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Antibacterial and virucidal activity of 28 extracts from plants endemic to Korea against *Bacillus cereus*, *Staphylococcus aureus*, and murine norovirus

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Abstract Antibacterial activity against foodborne bacteria (*Bacillus cereus*, *Staphylococcus aureus*, *Salmonella* Enteritidis) and inhibitory activity against murine norovirus, a human norovirus surrogate, of 28 extracts from plants endemic to Korea were investigated in this study. All plant extracts showed antibacterial activity only against gram-positive bacteria, *B. cereus* and *S. aureus*. Extracts from *Callistemon speciosus* and *Nymphaea tetragona* showed inhibition zones of 16.54 and 24.35 mm against *B. cereus* and *S. aureus*, respectively, presenting the highest antibacterial activities recorded in this study. Among all samples, *Ardisia japonica* extract at concentrations of 100 and 200 μg/mL showed the highest virucidal activities of 96.6 and 100.0%, respectively. *Ardisia japonica*, *Duchesnea indica*, *Polygonum aviculare*, and *Geum japonicum* extracts showed high antibacterial and virucidal activity simultaneously without Raw 264.7 cell cytotoxicity. These plant extracts may serve as potential antimicrobials to control foodborne infections.

Keywords Antibacterial · Foodborne pathogens · Plant extracts · Virucidal

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Introduction

Foodborne diseases are significant contributors to the global disease burden [1]. Outbreaks of food poisoning and incidences of food contamination, especially owing to foodborne bacteria and viruses, are reported in many countries every year, resulting in great social and economic losses as well as human health hazards [2,3]. The incidence of foodborne infections globally reaches 600 million cases and is estimated to cause 420,000 deaths per year [4]. In Korea, over the last 5 years (2018-2022), bacteria and viruses accounted for approximately 39.1 and 19.5% of cases, respectively, caused by all food poisoning-causative substances, with norovirus alone accounting for 18% of the cases, thus becoming the single most common causative agent [5]. Foodborne illnesses also entail a huge economic burden and are estimated to cost the US economy between $55.5 and $93.2 billion per year [6].

In practice, current antimicrobial techniques or chemotherapeutics to treat different microbial contaminations or infections are limited by low efficacy, multi-drug resistance, and many other side effects [7-11]. Annual deaths worldwide owing to antimicrobial resistance continue to climb to around 750,000 and are projected to reach as high as 10 million by the year 2050 [12]. Thus, there are constant demands for the discovery of alternative control methods and agents. Furthermore, as consumers become more concerned about food safety and environmental issues, they show an increasing need for ‘natural’ materials. Natural antimicrobials can be obtained from animals, plants, and microorganisms, and among them, plants have many advantages in that they are very abundant and safe in terms of antibiotic gene transfer and have good therapeutic potential and accessibility [10,13]. Additionally, naturally derived medicinal plant extracts are more easily absorbed by the body owing to their natural origin and show fewer side effects [14].

Various studies have regularly reported antimicrobial activities of medicinal plants all over the world [7]. Crude plants and

phytochemicals (secondary metabolites), including extracts, essential oils, and isolated compounds, play a key role in antimicrobial activities against foodborne pathogens; they have been extensively evaluated for their ability to prevent the invasion of pathogens responsible for food spoilage and, therefore, may be used to limit the spread of foodborne infections [15]. The antibacterial activity of various plant extracts against *Campylobacter* species, *Salmonella spp*, *Escherichia coli*, *Staphylococcus aureus*, *Shigella*, *Listeria monocytogenes*, *Clostridium* spp, *Bacillus cereus*, and *Vibrio cholerae* has been reported [7]. For example, the extracts of *Dryopteris erythrosora* (D.C. Eaton) Kuntze, *Siegesbeckia glabrescens* Makino leaf, *Morus alba* L. bark, *Carex pumila* Thunb. root, and *Citrus paradisi* Macfad. seed were reported to have potent antibacterial activity against *B. cereus* [16]. Several studies have demonstrated the antibacterial property of essential oils derived from *Petroselinum crispum* (Mill.) Fuss, *Cuminum cyminum* L, white mustard (*Sinapis alba* L.), and *Chamaecyparis obtusa* (Siebold & Zucc.) Endl against *S. aureus* [17-20]. Moreover, the antiviral activity of extracts from grape seeds, *Angelica gigas*, *Citrus aurantium*, and *Lindera obtusiloba*; essential oils from *Origanum vulgare*, *Pimenta dioica*, and *Thymus mastichina*; isolated compounds such as carvacrol, thymol, catechins, citral, and curcumin and juices from orange, cranberry, pomegranate, among others against murine norovirus (MNV) and feline calicivirus (FCV), which are human norovirus (HNV) surrogates, has also been reported [2]. Although many research reports are available on antibacterial materials, there is a relatively small amount of data available on the use of antiviral materials [21]. Since bacteria and viruses coexist in food substances and in the environment, it is necessary to explore these plant-based natural antimicrobials with a wide spectrum of both antibacterial and antiviral activities rather than those that have antibacterial or antiviral activity alone.

The purpose of this study was to explore extracts from plants endemic to Korea that exhibit antibacterial and virucidal activities against foodborne pathogenic bacteria, *B. cereus, S. aureus, S.* Enteritidis, and MNV as an HNV surrogate.

Materials and Methods

Materials and bacterial strains

A total of 28 plants endemic to Korea are listed in Table 1. The methanol extracts of these plants were purchased in the form of concentrates from the Natural Product Central Bank at the Korean Research Institute of Bioscience & Biotechnology (KRIBB; Daejeon, Korea). Brief extraction procedure of plant extracts is as follows: The plant (120 g) dried in the shade and powdered was added to 1 L of Methyl alcohol 99.9% (HPLC grade) and extracted through 30 cycles (40 KHz, 1500 W, 15 min. ultrasonication-120 min. standing per cycle) at room temperature using an ultrasonic extractor (SDN-900H, SD-Ultrasonic Co., Ltd., Seoul, Korea). After filtration (Qualitative Filter No. 100, Hyundai Micro Co., Ltd., Seoul, Korea) and drying under reduced pressure, plant extract with a yield of 10% was obtained. Bacterial strains used in this study were *B. cereus* (ATCC 14579), *S. aureus* (ATCC 25923), and *S.* Enteritidis (ATCC 13076). Kanamycin, ribavirin, and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Muller-Hinton agar (MHA) and tryptic soy broth (TSB) were bought from Becton Dickinson (Sparks, MD, USA). DMEM medium, fetal bovine serum (FBS), and penicillin/streptomycin were purchased from Welgene (Gyengsan, Korea).

Cell culture and virus preparation

Raw 264.7 cell line was kindly provided by Dr. So-young Lee from the Korea Food Research Institute. Cells were maintained in DMEM medium supplemented with 5% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin at 37 ºC in 5% CO_2. MNV was obtained from American type culture collection (ATCC, Manassas, VA, USA). The MNV stock was propagated by adding 100 μL of MNV stock and 4 mL DMEM to 70% confluent Raw 264.7 cells in a plate. After 2 h incubation, the medium was supplemented with 5% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin and incubated for 2 days to monitor cytopathic effect presentations. The culture was then centrifuged for 5 min at 4500 rpm and the supernatant was stored at −80 ºC. The virus titer was determined by using plaque assay.

Cell viability assay

Cell viability was measured using an MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] assay [22]. Raw 264.7 cells were seeded in a 96-well plate at a density of 1×10^4 cells/well, and incubated for 24 h at 37 ºC in an incubator at 5% CO_2. Cells were treated with 10 μL of plant extracts in concentrations of 100 and 200 μg/mL. After 24 h incubation, 5 mg/mL of MTT solution was added to each well, and the plates were incubated for another 4 h at 37 ºC. After the removal of the supernatant, 100 μL of DMSO was added to dissolve the formazan crystals. The absorbance was measured at 540 nm using a microplate reader (SpectraMax i3x, Molecular Devices Corp., CA, USA). Cell viability is expressed as the percentage of absorbance of the treated cells compared with that of the untreated cells.

Determination of antibacterial activity

The antibacterial activities of the plant extracts against three foodborne bacteria (*B. cereus*, *S. aureus*, and *S.* Enteritidis*)* were determined using the agar well diffusion assay [23]. Bacteria were incubated in 5 mL of TSB overnight at 37 ºC in a shaking incubator. Each bacterial suspension was diluted to 10^6 CFU/mL and then spread on MHA using a sterile cotton swab. A hole with a diameter of 6 mm was punched using a sterile tip on the MHA plate. Then, 50 μL of the extract, at a concentration of 20 mg/mL, was introduced into the well. DMSO and Kanamycin were used

Table 1 List of endemic plants screened in this study

Genus and species	Family	Part used	Collection Area
Duchesnea indica 1	Rosaceae	Whole	Chungnam
Duchesnea indica 2	Rosaceae	Whole	Jeju
Elaeocarpus sylvestris	Elaeocarpaceae	Leaf	Jeju
Ardisia japonica 1	Myrsinaceae	Leaf	Jeju
Ardisia japonica 2	Myrsinaceae	Whole	Jeju
Reynoutria sachalinensis	Polygonaceae	Fruit	Ulleung
Polygonum aviculare	Polygonaceae	Whole	Jeonbuk
Callistemon speciosus 1	Myrtaceae	Stem	Jeju
Callistemon speciosus 2	Myrtaceae	Leaf	Jeju
Cornus controversa 1	Cornaceae	Leaf, Stem	Ulleung
Cornus controversa 2	Cornaceae	Leaf, Stem	Jeonnam
Staphylea bumalda	Staphyleaceae	Leaf, Stem	Gangwon
Paeonia lactiflora	Paeoniaceae	Whole	Jeonbuk
Geum japonicum	Rosaceae	Whole	Jeonbuk
Geranium sibiricum	Geraniaceae	Whole	Gyeonggi
Nymphaea tetragona	Nymphaeaceae	Root	Jeonbuk
Mallotus japonicas 1	Euphorbiaceae	Leaf, Stem	Chungnam
Mallotus japonicas 2	Euphorbiaceae	Leaf, Stem	Jeonnam
Plantago major	Plantaginaaceae	Whole	Jeonbuk
Angelica tenuissima	Umbelliferae	Whole	Gangwon
Perilla frutescens	Labiatae	Whole	Jeonbuk
Agrimonia pilosa	Rosaceae	Whole	Jeonnam
Rhus chinensis 1	Anacardiaceae	Leaf, Stem	Gyeonggi
Rhus chinensis 2	Anacardiaceae	Leaf, Stem	Gangwon
Cannabis sativa	Cannabaceae	Whole	Jeonbuk
Bletilla striata	Orchidaceae	Whole	Jeonbuk
Paeonia suffruticosa	Paeoniaceae	Whole	Jeonbuk
Acer pictum	Aceraceae	Leaf, Stem	Gyeonggi

as negative and positive controls, respectively. After 24 h incubation at 37 ºC, antimicrobial activity of each extract was analyzed by measuring the growth inhibition zone (diameter) that appeared around the well on the MHA plate.

Determination of virucidal activity

The virucidal activity of plant extracts on MNV was evaluated by incubating an MNV suspension (1×10^7 PFU/mL) with plant extracts (100 and 200 μg/mL) for 3 h at 37 ºC [24]. Then, the titer of MNV was directly measured using plaque assay [25]. Briefly, Raw 264.7 cells were seeded on 6-well plates at a density of 1×10^6 cells/well. On the next day, the cells were incubated for 1 h and 10 min with the virus inoculum properly diluted in serum-free DMEM. The inoculum was then aspirated, and the fresh complete DMEM containing SeaPlaque agarose (1% w/v; Lonza) was overlaid. Plaque counting was performed after 48 h by visualization with crystal violet staining. Virucidal activity was expressed as plaque-formation percentage subtracted from 100 of the extract-treated group with respect to the extract-untreated group.

Statistical analysis

All data were presented as mean±SD of three repeated measurements, and *t*-tests were analyzed using GraphPad Prism 9 software (GraphPad Inc., La Jolla, CA, USA). The differences between the groups were considered significant when p values were less than 0.05.

Results and Discussion

Cytotoxicity of plant extracts

In the preliminary study, 44 extracts exhibiting antibacterial activity against foodborne bacteria (*B. cereus*, *E. coli*, and *S.* Enteritidis) were first identified from the extracts of more than 200 plants endemic to Korea, and the virucidal activity against MNV was measured for these 44 samples (data not shown). Finally, 28 extracts of plants endemic to Korea (Table 1) having both antibacterial and virucidal activities were selected for this study. Additionally, a preliminary experiment on cytotoxicity was

Table 2 Viability (%) of Raw 264.7 cells treated with extracts from plants endemic to Korea

Extracts	Concentration (μg/mL)		Extracts	Concentration (μg/mL)	
	100	200		100	200
Duchesnea indica 1	99.7±1.0[a]	94.6±2.4[b]	*Nymphaea tetragona*	24.1±0.4[a]	30.9±0.2[b]
Duchesnea indica 2	98.3±1.0[a]	94.1±1.7[b]	*Mallotus japonicas 1*	78.4±3.5[a]	14.2±1.2[b]
Elaeocarpus sylvestris	48.3±0.5[a]	36.4±1.8[b]	*Mallotus japonicas 2*	91.5±1.2[a]	51.5±1.2[b]
Ardisia japonica1	97.7±4.6[a]	93.5±4.2[a]	*Plantago major*	97.6±2.6[a]	75.6±4.4[b]
Ardisia japonica2	99.2±1.6[a]	93.6±2.2[b]	*Angelica tenuissima*	92.9±0.8[a]	25.8±3.3[b]
Reynoutria sachalinensis	99.6±1.0[a]	68.3±3.9[b]	*Perilla frutescens*	96.7±1.1[a]	65.7±4.1[b]
Polygonum aviculare	99.3±1.3[a]	93.6±3.6[b]	*Agrimonia pilosa*	94.7±3.1[a]	59.1±5.2[b]
Callistemon speciosus 1	14.7±0.6[a]	11.5±0.9[b]	*Rhus chinensis 1*	54.8±0.6[a]	6.9±0.7[b]
Callistemon speciosus 2	4.8±0.3[a]	7.7±0.4[b]	*Rhus chinensis 2*	54.8±3.1[a]	19.9±2.3[b]
Cornus controversa 1	80.4±1.2[a]	41.6±3.1[b]	*Cannabis sativa*	48.4±1.9[a]	3.2±0.6[b]
Cornus controversa 2	9.8±0.9[a]	8.3±0.3[b]	*Bletilla striata*	18.7±1.9[a]	3.6±0.2[b]
Staphylea bumalda	97.2±2.0[a]	74.3±0.6[b]	*Paeonia suffruticosa*	43.1±1.8[a]	35.1±1.7[b]
Paeonia lactiflora	50.0±2.2[a]	27.5±2.1[b]	*Acer pictum*	36.7±3.5[a]	20.8±1.3[b]
Geum japonicum	99.6±0.3[a]	96.8±1.3[b]	Ribavirin*	72.5±3.0[a]*	58.1±2.2[b]*
Geranium sibiricum	90.9±2.0[a]	40.6±1.5[b]			

*Viability (%) of Raw 264.7 cells when the concentration of ribavirin is 50 or 100 μM
[a,b]Letters indicate statistical difference between concentrations (p <0.05)

conducted using three concentrations (100, 200 μg/mL, and 2 mg/mL) of plant extracts to determine the effective and non-cytotoxic concentration. From these results, the concentration of 2 mg/mL, which was highly cytotoxic with 30% or less viability in most samples, was eliminated for this study (data not shown). The viability of Raw 264.7 cells treated with those plant extracts is shown in Table 2. The viability of Raw 264.7 cells decreased in the presence of plant extracts in a concentration-dependent manner. When treated with a concentration of 100 μg/mL of each extract, half the number of extracts resulted in cell viability of 90.9-99.7%. When treated with a concentration of 200 μg/mL, six extracts, including those of *Duchesnea indica* 1 & 2, *Ardisia japonica* 1 & 2, *Polygonum aviculare,* and *Geum japonicum* resulted in cell viabilities of 93.5-96.8% (Table 2). Ribavirin used as a positive control for virucidal activity resulted in viabilities of 72.5 and 58.1% at concentrations of 50 and 100 μM, respectively.

Antibacterial activity of plant extracts

Antibacterial activity of the 28 extracts, chosen by preliminary screening described above, from plants endemic to Korea from 19 different families was investigated *in vitro* against *B. cereus, S. aureus,* and *S.* Enteritidis, which are known foodborne pathogens. All extracts at a concentration of 1 mg/well showed antibacterial activity against gram-positive bacteria, *B. cereus and S. aureus,* but not against gram-negative bacteria, *S.* Enteritidis (Table 3). The inhibition zones ranged from 6.53 to 16.54 mm for *B. cereus* and from 7.93 to 24.35 mm for *S. aureus*; therefore, *S. aureus* can be considered slightly more susceptible than *B. cereus*. Kanamycin was used as a positive control at a concentration of 30 μg/well and exhibited inhibition zones of 22.22 mm against *B. cereus*, 31.44 mm against *S. aureus,* and 25.82 mm against *S.* Enteritidis, whereas DMSO used as a negative control did not show any inhibitory activity against the three pathogens (Table 3). The extracts *Callistemon speciosus 2* against *B. cereus, and Nymphaea tetragona* against *S. aureus* showed the highest inhibition zones of 16.54 and 24.35 mm, respectively, showing lower levels of inhibition than exhibited by Kanamycin (Table 3). In another study, the ethyl acetate fraction of *Nymphaea tetragona* extract demonstrated good antimicrobial activity (minimum inhibitory concentration, 781 μg/mL) against four out of five *S.* Typhimurium *strains* [26]. In contrast, the extract of *Nymphaea tetragona* used in this study did not show any antibacterial activity against *S.* Enteritidis, even though both belong to the genus *Salmonella*. This variation may be attributed to the difference in the solvent used for extraction, the kinds of bacterial strains, and the source of the plant.

This study showed that methanolic extracts of various plants have promising antibacterial activity. It is reported that the methanol extract generally has greater antimicrobial activity than other solvent extracts [27]. This may be attributed to the polarity of methanol and its capability to extract more compounds from the plant samples, especially phenolic and polyphenolic groups of compounds [28,29]. Nevertheless, as plant extracts obtained from nature exist as a mixture of various compounds, it may be necessary to examine the effect of extraction using various solvents. The efficacy of crude antibacterial plant extracts may vary depending on the components and the spectrum of bacteria to be controlled, and more than one antibacterial mechanism may

Table 3 Antibacterial activity of extracts from plants endemic to Korea

Extracts	Inhibition zone (mm)		
	B. cereus	*S. aureus*	*S.* Enteritidis
Duchesnea indica 1	7.07±0.31	11.11±1.83	ND
Duchesnea indica 2	6.99±0.26	17.32±1.01	ND
Elaeocarpus sylvestris	9.76±0.36	22.84±0.60	ND
Ardisia japonica 1	8.67±0.26	10.84±0.68	ND
Ardisia japonica 2	7.95±0.38	9.70±0.20	ND
Reynoutria sachalinensis	11.32±0.49	10.54±0.21	ND
Polygonum aviculare	9.20±0.60	13.38±0.63	ND
Callistemon speciosus 1	8.02±0.52	14.67±1.02	ND
Callistemon speciosus 2	16.54±0.89	19.13±1.10	ND
Cornus controversa 1	9.23±0.64	20.22±0.55	ND
Cornus controversa 2	7.82±0.07	19.67±0.64	ND
Staphylea bumalda	7.40±0.17	15.58±2.81	ND
Paeonia lactiflora	8.90±0.72	19.45±0.29	ND
Geum japonicum	8.08±0.46	19.94±1.27	ND
Geranium sibiricum	9.08±0.74	17.40±0.67	ND
Nymphaea tetragona	13.07±0.36	24.35±0.26	ND
Mallotus japonicas 1	8.31±0.73	20.67±1.05	ND
Mallotus japonicas 2	6.53±0.92	9.29±0.44	ND
Plantago major	6.98±0.89	7.93±0.15	ND
Angelica tenuissima	8.36±0.66	20.70±0.76	ND
Perilla frutescens	7.37±0.60	12.56±1.33	ND
Agrimonia pilosa	9.81±0.20	16.74±0.85	ND
Rhus chinensis 1	9.91±0.20	18.67±0.83	ND
Rhus chinensis 2	10.62±0.39	15.54±0.51	ND
Cannabis sativa	7.72±0.16	8.92±2.63	ND
Bletilla striata	10.89±0.83	11.52±0.53	ND
Paeonia suffruticosa	9.28±0.98	17.93±0.38	ND
Acer pictum	9.09±0.84	12.41±0.80	ND
Kanamycin*	22.22±1.86*	31.44±1.58*	25.82±1.32*

*Inhibition zones (mm) against *B. cereus, S. aureus,* and *S.* Enteritidis when the concentration of kanamycin is 30 μg/well

be involved [7].

Mahida and Mohan [30] reported that *S. aureus* was found to be more susceptible to 68% of test plant extracts than other pathogens. This report is consistent with our findings that *S. aureus* was more susceptible than *B. cereus* when treated with the 28 plant extracts. In addition, gram-positive bacteria, including *B. cereus* and *S. aureus*, were susceptible to the endemic plant extracts, while gram-negative bacteria, including *S.* Enteritidis, were resistant to them in our study. Other studies on the antimicrobial activity of plant extracts against pathogenic bacteria support our results that plant extracts are more active against gram-positive bacteria [31,32]. This may be attributed to the fact that gram-positive and gram-negative bacteria differ in the structure and components of the cell wall. As the outer membrane of gram-negative bacteria has an asymmetric distribution of lipids with phospholipids and lipopolysaccharide (LPS) located in the inner and outer leaflets, which act as a barrier to many environmental substances, gram-negative bacteria are more resistant [33,34].

Virucidal activity of plant extracts

As shown in Table 4, the plant extracts showed virucidal activities ranging from 40.5 to 97.1% at a concentration of 100 μg/mL, and virucidal activities ranging from 37.7 to 100%, at a concentration of 200 μg/mL. As a positive control, ribavirin, an antiviral drug used for the treatment of hepatitis C virus infection, reduced the MNV titer by 68.3 and 37.7% at concentrations of 50 and 100 μM, respectively. The following plant extracts showed a high virucidal activity of 90% (i.e., 1 log PFU/mL reduction) or more, without exhibiting Raw 264.7 cell cytotoxicity: *Ardisia japonica* 1 & 2, *Polygonum aviculare*, and *Geum japonicum* at a concentration of 100 μg/mL, and *Duchesnea indica 2*, *Ardisia japonica* 1 & 2, *Polygonum aviculare*, and *Geum japonicum* at a concentration of

200 μg/mL. Both *Ardisia japonica* 1 & 2 showed the highest virucidal activity of 94.0-100%. Ideally, a plant extract being considered for its antiviral/antibacterial activity should have no effect on the cell growth and/or cell morphology [35]. In this study, although *Callistemon speciosus 1* showed high antiviral activity of 94.1 and 99.0% at concentrations of 100 and 200 μg/mL, respectively (Table 4), this extract shall not be considered as a promising antiviral candidate owing to the low viability of 14.7% and 11.5%, respectively (Table 2).

MNV was used to screen plant extracts with virucidal activity in this study. As HNVs do not multiply *in vitro* in cell culture, HNV surrogates, such as MNV or FCV, which are devoid of an envelope, contain ssRNA, and show high resistance to both antimicrobial preparation and environmental conditions, are commonly used in laboratory tests [1,36]. MNV can be used as a model to study norovirus replication and pathogenesis and has facilitated a better understanding of the norovirus life cycle [37]. There are various factors that affect the antiviral activity of plant-derived extracts and compounds, such as the type of virus tested, the incubation time and temperature with the virus, the type and concentration of the extracts/compounds, among others [8,24]. *Ardisia japonica* and *Geum japonicum* showed high virucidal activity against MNV in this study. Interestingly, constituents of *Ardisia japonica* and triterpene acids from *Geum japonicum* have been reported to have *in vitro* anti-human immunodeficiency (HIV) virus activity [38,39,40]; however, there are no previous reports of antiviral activity against MNV. In order to measure the virucidal activity, the incubation time was set to 3 h at 37 ºC to ensure sufficient reaction between the plant extract and the virus in this study. It is reported that preincubation of norovirus with natural substances can be used to determine the capacity of these compounds to reduce infectivity [41]. Several studies have suggested that antiviral activity increases with the reaction time between the virus and natural substances, the concentration of natural compounds, and the reaction temperature [8,38,40-42].

We demonstrated virucidal activity against murine norovirus using methanol extracts of plant samples. Similar to our study, Oh et al. [43] demonstrated the antiviral activity of methanol extracts from spices, herbal teas, and medicinal herbs against FCV. The antiviral activity against norovirus surrogates may vary depending on the type of solvent used for extraction. Iloghalu et al. [44] indicated that there was a reduction of MNV when treated with 60% aqueous methanol extracts of plants. In another study, Seo and Choi [45] determined the antiviral activities of methanol and ethanol extracts of some Korean herbs against MNV and FCV. Aboubakr et al. [46] used aqueous extracts from clove, fenugreek, garlic, onion, ginger, and jalapeno, and reported that clove and ginger extracts deactivated 6.0 and 2.7 log PFU/mL of the initial viral titers, respectively.

Eggers et al. [47] reported that the virucidal activity of a test preparation against a specific virus is confirmed if the infectious virus has decreased by at least 4 logs in the titer compared with the control mixture. Among the samples used in this study, *Ardisia japonica* 1 is the most promising plant extract, because it showed the highest activity of 100%, i.e., more than 4 log PFU/mL reduction, at a concentration of 200 μg/mL (Table 4). In addition,

Table 4 Virucidal activity (%) of extracts from plants endemic to Korea on murine norovirus

Extracts	Concentration (μg/mL)		Extracts	Concentration (μg/mL)	
	100	200		100	200
Duchesnea indica 1	40.5±9.8^{a}	72.6±3.9^{b}	*Nymphaea tetragona*	72.7±7.4^{a}	80.9±3.4^{a}
Duchesnea indica 2	86.5±4.0^{a}	95.5±4.0^{b}	*Mallotus japonicas 1*	80.7±2.5^{a}	73.0±5.0^{a}
Elaeocarpus sylvestris	40.5±6.1^{a}	73.5±8.8^{b}	*Mallotus japonicas 2*	84.5±0.8^{a}	88.8±0.8^{b}
Ardisia japonica1	96.6±1.5^{a}	100.0±0.0^{b}	*Plantago major*	48.6±5.2^{a}	69.4±5.2^{b}
Ardisia japonica2	94.0±5.3^{a}	99.1±1.5^{a}	*Angelica tenuissima*	57.6±1.2^{a}	65.6±4.4^{b}
Reynoutria sachalinensis	78.8±2.5^{a}	88.3±6.7^{a}	*Perilla frutescens*	75.0±4.8^{a}	80.5±5.4^{a}
Polygonum aviculare	97.1±5.1^{a}	97.8±2.2^{a}	*Agrimonia pilosa*	86.0±4.6^{a}	90.5±1.7^{a}
Callistemon speciosus 1	94.1±0.9^{a}	99.0±1.2^{b}	*Rhus chinensis 1*	51.6±1.3^{a}	64.4±6.5^{a}
Callistemon speciosus 2	65.6±4.7^{a}	82.9±4.2^{b}	*Rhus chinensis 2*	74.4±4.8^{a}	82.9±7.4^{a}
Cornus controversa 1	77.9±4.0^{a}	91.3±0.6^{b}	*Cannabis sativa*	83.8±3.6^{a}	75.9±5.3^{a}
Cornus controversa 2	77.3±7.0^{a}	95.0±4.6^{b}	*Bletilla striata*	69.6±1.8^{a}	86.6±3.8^{b}
Staphylea bumalda	65.7±4.6^{a}	62.6±6.8^{a}	*Paeonia suffruticosa*	65.2±3.3^{a}	59.6±6.3^{a}
Paeonia lactiflora	76.3±1.7^{a}	70.7±2.3^{b}	*Acer pictum*	81.2±2.5^{a}	84.5±1.0^{a}
Geum japonicum	91.5±1.7^{a}	96.8±1.6^{b}	Ribavirin*	68.3±10.0^{a}*	37.7±9.1^{b}*
Geranium sibiricum	78.9±1.7^{a}	70.1±3.3^{b}			

Virucidal activity (%) was expressed as plaque-formation percentage subtracted from 100 of the extract-treated group with respect to the extract-untreated group

*Virucidal activity (%) when the concentration of ribavirin is 50 or 100 μM

^{a,b}Letters indicate statistical difference between concentration (p <0.05)

we used an experimental method where the virus directly reacted with the plant extracts to promptly select the extracts with direct virucidal activity. To investigate the antiviral mechanism of natural components, cells must undergo pre-, co-, and post-treatment with the plant extract. Plant extracts contain various types of phytochemicals, which exhibit various antiviral activities that employ different mechanisms [48,49]. It is reported that the mechanism of antiviral activity of natural biochemical compounds including polyphenols, proanthocyanins, saponin, etc involves the prevention of viral attachment to host cells or blocking/damaging of either the viral capsids or receptors on the cell membranes, although it is not fully understood [50]. The chemical constituents of *Ardisia japonica* with high virucidal activity against MNV in this study have been reported and include triterpenoid saponins, bergenin and analogues, benzoquinones, a dimeric lactone, ardimerin digallate, ardimerin, quercitrin, friedelin, bauerenyl acetate, epifriedelinol, and bauerenol [38,39]. Of these compounds, bergenin and norbergenin showed weak anti-HIV activity, and norbergenin was more effective when added prior to or at the time of virus infection, suggesting that it inhibits at an early stage of the virus infection [38]. Ardimerin digallate showed inhibitory activity against both HIV-1 and HIV-2 ribonuclease H, while ardimerin, which lacks a galloyl moiety, did not show any inhibition of ribonuclease H nor did the other compounds (quercitrin, friedelin, bauerenyl acetate, and epifriedelinol) tested, suggesting that the inhibitory effects of ardimerin digallate are mediated in part due to the presence of galloyl unit [39].

We demonstrated *in vitro* antibacterial and virucidal activities of methanol extracts from 28 plants endemic to Korea against foodborne pathogenic bacteria and viruses. The antibacterial activity results from agar well diffusion experiments indicated that the extracts from *Callistemon speciosus* and *Nymphaea tetragona* had the highest activities and were more effective against *B. cereus* and *S. aureus*, gram-positive bacteria than *S.* Enteritidis, gram-negative bacteria. The virucidal activity studies revealed that *Ardisia japonica* had the highest plaque reduction effects of 96.6 and 100.0% at concentrations of 100 and 200 μg/mL, respectively. *Ardisia japonica*, *Duchesnea indica*, *Polygonum aviculare,* and *Geum japonicum* revealed high antibacterial and virucidal activity simultaneously without cytotoxicity. Further studies should be followed by the identification and structural characterization of the active components of these plants that showed the best activity. The exploration of novel extracts showing antibacterial efficacy against gram-negative bacteria and research on the mechanism of action of antibacterial and antiviral compounds are also required. These bioactive extracts from endemic plants in this study have the potential to be used as natural antibacterial and antiviral agents against foodborne bacteria and viruses.

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