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Antiviral effects of black raspberry (Rubus coreanus) seed extract and its polyphenolic compounds on norovirus surrogates

Ji-Hye Lee1,†, Sun Young Bae2,†, Mi Oh2, Jong Hyeon Seok1, Sella Kim1, Yeon Bin Chung1, Giri Gowda K1, Ji Young Mun3, Mi Sook Chung2 and Kyung Hyun Kim1,*

1Department of Biotechnology & Bioinformatics, Korea University, Sejong, Korea; 2Department of Food and Nutrition, Duksum Women’s University, Seoul, Korea; 3Department of Biomedical Laboratory Science, College of Health Science, Eulji University, Gyeonggi-do, Korea

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Black raspberry seeds, a byproduct of wine and juice production, contain large quantities of polyphenolic compounds. The antiviral effects of black raspberry seed extract (RCS) and its fraction with molecular weight less than 1 kDa (RCS-F1) were examined against food-borne viral surrogates, murine norovirus-1 (MNV-1) and feline calicivirus-F9 (FCV-F9). The maximal antiviral effect was achieved when RCS or RCS-F1 was added simultaneously to cells with MNV-1 or FCV-F9, reaching complete inhibition at 0.1–1 mg/mL. Transmission electron microscopy (TEM) images showed enlarged viral capsids or disruption (from 35 nm to up to 100 nm) by RCS-F1. Our results thus suggest that RCS-F1 can interfere with the attachment of viral surface protein to host cells. Further, two polyphenolic compounds derived from RCS-F1, cyanidin-3-glucoside (C3G) and gallic acid, identified by liquid chromatography-tandem mass spectrometry, showed inhibitory effects against the viruses. C3G was suggested to bind to MNV-1 RNA polymerase and to enlarge viral capsids using differential scanning fluorimetry and TEM, respectively.

Key words: antiviral activity; murine norovirus; feline calicivirus; black raspberry seed extract; cyanidin-3-glucoside

Human norovirus is the major pathogen of epidemic acute gastroenteritis and is the leading cause of approximately 90% of the non-bacterial outbreaks worldwide.1–3 Noroviruses can be divided into six major genogroups designated GI through GVI.1) GI and GII contain the majority of norovirus strains associated with human disease. Since the mid-1990s, when active surveillance with molecular diagnostic techniques was initiated, a single genotype, GII.4, has been associated with the outbreaks. Norovirus is transmitted mainly via the fecal–oral route and by person-to-person contact.

The infectious oral dose of norovirus is estimated to be less than 20 viral particles.4) Noroviruses are highly tolerant of environmental changes and often lead to death in elderly and immunocompromised persons.5) Although human norovirus has been recently discovered to be grown in B cells, murine norovirus-1 (MNV-1) and feline calicivirus-F9 (FCV-F9) are still used as surrogates for elucidating the molecular modes of norovirus replication and pathogenicity.6–8) At present, no vaccine or effective antiviral drug is available to prevent norovirus infection or illness.

Recent studies have highlighted the antiviral effects of natural products, including black raspberry (Rubus coreanus), cranberry, mulberry, pomegranate, grape seed, persimmon extract, and chitosan against human norovirus surrogates, MNV-1 and FCV-F9.9–14) It has also been reported that Dongchimi, a type of fermented kimchi, reduces FCV and MNV activity.15) Black raspberry, a member of the Rosaceae family, is native to Korea, China, and Japan. It is rich in polyphenolic compounds, which are known to have health-promoting effects,16) and has been used in the production of traditional wine and beverages. Black raspberry has previously been shown to possess a bone-protective effect17) and to have antibacterial activity against food-borne bacterial pathogens such as Escherichia coli O-157:H7, Salmonella Typhimurium, Bacillus cereus, and Staphylococcus aureus.18) It was also reported to exhibit strong antiviral activity against hepatitis B virus.19)

The seed makes up approximately 10% of the black raspberry fruit in weight. It is a byproduct of wine and juice production and is known to contain large quantities of polyphenolic compounds. Its extract is a potent antioxidant, scavenging free radicals and chelating iron ions.16) The seed oil significantly lowers triglycerides and moderately affects cholesterol metabolism in hamsters.20) However, antiviral activities of black raspberry seed extract (RCS) against food-borne viruses have not been studied. In this study, we have evaluated the inhibitory effects of RCS, its fraction with molecular
weight less than 1 kDa (RCS-F1), and polyphenolic compounds from RCS-F1 against MNV-1 and FCV-F9.

Materials and methods

Viruses and cells. RAW 264.7 cells (mouse leu-

(50 kHz, Powersonic 420, Hwashin Instrument Co. was extracted in 70% ethanol using ultrasound sieved through a mesh with a 1-mm pore size, which was maintained in Dulbecco’s modified Eagle’s medium (DMEM) (Invitrogen, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Sigma-Aldrich Corp., St. Louis, MO, USA) and penicillin streptomycin (PS) (Invitrogen, Grand Island, NY, USA) at 37 °C in an atmosphere containing 5% CO₂. MNV-1 and FCV-F9 were propagated in RAW 264.7 and CRFK cells with DMEM-2% FBS-1% PS medium for 3–4 days, with the titers at 1 × 10^6 plaque-forming units (PFU)/mL and 4 × 10^5 PFU/mL, respectively, and stored at −80 °C.

Sample preparation. Samples of RCS and RCS-F1 were provided by S&D Co., Ltd. (Chungbuk, Korea) (Lot No. SD-RW-RC-001 and SD-RW-RC-002). Briefly, the seeds were ground to a fine powder and sieved with a mesh having a 1-mm pore size, which was extracted in 70% ethanol using ultrasound (50 kHz, Powersonic 420, Hwashin Instrument Co., Ltd., Seoul, Korea) for 20 min at 20 °C and centrifuged (9,500 × g, 60 min, 4 °C). The supernatant was concentrated by a rotary evaporator and lyophilized, which was termed as RCS. The yield of the lyophilized RCS was 1.9% (w/w). RCS was dissolved in distilled water, sterilized through a 0.20-μm filter, and diluted aseptically to the indicated concentration with DMEM. RCS was further fractionated through a molecular weight cut-off 1 kDa ultrafiltration cell (Millipore Corp., MA, USA), and the filtrate was lyophilized to obtain a molecular weight less than 1 kDa fractions, RCS-F1. It was then dissolved in distilled water (DW), diluted aseptically to the indicated concentration with DMEM, sterilized through a 0.20-μm filter, and used for subsequent antiviral assays.

Quantification of polyphenolic compounds. Polyphenolic compounds of RCS-F1 were quantitatively analyzed by the previously reported method with minor modifications.21) The liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis was carried out using LCMS-8040 spectrometer and Nexera UHPLC system (Shimadzu, Kyoto, Japan) equipped with a Shim-pack XR-ODSII column (2.0 × 75 mm, 2.2 μm). The mobile phase was composed of solvent A (0.1% formic acid) and B (0.1% formic acid in acetonitrile) and the gradient was 95:5 at 1.5 min to 65:35 at 8 min to 100:0 at 10 min, followed by washing and reconditioning of the column. The flow rate was 0.4 mL/min, and the eluent was detected at 280 nm. Gallic acid, caffeic acid, ellagic acid, quercetin, cyanidin-3-glucoside (C3G), cyanidin-3-glucuronide (C3R), resveratrol, and catechin (Sigma–Aldrich Corp., St. Louis, MO, USA) were used as standard compounds.

Cytotoxicity test. The cytotoxicity was examined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.22) RAW 264.7 and CRFK cells were seeded at a density of 1.5 × 10⁶ and 2 × 10⁵ viable cells per well, respectively, in 96-well tissue culture plates with DMEM-10% FBS-1% PS medium. After 12–24 h, 90 μL of DMEM-10% FBS-1% PS and 10 μL of RCS, RCS-F1, or a polyphenolic compound was added to the cells and incubated for 1–24 h. The MTT solution (10 μL) was added and incubated at 37 °C for 2 h, and 100 μL of DMSO was added and incubated for 30 min. The absorbance at 570 nm was measured using a microplate reader (SpectraMax M2, Molecular Devices Corp., CA, USA). The percentage of cell viability was calculated as follows: % cell viability = (Abs_treatment/Abs_control) × 100.

Plaque reduction assays. The antiviral activity of the inhibitor (RCS, RCS-F1, or polyphenolic compound) was assessed using plaque reduction assay using monolayers of cells grown in 24-well plates. To understand the mode of action of the inhibitor on MNV-1 or FCV-F9, pre-treatment of cells with inhibitor was evaluated by adding the inhibitor in DMEM-10% FBS-1% PS to confluent monolayers of cells and incubating at 37 °C in 5% CO₂ for 1 h.23,24 After complete aspiration of the cell media, 200-μL virus suspensions (2–3 log_{10}PFU/mL) with 10-fold serial dilutions prepared in DMEM-10% FBS-1% PS were inoculated into each well. After viruses were absorbed at 37 °C in 5% CO₂ for 1 h, the inocula were aspirated, and 1 mL of DMEM containing 1.5% agarose, 5% FBS, and 0.5% PS was added to each well. The cells were stained with 0.5% crystal violet after incubation at 37 °C in 5% CO₂ for 48 h, and plaques were counted. For co-treatment, the same experimental procedures as those used for the pre-treatment were used, except that confluent cell monolayers were infected with 200 μL of viral suspension (2–3 log_{10}PFU/mL) and were simultaneously mixed with inhibitor. For post-treatment, after the infection with viral suspension (200 μL) to the cells for 1 h, inocula were aspirated, and the cells were incubated with inhibitor for 1 h. After the inhibitor was aspirated, 1 mL of 1.5% agarose overlay prepared in culture medium was added to each well. Sterilized DW was used as untreated control and 2-thiouridine (2TU), which was previously reported as an effective anti-MNV-1 agent, was used at a 50 or 200 μM for MNV-1 and FCV-F9, respectively, as a positive control.24 Antiviral activities were evaluated as relative plaque formation % for control (untreated) group based on different PFU.25,26 The 50% effective concentration (EC_{50}) was calculated from plaque formation (%).

Pre-treatment of virus with an inhibitor was accomplished by mixing the inhibitor and virus (5 log_{10}PFU/mL) (at a volume ratio of 1:9) and incubating at room temperature for 1 h. After incubation, 10-fold serially diluted mixtures were inoculated for 1 h (final 50–100
PFU/well for MNV-1 and FCV-F9) and the remainder of the procedure was the same as described above. PFU reduction (log10PFU/mL) was calculated from recovered virus PFU using number of plaques observed.11

RT-PCR analysis. To examine the ability of RCS, RCS-F1, or C3G to prevent the replication of virus, the gene level of MNV-1 RNA-dependent RNA polymerase (RdRp: NS7), MNV-1 virion protein genomelinked (VPg: NS5), or FCV-F9 protease-polymerase (ProPol: NS6–7), which is a component of large precursor of non-structural protein,27 was measured by conventional PCR. RAW 264.7 and CRFK cells were seeded into 12-well plates with DMEM, 10% FBS, and 1% PS at a density of 1 × 10^5/well or 5 × 10^5/well, respectively. RCS or RCS-F1 was simultaneously added during MNV-1 or FCV-F9 inoculation (MOI 0.01) of the cells for 1 h and removed from the cells. DW and 2TU (50 and 200 μM for MNV-1 and FCV-F9) were used as untreated and positive controls, respectively. After incubation for 24 h, cells were harvested and viral RNA was extracted with an RNAeasy kit (QIAGEN, Valencia, CA, USA). After the synthesis of cDNA using high-capacity cDNA reverse transcription kits (RT-Master Mix kit, Applied Biosystemy, MA, USA), PCR was carried out using primers (supplied by Bioneer Inc., Daejeon, Korea) as follows: MNV-1 RdRp (GeneBank access No. No. 008311),24 forward 5′-GGAATTCCATATGCTTCCCCGCCCCTCAGGCACC-TAT-3′ and reverse 5′-GATATGGGAAAGAGGACAGAACAAGG-GG-3′ and reverse 5′-GGATCCTCACTCAAGTGTA-TCTTCTC-3′; FCV-F9 ProPol (GeneBank access No. M86379.1),27 forward 5′-GGCCAAGGTAATTGTCCGT-3′ and reverse 5′-TCACTAATCCTGGATCACCAA-3′; murine glyceraldehyde-3-phosphate dehydrogenase (GAPDH, GeneBank access No. M23599.1),28 forward 5′-AAGCAACCTCTTATGTGAC-3′ and reverse 5′-TCCACCGGATACTCAGC-3′; and feline β-actin (GeneBank access No. AB051104.1),29 forward 5′-ACGAGGGGTTCGCC-3′ and reverse 5′-GATCTT-GATCTTCATGTCG-3′. PCR products were subjected to 15% agarose gel electrophoresis and visualized by staining with ethidium bromide. The cytotoxicity of RCS and RCS-F1 on RAW 264.7 and CRFK cells was evaluated by MTT assay.25 The percentage of viability of RAW 264.7 and CRFK cells after treatment with RCS or RCS-F1 at 1 mg/mL was ≥ 85% (Fig. S1). The antiviral experiments using RCS and RCS-F1 were carried out at concentrations that were not cytotoxic. Pre-treatment of cells with RCS (1 mg/mL) produced 67% inhibition in plaque formation (Fig. 1(A)). Co-treatment with RCS also exhibited high antiviral activity against MNV-1, 70% inhibition at the same concentration. For comparison, 2TU as a positive control yielded 94% inhibition at 50 μM. 2TU had significant inhibitory effects on MNV-1 and FCV-F9 replication by blocking the active site of viral RdRp protein.25 With post-treatment, 68% inhibition was achieved by RCS at 1 mg/mL. The antiviral activity of RCS was represented as a relative quantitation (RQ = 2^−ΔΔCt) to control (untreated group). ΔCt and ΔΔCt were calculated as an average of duplicates in two independent experiments.

Differential scanning fluorimetry (DSF). MNV-1 RdRp is involved in the synthesis and amplification of the viral genome. To determine the shift in transition temperature upon ligand binding to RdRp,24,31 the protein prepared as described previously32 was incubated with RCS, RCS-F1, or polyphenolic compound at 25 °C for 1 h. The solution was mixed with 0.5 μL SYPRO Orange and incubated at 25 °C. The temperature was increased by 0.5 °C every 30 s for 50 min, and relative fluorescence units were recorded using Mx3005P DSF (Stratagene, CA, USA). The excitation and emission wavelengths were 492 nm and 610 nm, respectively. Transition temperature was calculated from the maxima of the first derivative of relative fluorescence units/temperature.

Transmission electron microscopy (TEM). In order to visualize virus particles using TEM, MNV-1 virus (6 log10PFU/mL in DMEM) was incubated with DMSO, RCS-F1 (1 mg/mL in DMSO), or polyphenolic compound (200 μM C3G or 50 μM each of 4 polyphenolic compounds in DMSO) for 1 h at room temperature, which was applied to a freshly charged carbon-coated grid, and stained with 1% uranyl acetate solution for 1 min. The stained viruses were observed with a Hitachi H7600 transmission electron microscopy system (Hitachi, Tokyo, Japan) at 80 kV at a magnification of × 20,000.

Statistical analysis. All measurements were performed in triplicates. The experimental data were expressed as the mean ± SD. The data were analyzed using ANOVA with SAS software (version 9.2, SAS Institute, Cary, NC, USA), and differences in the means were tested with Duncan’s multiple range test. A p value < 0.05 was considered significant.

Results

Antiviral effects of RCS against MNV-1 and FCV-F9

The cytotoxicity of RCS and RCS-F1 on RAW 264.7 and CRFK cells was evaluated by MTT assay. The percentage of viability of RAW 264.7 and CRFK cells after treatment with RCS or RCS-F1 at 1 mg/mL was ≥ 85% (Fig. S1). The antiviral experiments using RCS and RCS-F1 were carried out at concentrations that were not cytotoxic. Pre-treatment of cells with RCS (1 mg/mL) produced 67% inhibition in plaque formation (Fig. 1(A)). Co-treatment with RCS also exhibited high antiviral activity against MNV-1, reaching 87% inhibition at the same concentration. For comparison, 2TU as a positive control yielded 94% inhibition at 50 μM. 2TU has significant inhibitory effects on MNV-1 and FCV-F9 replication by blocking the active site of viral RdRp protein.25 With post-treatment, 68% inhibition was achieved by RCS at 1 mg/mL. The antiviral activity of RCS was...
concentration dependent. In the case of FCV-F9, 69% of plaque reduction was obtained after pre-treatment of cells with 1 mg/mL RCS (Fig. 1(B)). The co- and post-treatments with RCS at the same concentration gave 84 and 78% reductions in plaque formation, respectively. In contrast, 2TU produced a 25–65% inhibition against FCV-F9, even at a concentration of 200 μM.

Antiviral effects of RCS-F1 against MNV-1 and FCV-F9

RCS was further fractionated through an ultrafiltration membrane to produce a component of RCS with a molecular weight less than 1 kDa, RCS-F1. Complete inhibition of plaque formation by MNV-1 was achieved by RCS-F1 at 1 mg/mL at the co-treatment (Fig. 1(C)). The EC50 of RCS-F1 against MNV-1 was 0.04 mg/mL. In the case of FCV-F9, the co-treatment and post-treatment with RCS-F1 at 1 mg/mL also completely abolished plaque formation (Fig. 1(D)).

For pre-treatment of virus with an inhibitor, when it was evaluated as log10PFU/mL titer reduction, high titers of MNV-1 and FCV-F9 were reduced by 2.71 and 3.03 log10PFU/mL with RCS-F1 at the same concentration, respectively (Table 1). These results therefore demonstrate that both RCS and RCS-F1 were effective in reducing the plaque formations of MNV-1 and FCV-F9 in a dose-dependent manner, and the inhibitory effects of RCS or RCS-F1 were consistently high with the co-treatment of the viruses.

Antiviral activities of polyphenolic compounds of RCS-F1 against MNV-1 and FCV-F9

Antiviral activities of various polyphenolic compounds derived from plants have been reported.12,35,36)
The antiviral activity of polyphenolic compounds in RCS-F1 was evaluated against MNV-1 or FCV-F9 in the co-treatment mode in which RCS-F1 showed the maximal antiviral activity. Among the tested polyphenolic compounds, however, catechin (34.6 mg/g equivalent to ~120 μM in 1 mg/mL of RCS-F1) and ellagic acid (11.4 mg/g; ~40 μM), the major polyphenolic compounds in RCS-F1, exhibited negligible and weak antiviral activities (Fig. 2). In contrast, C3G (0.15 mg/g; ~0.3 μM) and gallic acid (1.3 mg/g; ~8 μM), minor polyphenolic compounds in RCS-F1, showed significant antiviral activities, producing 50–65% inhibition at 100 μM against MNV-1 and FCV-F9 (Fig. 2).

Inhibition of RCS-F1 and C3G on viral gene expression

In order to examine the inhibition of viral gene expression, RdRp, VPg, or ProPol gene, which is a component of large precursor of non-structural viral protein,27) was amplified and compared using PCR and qPCR. RCS at 0.1 mg/mL significantly reduced MNV-1 RdRp, and RCS-F1 produced an even greater inhibition (Fig. 3(A) and (B)). RCS-F1 at 0.01 mg/mL almost completely reduced RdRp gene expression of MNV-1, showing a comparable effect to that of 2TU at 50 μM. However, RCS or RCS-F1 had no inhibitory effect on the ProPol gene expression of FCV-F9. In addition, quantitative analysis (RQ) using ΔΔCt showed that RCS-F1 reduced the gene of MNV-1 RdRp in both co- and post-treatments (Fig. 3(C)). These results strongly suggest that MNV replication is inhibited by RCS-F1 at the RNA level in a dose-dependent manner. Importantly, C3G, one of the minor polyphenolic compounds in RCS-F1, inhibited the expression of virus genes, when infected cells were treated simultaneously. At the concentrations from 10 to 200 μM, C3G showed a significant reduction of noroviral gene expression, similar to that by RCS-F1 (10–100 μg/mL).

Binding of RCS-F1 or C3G to MNV-1 polymerase and virus particles

To evaluate possible interactions between the MNV-1 polymerase enzyme and RCS-F1 or its polyphenolic compounds, the effect of temperature on protein stability was monitored by DSF in denaturation profiles. The extent of temperature shifts is believed to be proportional to the affinity of the ligand for a given protein.34) The virally encoded RdRp, one of the key targets for the development of novel antiviral agents, was selected. The transition temperature of untreated MNV-1 RdRp was 39.5 °C, which shifted to 40.5 and 43 °C in the presence of 100 μM of C3G and 200 μg/mL of RCS-F1, respectively (Fig. 4(A)). However, there was no change in the presence of other polyphenolic..
compounds from RCS-F1: catechin, ellagic acid, C3R, gallic acid, caffeic acid, 3,4-dihydroxybenzoic acid, or quercetin (data not shown). These results indicate that C3G can bind to MNV-1 RdRp protein, suggesting that the direct binding to MNV-1 RdRp may reduce viral gene expression, resulting in inhibition of noroviral replication.

To further determine the mode of action of RCS-F1, MNV-1 virus particles were examined using TEM. Untreated MNV-1 virus particles were spherical (Fig. 4(B), upper panel), ranging in size approximately from 30 to 35 nm, as previously reported.9 After the treatment with 1% DMSO as a positive control, the morphology and size of the MNV-1 virus particles were not different from those of the untreated control. However, the treatment with RCS-F1 increased the size of virus particles to 80–100 nm in diameter, which indicates denatured and enlarged particles, and disrupted some of them. Moreover, MNV-1 virus particles were treated with C3G or four polyphenolic compounds (C3G + gallic acid + catechin + ellagic acid), and their effects on virus particles were determined using TEM.

C3G increased the size of some virus particles, indicating that it enlarged and disrupted the virus particles, (Fig. 4(B), lower panel). When the virus particles were treated with four polyphenolic compounds simultaneously, the effect was similar or slightly more significant. These results thus suggest that the structural disruption of MNV-1 particles induced by RCS-F1 or C3G can lead to noroviral replication inhibition.

Discussion
The antiviral effects of black raspberry, cranberry, mulberry, persimmon, and pomegranate juices, grape seed extract, and chitosan against human norovirus surrogates, MNV-1 and FCV-F9, were previously reported.9–14 In addition to the anti-MNV-1 activities of black raspberry juice,9 we further examined the antiviral effects of black raspberry seed extract (RCS) and its fraction with molecular weight less than 1 kDa (RCS-F1) against MNV-1 and FCV-F9. RC seeds that are left over from wine and juice production are a
RC and RCS-F1 have maximal antiviral activity against MNV-1 (EC\textsubscript{50} of 0.07 mg/mL) when they are added simultaneously to cells and against FCV-F9.

Fig. 4. Binding of RCS-F1 or C3G to MNV-1 polymerase and virus particles.

Notes: (A) Differential scanning fluorimetry profile of purified MNV-1 RdRp in the absence and presence of 200 μg/mL of RCS-F1 or 100 μM of C3G. Each sample was tested in triplicates. (B) Transmission electron microscopic images of MNV-1 in the absence or presence of RCS-F1, C3G, or four polyphenolic compounds. Upper panel: MNV-1 control (left), MNV-1 treated with 1% DMSO (middle) and MNV-1 viruses (6 log\textsubscript{10}PFU/mL) treated with 1 mg/mL RCS-F1 (right). Lower panel: MNV-1 treated with 200 μM C3G (left) and 50 μM each of 4 polyphenolic compounds (C3G+GA+CA+EA). C3G: cyanidin-3-glucoside, GA: gallic acid, CA: catechin, and EA: ellagic acid. White arrows indicate enlarged virus particles. Scale bars, 100 nm.

waste byproduct of black raspberry industry. Thus far, the antiviral activities of RC seed extracts against noro-viruses have not been explored.
(EC50 of 0.03 mg/mL) when the virus is treated with it prior to CRFK cell infection. Importantly, RCS-F1, a component of RCS with a molecular weight less than 1 kDa, was also very effective in the co-treatment of MNV-1 (2.44 log10PFU/mL reduction) as well as of FCV-F9 (2.17 log10PFU/mL reduction), when initial viral titer was used at 2–3 log10PFU/mL. RCS-F1 was also very effective (2.71–3.0 log10PFU/mL reduction) in the pre-treatment with virus, when high titer viruses (5 log10PFU/mL) were treated with RCS-F1 for 1 h. It was reported that grape seed extract showed antiviral effect on the adsorption of FCV-F9 and MNV-1, but only minor effects on replication.11) It reduced the infectivity of MNV-1 by 1.97 log10PFU/mL, when initial viral titer was used at 5 log10PFU/mL.11)

Norovirus ORF1 is translated as a large polyprotein precursor which consists of six to seven non-structural proteins including VPg, Pro, and RdRp.37) These protein genes were selected for characterization of the norovirus gene expression. In the co-treatment of virus with RCS-F1 in the cells, RCS-F1 significantly inhibited MNV-1 RdRp gene expression, but little inhibition was shown in FCV-F9. The reduction of MNV-1 RdRp gene was also found in the relative quantification. Plaque reduction and qPCR results in the post-treatment showed that RCS-F1 also inhibited the viral replication. In addition, it was found to bind to MNV-1 RdRp protein, with a Tm shift of higher than 3 °C, suggesting that the binding of RCS-F1 to MNV-1 RdRp may reduce viral gene expression, which results in inhibition of noroviral replication. TEM studies also demonstrated RCS-F1-induced structural disruption of MNV-1 particles, possibly due to a non-specific detergent effect, which can be one of the underlying mechanisms of noroviral replication inhibition. It was previously reported that condensed tannins from persimmon38,39) or cranberry40) and carvacrol and thymol41) from natural plant extracts disrupted the envelope or capsid viruses. However, we cannot exclude the possibility that other components in RCS-F1 can contribute to the antiviral activities of this low molecular weight seed extract, which requires further study.

RCS-F1 is known to contain large amounts of polyphenolic compounds. Among them, catechin and ellagic acid are the major polyphenolic compounds in RCS-F1. Namely, the amounts of these two compounds are all higher than 10 mg/g in dry weight in RCS-F1, from the quantitation based on the LC/MS/MS analysis. Various polyphenolic compounds derived from plants, proanthocyanidin, resveratrol, catechins, and CYSTUS052, have considerable antiviral activities, which can be one of the underlying mechanisms of noroviral replication inhibition. It was previously reported that condensed tannins from persimmon38,39) or cranberry40) and carvacrol and thymol41) from natural plant extracts disrupted the envelope or capsid viruses. However, we cannot exclude the possibility that other components in RCS-F1 can contribute to the antiviral activities of this low molecular weight seed extract, which requires further study.

In conclusion, black raspberry seed-derived RCS-F1 was significantly effective in reducing the replication of food-borne virus surrogates. One of the possible modes of action of antiviral RCS-F1 would include being able to disrupt MNV-1 viral particles and to bind to viral RdRp enzyme. C3G, a polyphenolic compound of RCS-F1, was also effective in reducing plaque formation and gene expression of noroviral surrogate and it was able to bind to MNV-1 RdRp and to enlarge the virus particle. Our study will provide a potential application for the utilization of black raspberry seed extract and its RCS-F1 or cyanidin derivatives in the prevention of food-borne viral outbreaks, including decontamination of food contact surfaces such as food dressings and edible films in wraps, or sanitization of processing water.

Disclosure statement

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Supplemental materials

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