

Received: July 11, 2011
Revised: August 22, 2011
Accepted: August 25, 2011
Published: August 25, 2011

pH, and total phenolic contents of grape tomatoes were determined as compared with chlorine treatments.

MATERIALS AND METHODS

Plant Material, Reagents, and Media. Powdered air-dried SBG roots were obtained from a pharmacy store in the Jiangsu Province, China, in January, 2009. Folin−Ciocalteu's phenol, resazurin sodium, baicalein, wogonoside, baicalin, wogonin, chrysin, *p*-coumaric acid, caffeic acid, chlorogenic acid, and ferulic aicd were purchased from Sigma Chemical Corp. (St. Louis, MO). Ethanol (HPLC grade), acetonitrile (HPLC grade), and sodium hypochlorite were obtained from Acros Co. (Morris Plains, NJ). Carvacrol was purchased from SAFC Supply Solutions (St. Louis, MO). Bacto Rappaport-Vassiliadis (RV) broth was obtained from Dickinson and Company (Detroit, MI). Bacto tryptic soy broth (TSB), Bacto tryptic soy agar (TSA), and Difco Xylose lysine deoxycholate (XLD) agar were purchased from Dickinson and Co. (Sparks, MD). Dulbecco's modified Eagle medium (DMEM), fetal bovine serum (FBS), penicillin/streptomycin, amphotericin B, sodium bicarbonate, and sodium pyruvate were obtained from Mediatech (Herndon, VA).

Preparation of SBG Ethanol Extract. Ten grams of powdered air-dried SBG root was extracted three times for 1 h each with 200 mL of 60% ethanol at 50 °C. The extracts were filtered, combined, and concentrated under vacuum at 50 °C using a rotary evaporator (Heidolph, Laborota 4000, Schwabach, Germany), yielding the SBG 60% ethanol extract (SBG60%EE) at 250 mg/mL based on the dry weight of SBG root. SBG80%EE and SBG100%EE were prepared according to the same procedure but with 80 and 100% ethanol, respectively.

Characterization of Bioactive Compounds from SBGEEs by HPLC-PDA. After centrifugation of the SBGEEs at 3000 relative centrifugal force (rcf) for 3 min, 10 μL was injected into and analyzed by a Shimadzu LC-20A automated liquid chromatographic system, which consists of a photodiode array (PDA) detector, a CBM system controller, LC-20AT pumps, a SIL-20AC autosampler with cooler, and a reverse-phase C18 (5 μm, 150 × 4.6 mm, 110 Å) column with a matched guard column. The flow rate was 1.0 mL/min at 35 °C. The mobile phase was composed of acetonitrile (A) and 0.1% trifluoroacetic acid (TFA) (v/v) (B) with a gradient elution program of 12−45% (A) from 0 to 22.00 min, 45−45% (A) from 22.01 to 31.00 min, and 45−12% (A) from 31.01 to 37.00 min. A 10 min re-equilibration time was used between HPLC runs. The detection wavelength of the samples was 285 nm. The analysis of the samples was compared to available authentic standards.[11]

Quantitative determinations of the peaks were performed by the external standard method. Standard solutions containing baicalein, wogonoside, baicalin, wogonin, and chrysin at 20, 40, 100, and 200 μg/mL were prepared in mobile phase, and 50 μL of each standard solution was injected in triplicate into the HPLC column. Calibration graphs for baicalein, wogonoside, baicalin, wogonin, and chrysin were constructed by plotting flavonoid peak area against concentration. Linearity was assessed by using the regression coefficient (R^2).

Analysis of Phenolic Acids from SBGEEs. For determination of chlorogenic acid and ferulic acid, 15 μL of each sample was analyzed by HPLC as described in the previous section using a reverse-phase C18 (5 μm, 150 × 4.6 mm, 110 Å) column with a matched guard column (Waters Corp., Milford, MA). The flow rate was 1.0 mL/min at 35 °C. The mobile phase consisted of water in 0.05% TFA (A) and acetonitrile (B) with the following gradient program: 0 min, 0% B; 3 min, 16% B; 22 min, 22% B; 23 min, 23% B; 25 min, 0% B. The absorbance of samples was scanned from 190 to 450 nm by the Shimadzu SPD-M10 V PDA detector. The analytes were detected at 280 nm. Chlorogenic acid, ferulic acid, *p*-coumaric acid, and caffeic acid in SBGEEs were identified by comparison with the authentic standards (retention time and spectrum) and quantified using five-point calibration curves. Chlorogenic acid, ferulic acid, *p*-coumaric acid, and caffeic acid standard solutions were prepared in concentrations of 10, 50, 100, 250, and 500 μg/mL. Each sample for HPLC analysis was injected three times. Linearity was assessed by using the regression coefficient (R^2) ($p < 0.05$).

Tests of Anticoliphage MS2. Coliphage MS2 (MS2), a bacterial virus infecting *E. coli*, is used as a process indicator and surrogate for HAV and other human enteric viruses in various environmental virology studies, including produce disinfection studies.[12] MS2 was kindly provided by Dr. Yan Jin of the Department of Plant and Soil Sciences at the University of Delaware (UD, Newark, DE). MS2 was grown on its corresponding bacterial host *E. coli* ATCC 15597B-1. One milliliter of freshly grown *E. coli* was transferred to 9 mL of Tryptone soy broth (TSB) containing 1% yeast extract (TSBYE) and grown at 37 °C with shaking. After the culture had been incubated for 4−5 h, it was inoculated with 1% of a lysate containing ~1×10^8 PFU/mL bacteriophage MS2 and incubated statically overnight at 37 °C or until the culture was completely cleared by phage infection. Bacterium cells were removed by centrifugation at 2000*g* for 10−15 min, and the supernatant was filter-sterilized using a 0.45 μm membrane filter.[13,14] Bacteriophage MS2 stock lysates were stored at −20 °C until use. SBG extracts (SBG60%EE, SBG80%EE, SBG100%EE) and two of their major components (chrysin, baicalin), and thymol, as well as their corresponding carrier solvents, were tested against phage using previously described methods with slight modification.[15] One hundred microliters of sample was mixed with 100 μL of diluted virus solution by vortexing for 10 min, and then 4 mL of melted TSA was added to the mixture. Finally, the mixture was poured onto prepared TSA plates. After the plates solidified, they were incubated overnight at 37 °C and plaques counted.

Anti-Hepatitis A Virus Test (TCID50). HAV (ATCC VR-1402) was propagated and assayed on fetal rhesus monkey kidney cells (FRhK-4 cells; ATCC CRL-1688) in DMEM. Growth media were supplemented with 2% (for maintenance) or 10% (for growth) of FBS, penicillin/streptomycin, amphotericin B, sodium bicarbonate, and sodium pyruvate. All cells were maintained at 37 °C in an atmosphere of 5% CO_2. For virus propagation, virus was added to confluent flasks, which were then subjected to three freeze−thaw cycles, and media were harvested at 14 days. Cellular debris was cleared by centrifugation and filtration (0.45 μm diameter pore size). Virus stocks were stored in media with 2% FBS at −80 °C until used. HAV infectious titer was evaluated by tissue culture infectious dose 50 ($TCID_{50}$) using FRhK-4 cells grown in 96-well plates. HAV was mixed with samples such as SBGEEs, chrysin, or bacicalin in a 2:1 ratio. The plates were incubated with CO_2 at 37 °C for 2 h before the addition of 100 μL of 2% growth media to each well. Finally, the plates were incubated for 14 days, the cytopathic effect (cpe) of the virus in each well was evaluated microscopically, and the $TCID_{50}$ was determined on the basis of the number of wells displaying positive cpe.[16]

Antibacterial Activity. *Bacteria Strains.* The bacteria used for this study were obtained from the culture collection in the Department of Animal and Food Sciences at UD and included *L. monocytogenes* ATCC 19115, *S. aureus* ATCC 27217, and *S. enterica* serovars Typhimurium, Kentucky, Senftenberg, and Enteritidis. Stock cultures on TSA were stored at 4 °C.

Determinations of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). The MICs of the samples against the test bacteria were determined according to a modified method of Hansen et al.[17] Inocula of the microorganisms were prepared from 24 h cultures, and the suspensions were adjusted to 10^4 CFU/mL by dilution in TSB; then 50 μL of the bacteria was added into each well of a sterile cell culture 96-well plate. SBG60%EE, SBG80%EE, and SBG100%EE were diluted with TSB to prepare a range of concentrations (1, 5, 12.5, 25, 50, and 100 mg/mL) and major compounds in SBGEE (baicalin, baicalein, wogonin, wogonoside, chrysin, chlorogenic acid, and ferulic acid) at a range of concentrations (0.1−40 mg/mL); then

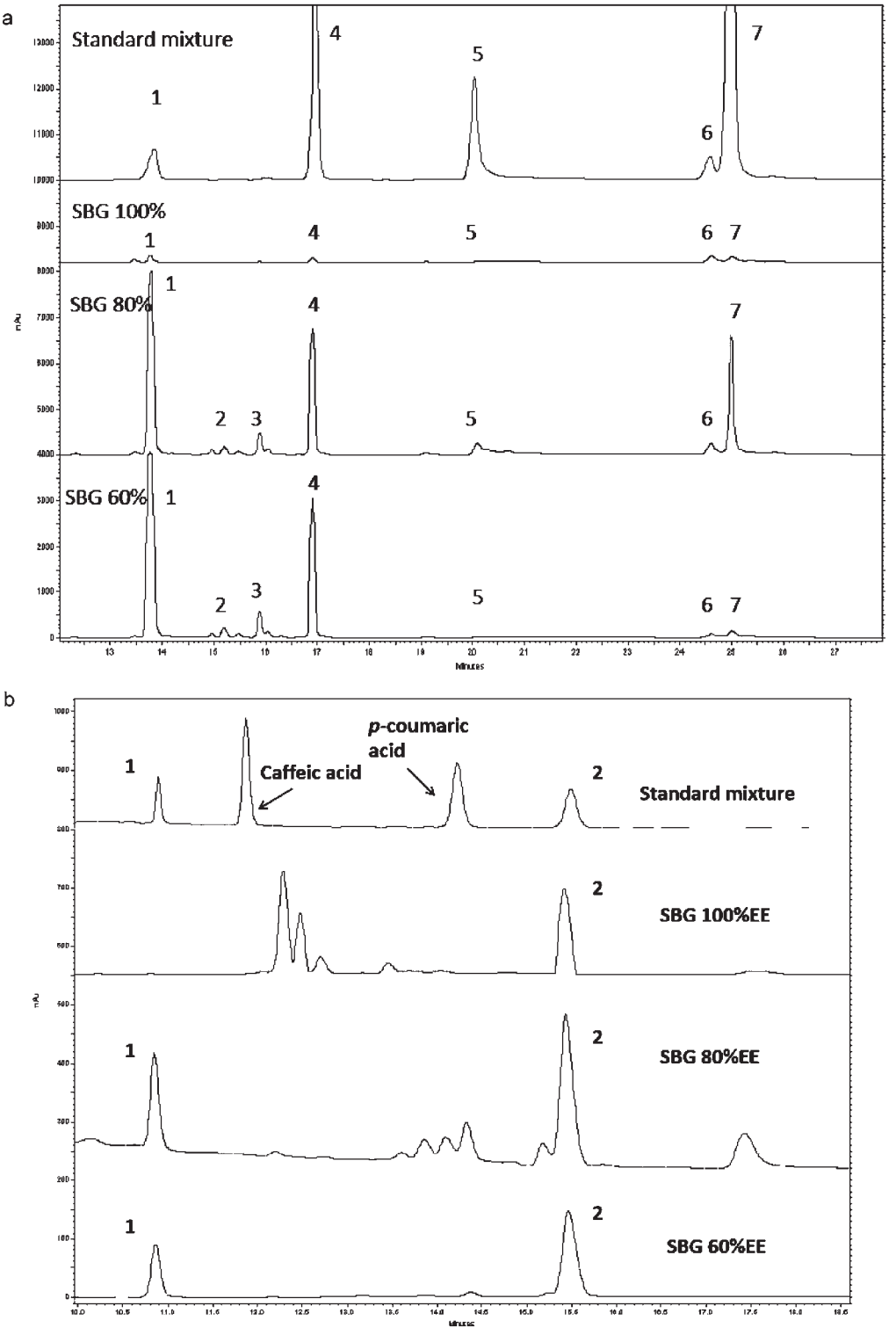

Figure 1. HPLC chromatograms of (a) flavonoids and (b) phenolic acids from SBG 60%, 80% and 100% ethanol extracts and standard mixture.

100 μL of samples was added into each test well containing the bacteria. Positive control TSB, negative control kanamycin (300 μg/mL), and ethanol solutions corresponding to the ethanol percentages in the test samples were also tested at 100 μL in wells containing 50 μL of bacteria. TSB was added at 150 μL in wells containing no cells to test the effects of the medium itself. Finally, 15 μL of resazurin (1 mg/mL) was added into each well. For comparative purposes, the MIC and MBC of carvacrol were also determined. The MIC was defined as the lowest concentration of the samples at which the microorganism did not demonstrate visible growth after 24 h of incubation at 37 °C. Microorganism growth was indicated by color change of the resazurin from blue to pink. Twenty microliters of the content of wells without any definitive color change from blue to pink was plated onto TSA. The plates were incubated at 37 °C for an additional 24 h to determine the count of

viable cells. The MBC of the samples was defined as the minimum concentration at which there was no growth of microorganisms on the TSA plates.

Effects of SBGEEs on Foodborne Pathogens on Grape Tomatoes. Grape tomatoes were obtained from a local market on the day of experimentation. Inocula were prepared from static cultures grown at 37 °C in TSB for 24 h. *S. enterica* Typhimurium, Senftenberg, Kentucky, and Enteritidis final inoculation was 10^8 CFU/g. Intact surfaces were selected for spot inoculation (25 μL). The inoculum suspension droplets were placed on the surfaces and allowed to air-dry at 25 °C for about 2 h in a biological safety cabinet.

On the basis of the results of the MIC assay, seven rinse solutions were tested and included sterile water, 4% ethanol, carvacrol (0.2 mg/mL; MIC value against *Salmonella* strains), 200 ppm chlorine, and SBG60%EE, SBG80%EE, and SBG100%EE (12.5 mg/mL). Ethanol at 4% was included because it was the highest ethanol percentage in our SBGEEs and carvacrol washing solutions. One milliliter of SBG60%EE (250 mg/mL) was transferred to 19 mL of sterile water to obtain a concentration of SBG60%EE of 12.5 mg/mL. SBG80%EE and SBG100%EE were prepared the same way. Two grape tomatoes in a beaker were submerged in 20 mL of the rinse solution with continuous agitation. Two wash times, 5 and 10 min, were tested. After rinsing, the treatment solutions were decanted, and the tomatoes were placed in a sterile stomacher sample bag containing 10 mL of elution buffer (PBS) and homogenized in a stomacher (Colworth, Stomacher 400, U.K.) for 1 min at medium speed. For the determination of viable bacteria, a 100 μL aliquot of the homogenate was plated on XLD agar. Plates were incubated at 37 °C for 24 h before presumptive positive colonies were counted.

A modified *Salmonella* enrichment experiment was carried out due to limited incubation space in the laboratory. The grape tomato homogenates in PBS (1 mL) were transferred to 9 mL of pre-enrichment broth (TSB) and incubated at 37 °C for 24 h. After mixing, 0.1 mL of TSB was transferred to 10 mL of RV broth (preheated to the incubation temperature). The RV broths were incubated in a water bath at 42 °C for 24 h. After incubation, 10 μL of cultures (RV broths) was streaked on XLD agar. Plates were incubated at 37 °C for 24 h and examined for the presence of *Salmonella* colonies. Colonies of presumptive *Salmonella* strains formed on XLD agar were randomly selected for confirmation. The microbial population detection limit was 2.0 $\log_{10}$ CFU/mL.

Color and pH Measurement of Grape Tomato after Washing by SBGEE Solutions. SBGEEs had yellow color, which might affect the color of the treated grape tomato, so the color of treated and untreated grape tomato was determined by direct reading with a color reader (Minolta model CR-10; Minolta Camera Co., Ltd., Osaka, Japan) to obtain the color values: L^* (brightness/darkness), a^* (redness/greenness), and b^* (yellowness/blueness).[18] Measurements were taken from treated and untreated grape tomatoes without inoculation of *Salmonella* at three different parts of a grape tomato and averaged. Untreated and treated grape tomatoes without inoculation of *Salmonella* were placed in sterile stomacher sample bags and homogenized in a stomacher for 1 min at medium speed. The pH of the homogenates of those grape tomatoes was measured by a pH-meter (FiveEasy FE20; Mettler-Toledo AG, Switzerland).

Effect of SBGEE Washing Solutions on Total Phenolic Contents of Grape Tomato. A total phenolic content assay was used to determine the total phenolics of grape tomato before and after washing by SBGEE,[19] with a range of gallic acid used as standards. The reaction mixture was composed of 20 μL of SBG60%EE, SBG80%EE, SBG100%EE, or BHT at a range of concentrations, 180 μL of distilled water, 100 μL of Folin−Ciocalteu reagent, and 0.5 mL of a 20% sodium carbonate solution in a microcentrifuge tube. The reaction mixtures were covered and allowed to stand at room temperature for 2 h in microcentrifuge tubes, and then 200 μL of each was transferred into each blank well of a clear 96-well plate after they were vortexed vigorously. The absorbance was measured at 765 nm using the Synergy 2 multimode microplate reader (BioTek Instruments, Inc., Winooski, VT). Total phenolic conetents were expressed as micrograms of gallic acid equivalents (GAEs) per milligram of fresh weight (FW).

Statistical Analysis. Three independent trials were evaluated, and all experiments were performed in triplicate in each trial. The data were recorded as the mean ± standard deviation. Microbial reductions, color, pH measurements, and nutrient contents were analyzed for significant treatment differences using one-way analysis of variance (ANOVA), fit model, and Tukey's HSD test of JMP (v. 7.0, SAS Institute, Inc., Cary, NC). The main effects and interaction effects in washing experiments were assessed by fit model. Significance was determined at $p < 0.05$.

RESULTS

Effect of Ethanol Concentrations on the Bioactive Compounds Profile in SBG. In the present study, different concentrations (60, 80, and 100%) of ethanol solutions were examined to extract bioactive compounds from the root of SBG. The results indicated that the contents of tested chemicals gradually increased with the increase of the concentrations of ethanol from 60 to 80%. High concentrations of ethanol (100%) did not benefit efficient extraction of the identified compounds. Eighty percent aqueous ethanol extracted the highest amounts of the compounds ($p < 0.05$).

The phytochemical compositions of the different ethanol extracts of SBG are shown in Figure 1. Seven of the compounds were identified as baicalin, wogonoside, baicalein, wogonin, chrysin, chlorogenic acid, and ferulic acid; the two other unidentified compounds might be apigenin-7-*O*-β-D-glucuronide and chrysin-7-*O*-β-D-glucuronide when compared to published data.[20]

Linear calibration graphs with good correlation coefficients were obtained for these flavonoids in the range of 20−200 μg/mL. The contents of baicalin, wogonoside, baicalein, wogonin, and chrysin from SBGEEs are shown in Table.1. SBG80%EE had the highest contents of baicalin, wogonoside, baicalein, wogonin, and chrysin: 270.49 ± 12.79, 119.88 ± 11.96, 45.61 ± 5.50, 49.5 ± 2.97, and 28.45 ± 2.19 μg/mL, respectively. SBG60%EE had lower contents of those compounds than SBG80%EE, but higher than those of SBG100%EE. The HPLC quantification of the flavonoid contents for the SBGEEs decreased in the order SBG80%EE > SBG60%EE > SBG100%EE. There were two fewer peaks in the chromatograph for SBG 100% ethanol extract. These two peaks might be apigenin-7-*O*-β-D-glucuronide and chrysin-7-*O*-β-D-glucuronide.

Linear calibration graphs with good correlation coefficients were obtained for the tested phenolic acids in the range of 10−500 μg/mL. Phenolic acids of *p*-coumaric acid and caffeic acid were not found in SBGEEs because they were under the HPLC detection limits. The contents of chlorogenic acid and ferulic acid from SBGEEs are shown in Table 1. SBG80%EE had the highest contents of chlorogenic acid and ferulic acid at 16.83 ± 0.84 and 78.73 ± 2.22 μg/mL, respectively. Chlorogenic acid was not detected in SBG100%EE, but the extract had 37.90 ± 10.89 μg/mL ferulic acid. HPLC quantification of the phenolic acid contents for the SBGEEs decreased in the order SBG80%EE ≥ SBG60%EE > SBG100%EE.

Anti-Coliphage MS2 Activities of Natural Agents. The reductions of MS2 after treatment with different samples are shown in Table 2. On the basis of our current results, SBG80%EE and SBG100%EE at 6.25 mg/mL could achieve around 0.2 log PFU/mL reductions of MS2, and there was no significant

Table 1. Bioactive Compound Profile from SBG Ethanol Extracts by HPLC-PDA

compound	retention time (min)/content[a] (μg/mL)				regression eq[b]	R^{2c}
	SBG60%EE	SBG80%EE	SBG100%EE	standard mixture		
Figure1a (1), baicalin	13.776/(226.07 ± 55.51)	13.766/(270.49 ± 12.79)	13.768/(12.06 ± 2.14)	13.784/(500)	$y = 252662x - 1 \times 10^5$	0.996
Figure 1a (2), unidentified 1	15.192	15.215				
Figure 1a (3), unidentified 2	15.88	15.891				
Figure 1a (4), wogonoside	16.9/(81.99 ± 19.96)	16.902/(119.88 ± 11.96)	16.904/(6.16 ± 4.25)	16.996/(500)	$y = 257320x + 5 \times 10^6$	
Figure 1a (5), baicalein	20.162/(25.83 ± 0.95)	20.08/(45.61 ± 5.50)	20.184/(5.35 ± 0.35)	20.052/(200)	$y = 127781x - 2 \times 10^6$	0.996
Figure 1a (6), wogonin	24.612/(29.18 ± 3.08)	24.6/(49.5 ± 2.97)	24.608/(7.6 ± 1.70)	24.592/(200)	$y = 27743x - 9 \times 10^4$	0.999
Figure 1a (7), chrysin	25.008/(10.18 ± 0.31)	24.992/(28.45 ± 2.19)	25.008/(9.1 ± 1.83)	24.984/(200)	$y = 283177x - 2 \times 10^6$	0.998
Figure 1b (1), chlorogenic acid	10.879/(13.50 ± 4.95)	10.845/(16.83 ± 0.84)		10.921/(500)	$y = 5 \times 10^6x + 2 \times 10^6$	0.987
Figure1b (2), ferulic acid	15.469/(30.18 ± 17.09)	15.428/(78.73 ± 2.22)	15.406/(37.90 ± 10.89)	15.501/(500)	$y = 4 \times 10^6x + 3 \times 10^6$	0.977

[a] The results are expressed as mean ± SD. [b] In the regression equation $y = ax + b$, x refers to the concentration (μg/mL) and y indicates the peak area, and [c] R^2 is the correlation coefficient of the equation.

Table 2. Anti-Coliphage MS2 Activities of Antimicrobial Agents

sample	concentration (mg/mL)	log reduction of MS2[a] (PFU/mL)
control		0.00 ± 0.00 e
chrysin	1.25	1.03 ± 0.04 b
thymol	2.50	1.54 ± 0.09 a
baicalin	1.25	0.54 ± 0.03 c
SBG 60%EE	6.25	0.01 ± 0.01 e
SBG 80%EE	6.25	0.18 ± 0.02 d
SBG 100%EE	6.25	0.22 ± 0.03 d

[a] The results are expressed as the mean ± SD. Entries followed by the same letter are not significantly different ($p > 0.05$). The log reductions of MS2 by antimicrobial agents were compared to MS2 reductions of their corresponding solvents, respectively.

difference between them ($p > 0.05$). SBG60%EE obtained no significant log reduction of MS2 ($p > 0.05$). Also, 1.25 mg/mL chrysin and 1.25 mg/mL baicalin could achieve 1.03 and 0.54 log PFU/mL reductions of MS2, respectively, which were less effective than 2.5 mg/mL thymol, a natural compound used as negative control.

Anti-HAV Activities of Antimicrobial Agents. The HAV strain gave a clearly visible cpe on FRhK-4 cells, and this allowed microscopic evaluation of virus replication and enabled the establishment of a $TCID_{50}$ titration system. The $TCID_{50}$ titer determination was validated by titration of a virus stock. Our results showed that SBGEEs at 6.25 mg/mL and chrysin at 1.25 mg/L attained no anti-HAV activities of HAV in comparison to their solvents (data not included). Only 1.25 mg/mL baicalin could achieve around 0.1 log reduction of HAV.

Antibacterial Activity of SBGEEs with Their Major Components in Growth Media and Their Application as Washing Solutions for Grape Tomatoes. The MICs and MBCs of SBGEEs and their phytochemical compositions are shown in Table 3. Ferulic acid had the lowest MIC and MBC values, followed by baicalein and baicalin. Because of higher contents of ferulic acid, baicalein, and baicalin, SBG80%EE may have a better antibacterial activity among SBGEEs against all six foodborne pathogenic bacteria. For example, the MIC and MBC of SBG60% against all four *Salmonella* strains were 50 mg/mL, which was 2 times those of SBG80%EE (25 mg/mL).

The reductions of *Salmonella* on tomatoes after washing with SBGEEs and other washing treatments are shown in Figure 2. Compared with 4% ethanol, SBG80%EE at 12.5 mg/mL resulted in >1 log reductions of *Salmonella* serovars Typhimurium, Senftenberg, Kentucky, and Enteritidis isolates in 5 and 10 min. SBG60%EE at 12.5 mg/mL reduced the counts by approximately around 1.0 log and had a better performance in achieving log reduction of *Salmonella* strains than SBG100%EE. Through enrichment there were no actual *Salmonella* viable cells on inoculated grape tomatoes that were treated by 200 ppm chlorine, and the obtained results were below the microbial detection limit ($2 \log_{10}$ CFU/mL) in 5 and 10 min washings. Sanitizing solution containing 200 ppm aqueous chlorine achieved >4 log reductions of *Salmonella*. SBG60%EE, SBG80%EE, and carvacrol at 0.2 mg/mL were effective in inactivating *S. enterica* on grape tomato, with population reduction not significantly greater than 1 log ($p > 0.05$). The statistical analysis of the seven washing solution in both 5 and 10 min washings revealed no significant interaction between the washing time and concentrations of treatments ($p > 0.05$). Longer wash times (5−10 min) did not increase the efficacy of wash solution against *S. enterica* on grape tomato.

Color and pH Values of Treated and Untreated Grape Tomatoes. Color parameters did not change significantly after

Table 3. Antibacterial Activities of the SBG Ethanol Extracts against Six Foodborne Pathogens As Compared to Carvacrol and Chlorine

		Gram-positive		Gram-negative			
		S. aureus ATCC 19115	*L. monocytogenes* ATCC 27217	*S. enterica* Kentucky	*S. enterica* Senftenberg	*S. enterica* Enteritidis	*S. enterica* Typhimurium
SBG60%EE	MIC[a]	50	50	50	50	50	50
	MBC[b]	50	50	50	50	50	50
SBG80%EE	MIC	12.5	25	25	25	25	25
	MBC	25	25	25	25	25	25
SBG100%EE	MIC	>100	>100	>100	>100	>100	>100
	MBC	>100	>100	>100	>100	>100	>100
baicalein	MIC	0.5	1	1	1	1	1
	MBC	1	2	2	2	2	2
wogonoside	MIC	1	5	10	10	10	10
	MBC	2	10	20	20	20	20
baicalin	MIC	0.5	2	2	2	2	2
	MBC	1	5	5	5	5	5
wogonin	MIC	1	5	10	10	10	10
	MBC	2	10	20	20	20	20
chrysin	MIC	10	10	10	10	10	10
	MBC	20	20	20	20	20	20
chlorogenic acid	MIC	5	5	5	5	5	5
	MBC	10	10	10	10	10	10
ferulic acid	MIC	0.4	0.4	0.4	0.4	0.4	0.4
	MBC	1	1	1	1	1	1
carvacrol	MIC	0.1	0.1	0.2	0.2	0.2	0.2
	MBC	0.2	0.4	0.4	0.4	0.4	0.4
chlorine	MIC[c]	40	40	40	40	40	40
	MBC[d]	80	80	80	80	80	80

[a] MIC, minimum inhibitory concentration in mg/mL. [b] MBC, minimum bactericidal concentration in mg/mL. [c] MIC, minimum inhibitory concentration in ppm. [d] MBC, minimum bactericidal concentration in ppm.

washing. There was no significant change in color between untreated tomato and treated tomato for any of the color parameters (L^*, a^*, and b^*) ($p > 0.05$). The pH value of untreated grape tomato was 4.14 ± 0.03, and the pH values of treated tomatoes were around 4.20. There was no statistically significant difference of pH value between untreated and treated grape tomato ($p > 0.05$).

Total Phenolic Contents of Treated and Untreated Grape Tomatoes. The total phenolic content of untreated tomato was 42.46 ± 5.38 μg GAE/mg FW, and the total phenolic contents of grape tomatoes treated by SBG60%EE, SBG80%EE, SBG100%EE, and 4% ethanol (positive control) were 39.33 ± 7.91, 35.44 ± 0.59, 45.08 ± 2.34, and 37.38 ± 3.16 μg GAE/mg FW, respectively. The total phenolic content of grape tomato without washing was 41.74 ± 2.27 μg GAE/mg FW. None of the tested washing solutions affected significantly the total phenolic contents of grape tomatoes ($p > 0.05$).

DISCUSSION

Different ethanol concentrations used for extraction of SBG affected the content of major flavonoids and phenolic acids in SBG extracts. SBG80%EE contained the highest contents of seven identified bioactive compounds (baicalin, wogonoside, baicalein, wogonin, chrysin, chlorogenic acid, and ferulic acid), even 2 times more than SBG60%EE. Although wogonin isolated from SBG

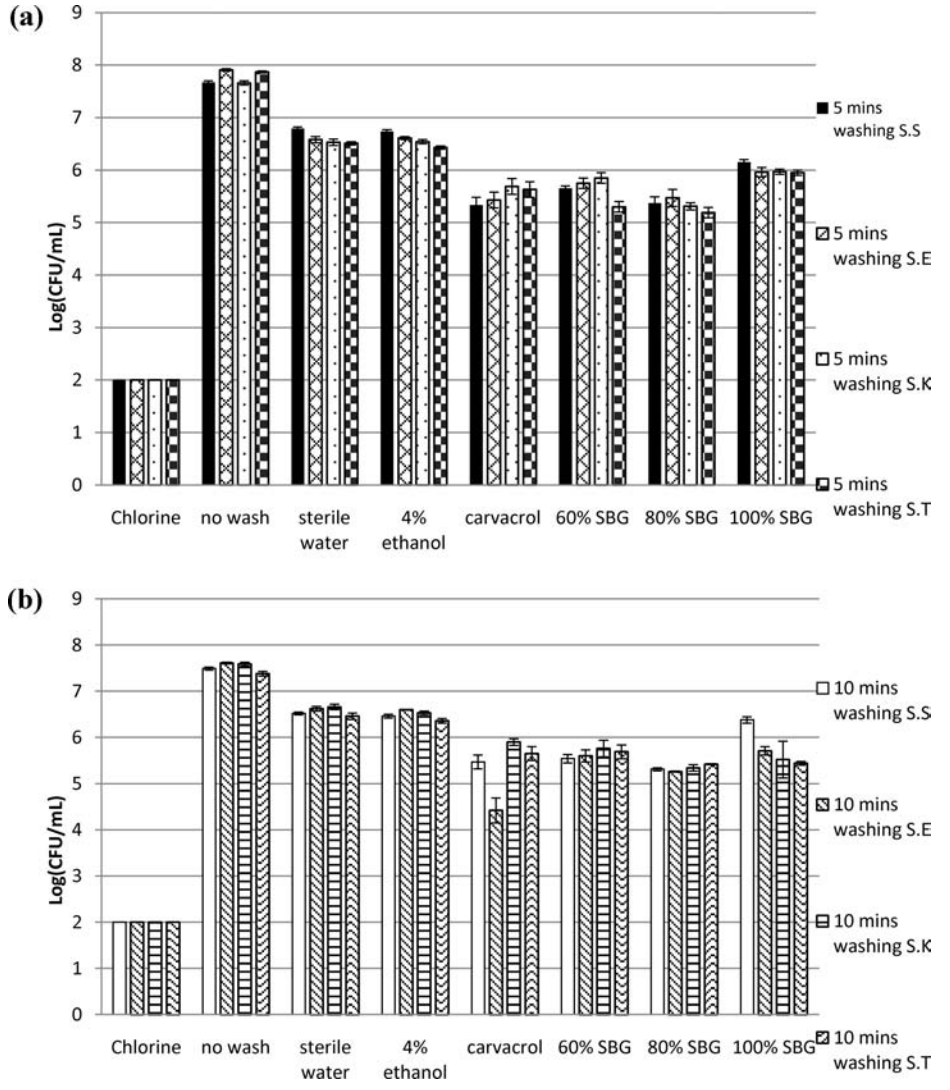

Figure 2. Recovery of four strains of *Salmonella enterica* from grape tomatoes following (a) 5 and (b) 10 min rinses and plating on XLD agar. S.S, *Salmonella enterica* Senftenberg; S.E, *Salmonella enterica* Enteritidis; S.K, *Salmonella enterica* Kentucky; S.T, *Salmonella enterica* Typhimurium. The *Salmonella* population on inoculated grape tomatoes which was treated by 200ppm chlorine was below the microbial detection limits (2 log10 CFU/mL) after 5 min and 10 min washing. Chlorine, chlorine at 200ppm; Carvacrol, carvacrol at 0.2 mg/mL; 60%SBG, SBG60%EE at 12.5 mg/mL; 80%SBG, SBG80%EE at 12.5 mg/mL; 100%SBG, SBG100%EE at 12.5 mg/mL.

inhibited hepatitis B virus in a previous study,[4] SBGEEs at 6.25 mg/mL had only limited antiviral activities against HAV and coliphage MS2. Shown in our MIC and MBC tests, the SBG ethanol extracts had good antibacterial activities against *L. monocytogenes* ATCC 19115, *S. aureus* ATCC 27217, and *S. enterica* serovars Typhimurium, Kentucky, Senftenberg, and Enteritidis, which is in agreement with previous results.[7,21] Among the three SBG ethanol extracts, SBG80%EE demonstrated the lowest MICs and MBCs against six foodborne pathogens. In addition, ferulic acid had the lowest MIC (0.4 mg/mL) and MBC (1 mg/mL) values among the identified bioactive compounds, followed by baicalein and baicalin; all of these data might explain why SBG80%EE had stronger antibacterial activities than SBG60%EE and SBG100%EE. To our best knowledge, this is the first time the phytochemical composition and antimicrobial effects of different ethanol extracts for SBG have been compared.

Baicalein, wogonin, and baicalin might be the major antibacterial substances in SBG crude extracts that inhibit the growth of *S. aureus*, *E. coli*, and *Bacillus subtilis*.[22,23] However, it is possible that the phenolic acids in SBGEEs, such as ferulic acid and chlorogenic acid, might also contribute to the strong antibacterial activities of SBG, as observed in our studies. Some previous studies have reported that chlorogenic acid in *Cynara scolymus* L. and *Cydonia oblonga* Miller[24,25] and ferulic acid in *Lomatium californicum* and *Onosma hispidum*[26,27] are potential natural antimicrobial agents against *S. enterica*, *S. aureus*, and *E. coli*. With lower MIC and MBC associated with ferulic acid, the content of this phenolic acid might be very important for the antimicrobial effects of SBG extracts. As a result, the quantification of these flavonoids and phenolic acids is important to maintain the quality and antimicrobial efficacy of SBG extracts, which might be varied with other batches of raw materials.

Postharvest treatment using sanitizers such as chlorine-based sanitizers are widely used in the fresh produce industry.[28] However, besides the limited efficacy for fresh produce, there are risks associated with organic matter byproducts such as chloroform

($CHCl_3$) and trihalomethanes (THM). These byproducts have known or suspected carcinogenic or mutagenic effects and may cause new regulatory restrictions for usage of chlorine-based sanitizers in the future.[29] Safer natural antimicrobials are highly desirable because of the general public's acceptance.[30] Our study thus evaluated SBGEEs' ability as safe and effective natural antimicrobial alternatives to inactivate the foodborne pathogen *Salmonella enterica* on tomatoes. Even though our concentration of SBGEEs was calculated on the basis of dry weight of SBG and not on the extract weight, only SBGEE at 12.5 mg/mL was evaluated. Higher concentrations were not tested because of the consideration of cost-effectiveness.

To our surprise, no significant differences were observed in the abilities of SBG60%EE and SBG80%EE to inactivate *S. enterica* on grape tomato. This is different from the results obtained for the MIC and MBC tests. Compared with SBG60%EE, SBG80% EE with smaller MIC and MBC did not increase inhibitory effects against *Salmonella* inoculated on the surface of tomatoes after 5 or 10 min of application. Carvacrol was used as another positive control in the washing test because it has been found to possess antimicrobial activity in vitro against a broad spectrum of bacteria including *S. enterica* and *L. monocytogenes*.[31−33] At its MIC in the washing solution, carvacrol inhibited the growth of *Salmonella* on the surface inoculated tomatoesonly by 1 log, which is much less than the log reduction achieved by 200 ppm chlorine, which is widely used in the tomato industry. However, 200 ppm of chlorine was 5 times its MIC because its MIC and MBC were 40 ppm and 80 ppm (unpublished data), respectively. In other words, a much higher concentration of chlorine than its MBC could receive more log reduction of *Salmonella* on tomatoes. The 1 log reduction associated with SBG extracts and carvacrol might be due to their concentrations being still not effective enough. Higher contents of SBG60%EE and SBG80%EE might have better inactivation. However, cost-effectiveness can be a significant challenge.

It is not surprising that none of the tested natural antimicrobials decreased the total phenol contents of grape tomatoes ($p > 0.05$). This result was consistent with Fukumoto et al.'s study that washing solutions such as chlorine did not decrease the total phenolic content of iceburg lettuce.[34] On the basis of our results, none of the treatments significantly affected the pH and color of the grape tomato ($p > 0.05$), which might be due to low residual antimicrobials on the surface of grape tomatoes and short contact time during the washing process.

In our previous study, SBG80%EE also demonstrated higher antioxidant capacities than SBG60%EE and SBG100%EE using an ORAC assay, which was modified on the basis of Wu et al.[35] (data not reported here). Ethanol concentration also significantly affected total phenolic contents of SBG samples, with 80% ethanol extracting more total phenolic compounds than 60 and 100% ethanol. SBG80%EE contained more bioactive compounds as identified by HPLC methods, which might partially explain the greater oxygen radical absorbance capacity and higher total phenolic content. Therefore, the application of SBGEE in washing solution might have additional protection against oxidation, which can be another benefit over the chlorine-based solution.

In conclusion, our study showed that flavonoids (baicalin, wogonoside, baicalein, wogonin, and chrysin) and phenolic acids (chlorogenic acid and ferulic acid) in SBG were affected by extraction solvent. SBG80%EE contained the highest contents of these compounds so that it had better antioxidant and antibacterial activities by our bioassays. SBGEEs at test level showed no significant effects on reducing MS2 and HAV. The use of SBG60%EE and SBG80%EE solutions at 12.5 mg/mL could reduce the *Salmonella* population on grape tomatoes by 1 log, which was similar to carvacrol at its MIC (0.2 mg/mL), but lower than 200 ppm chlorine solution. None of SBGEE treatments changed the total phenolic content, color, or pH value of grape tomato ($p > 0.05$). Some practical methods such as combination of SBGEE with heat or ultrasound might increase the antimicrobial efficacy without increasing the concentrations of SBGEEs, but challenges remain to make these approaches cost-effective.

AUTHOR INFORMATION

Corresponding Author

*E-mail: changwu@udel.edu. Phone: (302) 831-3029. Fax: (302) 831-2822.

REFERENCES

(1) Li, H. B.; Chen, F. Isolation and purification of baicalein, wogonin and oroxylin A from the medicinal plant *Scutellaria baicalensis* by high-speed counter-current chromatography. *J. Chromatogr., A* **2005**, *1074* (1), 107–110.

(2) Chan, S. W.; Li, S.; Kwok, C. Y.; Benzie, I. F. F.; Szeto, Y. T.; Guo, D. J.; He, X. P.; Yu, P. H. F. Antioxidant activity of Chinese medicinal herbs. *Pharm. Biol.* **2008**, *46* (4), 587–595.

(3) Huang, K. C. Antibacterial, antiviral, and antifungal herbs. *The Pharmacology of Chinese Herbs*; CRC Press: Boca Raton, FL, 1999; Vol. 3, pp 385−386.

(4) Huang, R. L.; Chen, C. C.; Huang, H. L.; Chang, C. G.; Chen, C. F.; Chang, C. Anti-hepatitis B virus effects of wogonin isolated from *Scutellaria baicalensis*. *Planta Med.* **2000**, *66* (8), 694–698.

(5) Huang, W. H.; Lee, A. R.; Yang, C. H. Antioxidative and anti-inflammatory activities of polyhydroxyflavonoids of *Scutellaria baicalensis* Georgi. *Biosci., Biotechnol., Biochem.* **2006**, *70* (10), 2371–2380.

(6) Shan, B.; Cai, Y.-Z.; Brooks, J. D.; Corke, H. The in vitro antibacterial activity of dietary spice and medicinal herb extracts. *Int. J. Food Microbiol.* **2007**, *117*, 112–119.

(7) Bruzewicz, S.; Malicki, A.; Oszmianski, J.; Jaroslawska, A.; Jarmoluk, A.; Pawlas, K. Baicalin, added as the only preservative, improves the microbiological quality of homemade mayonnaise. *Pakistan J. Nutr.* **2006**, *5* (1), 30–33.

(8) FAO/WHO. *Microbiological Hazards in Fresh Fruits and Vegetables*; Microbiological Risk Assesment Series; Rome, Italy, 2008 (meeting report).

(9) Harris, L. J.; Farber, J. N.; Beuchat, L. R.; Parish, M. E.; Suslow, T. V.; Garrett, E. H.; Busta, F. F. Outbreaks associated with fresh produce: incidence, growth, and survival of pathogens in fresh and fresh-cut produce. *Compr. Rev. Food Sci. Food Saf.* **2003**, *2* (1), 78–141.

(10) Petrignari, M.; Verhoef, L.; Van Hunen, R.; Swann, C.; Van Steenbergen, J.; Boxman, I. A possible foodborne outbreak of hepatitis A in the Netherlands. *Euro Surveill.* **2010**, *15* (11), 19512.

(11) Han, J.; Ye, M.; Xu, M.; Sun, J. H.; Wang, B. R.; Guo, D. Characterization of flavonoids in the traditional Chinese herbal medicine-Huangqin by liquid chromatography coupled with electrospray ionization mass spectrometry. *J. Chromatogr., B* **2007**, *848* (2), 355–362.

(12) Mariam, T. W.; Cliver, D. O. Small round coliphages as surrogates for human viruses in process assessment. *Dairy Food Environ. Sanit.* **2000**, *20* (9), 684–689.

(13) Fino, V. R.; Kniel, K. E. UV light inactivation of hepatitis A virus, Aichi virus, and feline calicivirus on strawberries, green onions, and lettuce. *J. Food Prot.* **2008**, *71* (5), 908–913.

(14) Guan, D.; Kniel, K.; Calci, K. R.; Hicks, D. T.; Pivarnik, L. F.; Hoover, D. G. Response of four types of coliphages to high hydrostatic pressure. *Food Microbiol.* **2006**, *23* (6), 546–551.

(15) Gulati, B. R.; Allwood, P. B.; Hedberg, C. W.; Goyal, S. M. Efficacy of commonly used disinfectants for the inactivation of calicivirus

on strawberry, lettuce, and a food-contact surface. *J. Food Prot.* **2001**, *64* (9), 1430–1434.

(16) Barrett, P. N.; Meyer, H.; Wachtel, I.; Eibl, J.; Dorner, F. Determination of the inactivation kinetics of hepatitis A virus in human plasma products using a simple $TCID_{50}$ assay. *J. Med. Virol.* **1996**, *49* (1), 1–6.

(17) Hansen, L. T.; Austin, J. W.; Gill, T. A. Antibacterial effect of protamine in combination with EDTA and refrigeration. *Int. J. Food Microbiol.* **2001**, *66* (3), 149–161.

(18) Ashebir, D.; Jezik, K.; Weingartemann, H.; Gretzmacher, R. Change in color and other fruit quality characteristics of tomato cultivars after hot-air drying at low final-moisture content. *Int. J. Food Sci. Nutr.* **2009**, *60* (7), 308–315.

(19) Tsai, T. H.; Tsai, T. H.; Chien, Y. C.; Lee, C. W.; Tsai, P. J. In vitro antimicrobial activities against cariogenic streptococci and their antioxidant capacities: a comparative study of green tea versus different herbs. *Food Chem.* **2008**, *110* (4), 859–864.

(20) Zhang, L.; Zhang, R. W.; Li, Q.; Lian, J. W.; Liang, J.; Chen, X. H. Development of the fingerprints for the quality evaluation of Scutellariae Radix by HPLC-DAD and LC-MS-MS. *Chromatographia* **2007**, *66* (1), 13–20.

(21) Woo, I. T.; Park, K. N.; Lee, S. H. Antimicrobial activities of *Scutellaria baicalensis* Georgi against various pathogens and spoilage bacteria isolated from tofu. *J. Korean Soc. Food Sci. Nutr.* **2007**, *36* (4), 470–475.

(22) Li, J.; Khan, I. The advanced bioactivity studies of *Scutellaria baicalensis* Georgi and its phenolic compounds. *Acta Hortic.* **2006**, *720*, 157–169.

(23) Zhao, T. H.; Deng, S. H.; Yang, H. S.; Chen, S. P. Study of antibacterial activity of active fraction from stems and leaves of *Scutellaria baicalensis* Georgi. *Chinese Pharm. Bull.* **2007**, *23* (7), 882–886.

(24) Fattouch, S.; Caboni, P.; Coroneo, V.; Tuberoso, C. I.; Angioni, A.; Dessi, S.; Marzouki, N.; Cabras, P. Antimicrobial activity of Tunisian quince (*Cydonia oblonga* Miller) pulp and peel polyphenolic extracts. *J. Agric. Food Chem.* **2007**, *55* (3), 963–969.

(25) Zhu, X.; Zhang, H.; Lo, R. Phenolic compounds from the leaf extract of artichoke (*Cynara scolymus* L.) and their antimicrobial activities. *J. Agric. Food Chem.* **2004**, *52* (24), 7272–7278.

(26) Chou, S. C.; Everngam, M. C.; Sturtz, G.; Beck, J. J. Antibacterial activity of components from *Lomatium californicum*. *Phytother. Res.* **2006**, *20* (2), 153–156.

(27) Naz, S.; Ahmad, S.; Ajaz Rasool, S.; Asad Sayeed, S.; Siddiqi, R. Antibacterial activity directed isolation of compounds from *Onosma hispidum*. *Microbiol. Res.* **2006**, *161* (1), 43–48.

(28) Yuk, H. G.; Bartz, J. A.; Schneider, K. R. Effectiveness of individual or combined sanitizer treatments for inactivating *Salmonella* spp. on smooth surface, stem scar, and wounds of tomatoes. *J. Food Sci.* **2005**, *70* (9), 409–414.

(29) Artes, F.; Gomez, P.; Aguayo, E.; Escalona, V.; Artes-Hernundez, F. Sustainable sanitation techniques for keeping quality and safety of fresh-cut plant commodities. *Postharvest Biol. Technol.* **2009**, *51* (3), 287–296.

(30) Ito, N.; Fukushima, S.; Tamano, S.; Hiro, M.; Hagiwara, A. Dose response in butylated hydroxyanisole induction of forestomach carcinogenesis in F 334 rats. *J. Natl. Cancer Inst.* **1986**, *77* (6), 1261–1265.

(31) Lis-Balchin, M.; Deans, S. T. Bioactivity of selected plant essential oils against *Listeria monocytogenes*. *J. Appl. Microbiol.* **1997**, *82* (6), 759–762.

(32) Ravishankar, S.; Zhu, L.; Reyna-Granados, J.; Law, B.; Joens, L.; Friedman, M. Carvacrol and cinnamaldehyde inactivate antibiotic-resistant *Salmonella enterica* in buffer and on celery and oysters. *J. Food Prot.* **2010**, *73* (2), 234–240.

(33) Si, W.; Gong, J.; Chanas, C.; Cui, S.; Yu, H.; Caballero, C.; Friendship, R. M. In vitro assessment of antimicrobial activity of carvacrol, thymol and cinnamaldehyde towards *Salmonella* serotype Typhimurium DT104: effects of pig diets and emulsification in hydrocolloids. *J. Appl. Microbiol.* **2006**, *101* (6), 1282–1291.

(34) Fukumoto, L.; Toivonen, P.; Delaquis, P. Effect of wash water temperature and chlorination on phenolic metabolism and browning of stored iceberg lettuce photosynthetic and vascular tissues. *J. Agric. Food Chem.* **2002**, *50* (16), 4503–4511.

(35) Wu, C. Q.; Duckett, S. K.; Neel, J. P. S.; Fontenot, J. P.; Clapham, W. M. Influence of finishing systems on hydrophilic and lipophilic oxygen radical absorbance capacity (ORAC) in beef. *Meat Sci.* **2008**, *80* (3), 662–667.