Antiviral Effect of Extract from Fagopyrum buckwheats Against Two Nonenveloped Viruses

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ABSTRACT
Nutraceuticals have been a rich source of prophylactic and therapeutic benefits to humans against various diseases. Golden buckwheat (Fagopyrum dubotrys [F. dubotrys or F. dibityo]) has been used in Chinese traditional medicine and herbal medicine to treat inflammatory conditions. Extracts from the leaves and roots of the plant contain unique combinations of compounds that may have potential for future medications due to their anti-cancer, anti-oxidant, anti-inflammatory, anti-aging, and hepatoprotective properties. However, the potential antiviral activity of the plant extract has not been explored. The purpose of the current study is to investigate if Golden buckwheat or 5,7,3‘,4‘-tetrahydroxyflavan-3-ol C4-C8 dimer, the major compound of Golden buckwheat, possess antiviral activity against alcohol-resistant nonenveloped viruses such as norovirus and hepatitis A viruses. Methods used in the study include cytotoxicity assay (MTT assay), viral infection assays, and TCID50 assay. The results demonstrate that a single dose of both the extract of Golden buckwheat and 5,7,3‘,4‘-tetrahydroxyflavan-3-ol C4-C8 dimer (双聚原矢车菊甘元 in Chinese) are able to inhibit feline calicivirus (a surrogate for human norovirus) and human hepatitis A virus, when added either before viral infections or after viral infections without cytotoxicity. To the best of our knowledge, this is the first discovery that Golden buckwheat and its major component 5,7,3‘,4‘-tetrahydroxyflavan-3-ol C4-C8 dimer exhibit strong antiviral activities against nonenveloped viruses causing humans acute symptoms. As of today, there is no therapeutic method to treat norovirus or hepatitis A virus infection, these nutraceuticals may provide solutions for future prophylactic and therapeutic methods, pending future research and development.

Keywords
Golden buckwheat, Fagopyrum, Nutraceuticals, 5,7,3',4'-tetrahydroxyflavan-3-ol C₄-C₈ dimer, Norovirus, Hepatitis A virus.

Introduction
Nutraceuticals, a word invented by Dr. Stephen L. DeFelice, has been defined as naturally occurring dietary substances that could provide medicinal or health benefits to prevent and treat diseases [1]. Nutraceuticals may include food and foodstuffs, dietary regimen, nutrition supplements, herbal products, functional foods, fortified foods, and dietary supplements [2]. Many plant extracts belong to this category of naturally occurring compounds that possess medicinal properties. The genus Fagopyrum belongs to the flowering plant family Polygonaceae, which comprises 15 species mainly found in the northern hemisphere. There are 10 buckwheat species present in China, with three important species: Fagopyrum esculentum (F. esculentum) Moench. (common buckwheat), Fagopyrum tataricum (F. tataricum) (L.) Gaertn. (tartary buckwheat), and Fagopyrum dubotrys (F. dubotrys or F. dibityo) (D. Don) Hara. (perennial buckwheat or Golden buckwheat) [3]. These Fagopyrum buckwheats contain flavonoids, phenolics, fagopyritols, triterpenoids, steroids and fatty acids, and have been used in traditional Chinese medicine for multiple ailments and conditions [3]. Previous studies have demonstrated that Fagopyrum buckwheats and their extracts possess many bioactive properties including anti-tumor, anti-oxidant, anti-inflammatory, anti-aging, hepatoprotective, hypoglycemic, anti-allergic, and anti-fatigue activities [4-11]. In 1974, Fagopyrum buckwheats (F. dubotrys or F. dibityo, Golden buckwheats) was reported to...
treat acute inflammation in a clinical trial, and the major active ingredient was identified as 5,7,3′,4′-tetrahydroxyflavan-3-ol C_7-C_8 dimer [12], and referred to as a new flavonoid in 1987 [13]. Other major compounds found in golden buckwheat include rutin, quercetin, and hecogenin [3,14].

However, the potential anti-viral effects of Golden buckwheat in either extract form or purified compound form of the major component were not reported. The purpose of the current study is to investigate if Golden buckwheat extract (referred to as GBE hereafter) or 5,7,3′,4′-tetrahydroxyflavan-3-ol C_7-C_8 dimer exhibit antiviral activity that may provide potential therapeutic methods for viral infections. The goal of the current study is to evaluate potential antiviral activities of GBE and 5,7,3′,4′-tetrahydroxyflavan-3-ol C_7-C_8 dimer against feline calicivirus (FCV) and human hepatitis A virus (HAV).

The rationale for testing FCV and HAV is that FCV is a well-recognized test surrogate for human norovirus. Both norovirus and HAV are nonenveloped viruses, and they both cause acute illness in humans. Currently, there is no vaccine or therapeutic for treatment of norovirus infection. If GBE or 5,7,3′,4′-tetrahydroxyflavan-3-ol C_7-C_8 dimer demonstrates antiviral activity against norovirus, testing another nonenveloped virus causing human acute disease could unveil the potential broad-spectrum antiviral activities for the nutraceuticals. If GBE or 5,7,3′,4′-tetrahydroxyflavan-3-ol C_7-C_8 dimer possess antiviral activity against HAV, which infection also lacks treatment method, they could have a broad-spectrum of antiviral activities against hepatitis viruses and other viruses. Therefore, the class of plants and their phytochemicals warrant further studies to discover the potential antiviral properties for the use of treatment and prevention of viral infection associated with human illnesses.

Material and Methods

Cells and viruses
Human hepatitis A virus (VR-1402), feline calicivirus (VR-782), FRhk-4 feline rhesus monkey kidney cells (CRL-1668) and CRFK cat kidney cells (CCL-94), DMEM medium (30-2002), and EMEM medium (30-2003) were purchased from ATCC. Cell culture, viral propagation and harvesting were performed according to supplier’s protocols, which received approval from Augusta University Institutional Biosafety Committee. GBE was purchased from Xi’an Orient Biotechnology, Co., Ltd., China. Purified 5,7,3′,4′-tetrahydroxyflavan-3-ol C_7-C_8 dimer (referred to “dimer” here after) were provided from Changxing Sanju Biotechnology Ltd, China.

MTT assay
Cell viabiliy assay (MTT assay) was performed according to the method previously described [24]. Briefly, cells were cultured in a 96-well plate until confluent. Cell culture medium containing specific agents were incubated with the monolayer of the cells for 1 h before the medium was changed. After overnight incubation, the plate was removed from the cell culture incubator and an MTT assay was performed as described [24]. To determine if GBE is associated with cytotoxicity, GBE was dissolved in either EMEM or DMEM for CRFK or FRhK cells respectively. Cells were grown in 96 well plate until 90% confluent. GBE in EMEM or DMEM was added to the wells in quadruplets at 0, 0.1, 1, and 2%, followed by incubation overnight. MTT assay was performed on the cells, and cell viability was calculated as previously described [24].

Viral infectivity assays
TCID50 assay (50% tissue culture infectious dose assay) was used to determine viral titers and the inhibitory effects of the agents. The infectivity of HAV and FCV was measured with or without treatment of the plant agents to determine three aspects of the antiviral capabilities: pre-infection, simultaneous infection, and post-infection. Pre-infection experiments test the viral infectivity after cells are pre-treated with the plant agents for 1 h, followed by viral infection at different dilutions for 1 h, before TCID50 assay. Simultaneous infection experiments test the effect of the plant agent in direct contact with the virus, when they were mixed prior to infecting the cells for 1 h, in different dilutions, followed by TCID50 assay. Post-infection experiments test the viral infectivity with the addition of the plant agents after 1 h of viral infection at different dilutions, followed by TCID50 assay. Controls of the assay were uninfected (negative) and infected but untreated cells (positive). All assays were repeated three times independently.

Pretreatment of CRFK cells with different concentrations of GBE for 1 h before TCID50 assay
CRFK cells were plated in 96-well tissue culture plates in EMEM culture medium with 10% fetal bovine serum (FBS) and antibiotics at 37°C, 5% CO_2_. When cells covered the surface of each well to >90%, GBE dissolved in EMEM culture medium with 10% FBS was added at 0, 0.1, 0.2 and 1% in triplicates of 100 µl/well, followed by incubation for 1 h. GBE medium then was removed and FCV was added at different dilutions by Hanks balanced salt solution (HBSS) from 10^{-5} to 10^{-8}. After 1 h of absorption, virus was removed and EMEM culture medium with 0.2% FBS was added to each well. Cytotoxic effect (CPE) was recorded and result was calculated after 5 days.

GBE and FCV added to cells simultaneously
GBE was dissolved in EMEM containing 10% FBS. To 0.45 ml of the medium containing GBE at 0, 0.1, 0.2 or 1%, 50 µl FCV was added and incubated for 1 h at room temperature. 100 µl of the mix was added to 0.9 ml EMEM containing 10% FBS. This is a 10^{-2} viral/GBE mix. A series of dilutions of this mix at 10^{-3} to 10^{-6} was made, and 100 µl from each dilution in quadruplets was added to the wells and incubated for 1 h. The mix was replaced with EMEM containing 0.2% FBS, and the plate was incubated for at least 5 days in a tissue culture incubator with 5% CO_2_ at 37°C, until CPE was observed for TCID50 assay.

GBE added after FCV viral infection of CRFK cells
CRFK cell monolayer in a 96-well plate was infected with FCV in a series dilution from 10^{-3} to 10^{-6} in quadruplets in HBSS. After 1 h incubation, the virus/HBSS was replaced with GBE-containing
EMEM with 10% FBS, and incubated for 1 h prior to medium change of EMEM containing 0.2% FBS. CPE was observed from day 5 under a microscope and TCID50 values were calculated.

HAV infectivity assay was conducted in 48-well plates. FRhK-4 cells in complete DMEM medium containing 10% FBS were plated in each well to allow the cells to form a monolayer. To measure the viral titer, 50 µl HAV virus was add to 450 µl HBSS. This is 10^-1 dilution of viral mix. A series of dilutions by adding 100 µl of the mix to 900 µl of HBSS up to 10^-6. To a 48-well plate, 250 µl from each dilution (10^-3 to 10^-6) was loaded to the designated three repeating wells per dilution. After 1 h absorption, the virus/HBSS mix was removed and DMEM containing 2% FBS was added to each well. The plate was incubated for at least 8 days in a tissue culture incubator with 5% CO_2 at 35°C until CPE was observed for TCID50 assay calculation.

Pre-infection viral infectivity assay
GBE dissolved in DMEM containing 2% FBS was added to FRhK cell monolayer in a 48 well plate at 0, 0.1, 0.2 and 1% and incubated for 1 h. The GBE/medium was then replaced with a series dilution of HAV from 10^-3 to 10^-6 in HBSS in triplicates per each dilution, and the virus was allowed to be absorbed for 1 h before the virus was removed, and DMEM containing 2% FBS was added to each well. The plates were incubated at 35°C with 5% CO_2. On day 5, the medium was changed. CPE was observed from day 8 under a microscope and TCID50 result was calculated.

GBE and HAV virus were added to cells at the same time
GBE was dissolved in DMEM containing 2% FBS. To 0.45 ml of the medium containing 0, 0.1, 0.2, and 1% GBE, 50 µl HAV was added, and incubated for 1 h at room temperature. 100 µl of the mix was added to 0.9 ml DMEM containing 2% FBS. This is 10^-2 viral/GBE mix. A series of dilutions of this mix were made at 10^-3 to 10^-6. 250 µl of each dilution was loaded to each well in triplicates and incubated for 1 h. The mix was replaced with DMEM containing 2% FBS, and the plate was incubated for at least 8 days in a tissue culture incubator with 5% CO_2 at 35°C until CPE was observed for TCID50 assay.

Post-infection assays
FRhK cell monolayer in a 48-well plate was infected with HAV in a series dilution from 10^-3 to 10^-6 in triplicates in HBSS. After 1 h incubation, the virus/HBSS was replaced with GBE-containing DMEM and incubate for 1 h prior to medium change of DMEM containing 2% FBS. CPE was observed from day 8 under a microscope and TCID50 was calculated.

Infectivity assays of 5,7,3’,4’-tetrarydroxyflavon-3-ol C_4-C_8 dimer against FCV and HAV
Procedures are identical to the infectivity assays using GBE except the concentration used was 0.1%.

Statistical analysis
All assays were performed three times. The paired t tests were used to analyze the data between treatment and control at the p < 0.05 level of significance. One-way analysis of variance (ANOVA) was carried out to analyze the samples treated with different concentrations before, simultaneously, or after viral infection, respectively.

Results
Cell viability test results after incubation with GBE
To determine if GBE is associated with cytotoxicity, GBE was dissolved in either EMEM or DMEM for FCV F9 or HAV viral infections in CRFK or FRhK cells, respectively. Figure 1 demonstrates that GBE did not reduce cell viability in CRFK cells even at 2%. Statistical analysis was performed using one-way ANOVA and t-test. The results demonstrate that there is a significant difference among all samples tested (ANOVA p=0.015). Result from t test indicates that the only differences among samples are between 2% and control (0%), and 2% and 0.5% (p<0.05). That is, GBE at 2% significantly increased cell viability in CRFK cells.

GBE and HAV virus were added to cells at the same time
GBE was dissolved in DMEM containing 2% FBS. To 0.45 ml of the medium containing 0, 0.1, 0.2, and 1% GBE, 50 µl HAV was added, and incubated for 1 h at room temperature. 100 µl of the mix was added to 0.9 ml DMEM containing 2% FBS. This is 10^-2 viral/GBE mix. A series of dilutions of this mix were made at 10^-3 to 10^-6. 250 µl of each dilution was loaded to each well in triplicates and incubated for 1 h. The mix was replaced with DMEM containing 2% FBS, and the plate was incubated for at least 8 days in a tissue culture incubator with 5% CO_2 at 35°C until CPE was observed for TCID50 assay.

GBE and HAV virus were added to cells at the same time
GBE was dissolved in DMEM containing 2% FBS. To 0.45 ml of the medium containing 0, 0.1, 0.2, and 1% GBE, 50 µl HAV was added, and incubated for 1 h at room temperature. 100 µl of the mix was added to 0.9 ml DMEM containing 2% FBS. This is 10^-2 viral/GBE mix. A series of dilutions of this mix were made at 10^-3 to 10^-6. 250 µl of each dilution was loaded to each well in triplicates and incubated for 1 h. The mix was replaced with DMEM containing 2% FBS, and the plate was incubated for at least 8 days in a tissue culture incubator with 5% CO_2 at 35°C until CPE was observed for TCID50 assay.

Figure 1: Cell viability assay result of CRFK cells treated with different concentrations of GBE.

Figure 2: Cell viability assay result of FRhK cells treated with different concentrations of GBE.

Statistical analysis
All assays were performed three times. The paired t tests were used to analyze the data between treatment and control at the p < 0.05 level of significance. One-way analysis of variance (ANOVA) was carried out to analyze the samples treated with different concentrations before, simultaneously, or after viral infection, respectively.
Results of inhibitory effects of GBE on feline calicivirus (FCV) F9

Pretreatment of CRFK cells with different concentrations of GBE for 1 h before TCID50 assay. Figure 3 demonstrates that results from three independent experiments indicate a significant inhibition of viral infection at all concentrations of GBE, even though there was no direct contact of GBE and virus. Specifically, GBE at all concentrations reduced FCV F9 infection by more than 50% if GBE was incubated with CRFK cells for 1 h before FCV F9 infection (n=3, p<0.01). There is no statistical difference among all concentrations (ANOVA, p=0.62).

**Figure 3:** Result of the inhibitory effect of GBE on CRFK cells after the cells were pre-treated with different concentrations of GBE for 1 h. Reduction of viral infectivity values are: 0.1% GBE, 48.00% ± 14.16, 0.2% GBE, 48.00% ± 14.16, and 1% GBE, 37.86% ± 13.88.

**GBE and FCV were added to cells at simultaneously**

Figure 4 shows that GBE at all concentrations significantly inhibited FCV viral infection in CRFK cells. Data was obtained from 3 independent experiments. The interesting observation is that lower concentration of GBE has significant higher efficacy than higher concentrations. Specifically, GBE at all concentrations significantly reduced FCV F9 infection (n=3, p<0.01, two tailed t-test). On the other hand, 0.1% GBE showed higher efficacy (17.79% ± 0 infectivity) than other concentrations (48.00% ± 14.16 and 52.58% ± 4.10 infectivity). ANOVA indicate the differences are statistically significant (p=0.0048). There is no statistical difference between 0.2% and 1%.

**Figure 4:** Result of the inhibitory effect of GBE on CRFK cells after the cells were infected by FCV F9 in the present of different concentrations of GBE.

GBE added after FCV F9 viral infection of CRFK cells

Figure 5 shows the results from three independent experiments that without direct contact with the virus, GBE significantly lowered FCV F9 infection. Specifically, GBE at all concentrations significantly reduced FCV F9 infection (n=3, p<0.001). There is no statistical difference among the concentrations.

**Figure 5:** Result of the inhibitory effect of GBE on CRFK cells after the cells were infected by FCV F9 in the present of different concentrations of GBE. At 0.1%, the infectivity was reduced to 43.38% ± 22.16. At 0.2%, the viral infectivity was reduced to 36.56% ± 21.65, while 1% GBE reduced the viral infectivity to 30.56% ± 0.94.

In summary, FCV F9, a surrogate of human norovirus that is resistant to alcohol, can be effectively inhibited by GBE with different incubation methods.

Results of Effects of GBE on human hepatitis A virus (HAV)

Pretreatment of FRhK cells with different concentrations of GBE for 1 h before TCID50 assay. Figure 6 demonstrates the results from three independent experiments. GBE at all concentrations led to a significant inhibition of HAV viral infection, even though there was no direct contact of GBE and virus. Statistical analysis shows that GBE at 0.2% and 1% significantly reduced HAV infection rate in FRhK cells (n=3, p<0.05). At 1%, pre-incubation of GBE with FRhK cells for 1 h reduced HAV infection rate to 26.59%.

**Figure 6:** Result of the inhibitory effect of GBE on HAV infection of
FRhK cells after the cells were pre-treated with different concentrations of GBE for 1 h. Reduction of viral infectivity values are: 0.1% GBE, 59.09% ± 23.15, 0.2% GBE, 34.94% ± 18.57, and 1% GBE, 26.59% ± 20.64.

On the other hand, there is no statistical difference among the concentrations on the effect of HAV (ANOVA, p=0.221). The result indicates that GBE is effective in reducing HAV infection of FRhK cells if GBE is incubated with FRhK cells prior to HAV infection, and the apparent dose response is statistically insignificant.

GBE and HAV virus were added to cells simultaneously. Figure 7 shows that GBE at all concentrations significantly inhibited FCV F9 viral infection in CRFK cells. Data was obtained from three independent experiments. The interesting observation is that lower concentration of GBE has a higher efficacy than higher concentrations. Statistical analysis using t-test shows that GBE at all concentrations significantly reduced HAV infection rate in FRhK cells (n=3, p<0.05). There is no statistical difference among the concentrations on the effect of HAV (ANOVA, p=0.426). The result indicates that GBE is effective in reducing HAV infection of FRhK cells when GBE was incubated with HAV and FRhK cells during HAV infection.

GBE added after HAV infection of FRhK cells
Figure 8 shows the results from three independent experiments. Without direct contact with the virus, GBE at all concentrations significantly lowered HAV infection. Statistical analysis shows that GBE at all concentrations significantly reduced HAV infection rate in FRhK cells (n=3, p<0.05). Result from one way ANOVA shows there is no difference among GBE concentrations (p=0.183). The result indicates that GBE is effective in reducing HAV infection of FRhK cells after HAV infection.

In summary, HAV can be effectively inhibited by a single dose of GBE using different incubation methods. HAV is one of the most difficult to inactivate virus due to its size and nonenveloped structure similar to poliovirus and feline calcivirus. Thus, GBE is a strong inhibitor of HAV.

Results of the Effect of C. 5,7,3’,4’-tetrarydroxyflavon-3-ol C4-C8 dimer on FCV and HAV
We used purified 5,7,3’,4’-tetrarydroxyflavon-3-ol C4-C8 dimer to test on the inhibitory effect of human hepatitis A virus (HAV) to see if they are able to inhibit this non-enveloped virus. The method used for viral inhibition was identical to the method described above, except the concentration used for the purified compounds was 0.1%.

Figure 9 demonstrates that 5,7,3’,4’-tetrarydroxyflavon-3-ol C4-C8 dimer is effective against FCV infection comparable to quercetin, better than rutin. ANOVA indicates there is no statistical difference between the control and pre-treatment (p=0.071). But both post-treatment and simultaneous treatment significantly inhibited viral infectivity (p<0.002).

Figure 9: Results of the inhibitory effects of 5,7,3’,4’-tetrarydroxyflavon-3-ol C4-C8 dimer on FCV F9 using three different treatment methods. Before, CRFK cells were pre-treated with 5,7,3’,4’-tetrarydroxyflavon-3-ol C4-C8 dimer for 1 h prior to FCV F9 infection with reduction of 33.87% ± 45.76%. Sametime, the dimer was mixed with the virus prior to dilution and infection, infectivity reduced by 38.11% ± 14.54. After, the dimer was added after viral infection, 28.44% ± 15.82. *only two data points available with large variation.
and 1.1 million episodes of pediatric gastroenteritis annually in the United States. On a worldwide scale, noroviruses cause 570 to 800 deaths, mostly among young children and the elderly [18]. Transmission of these highly infectious plus-stranded RNA viruses occurs primarily through contaminated food or water, but also through person-to-person contact and exposure to objects that have been contacted with the virus. Symptoms of norovirus include fever, cramps, head and body aches, along with profound gastroenteritis, diarrhea and vomiting. Symptoms can arise gradually or abruptly and usually resolve within 48 to 72 h. Currently there is no treatment for norovirus [18]. During an active norovirus infection, it is important for the infected person to intake a sufficient amount of fluids to avoid dehydration. Intravenous fluid delivery is necessary if the infected person is not able to drink sufficient fluids. Loss of fluid due to vomiting and diarrhea can lead to severe dehydration, and if untreated, it may lead to more severe complications and even death [19].

Hepatitis is an inflammation of the liver caused by hepatitis viruses and other infections, toxic substances like alcohol and drugs, and autoimmune diseases. The five main hepatitis viruses are: hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), and hepatitis E virus (HEV). HAV and HEV cause acute hepatitis, while HBV, HCV, and HDV are the cause of chronic viral hepatitis. Chronic infection with hepatitis can lead to chronic liver disease, cirrhosis, and hepatocellular carcinoma if left untreated [20]. HBV and HCV are responsible for 96% of mortality from viral hepatitis. According to the World Health Organization (WHO), in 2015, viral hepatitis was estimated to have caused 1.34 million deaths worldwide, which is a 22% increase since 2000. HAV infection causes acute hepatitis as one of the most common infectious diseases worldwide. According to WHO, HAV infection resulted in 13.7 million illnesses and 28,000 deaths in 2010 [21,22]. Unlike HBV and HCV, HAV is a positive single-stranded, nonenveloped ribonucleic acid (RNA) virus [23].

To the best of our knowledge, we report for the first time that GBE and its major component 5,7,3',4'-tetrahydroxyflavan-3-ol C4-C8 dimer possess significant antiviral activities against FCV, a surrogate for human norovirus, and HAV, which causes acute hepatitis in humans. In addition, GBE is not cytotoxic to mammalian cells at or under 2% (Figures 1 and 2). At the concentration range of 0.1% to 1%, a single dose of GBE significantly reduced FCV infection regardless if administered before, simultaneously, or after FCV infection in CRFK cells (Figures 3, 4, 5). FCV F9, as well as human norovirus, are among the most difficult to inactivate viruses due to their sizes and nonenveloped structure similar to poliovirus. Thus, GBE can be categorized as a strong inhibitor of FCV/norovirus. Treatment of CRFK cells for 1 h without direct contact to the virus led to >50% reduction in infected cells by FCV (Figures 3). When GBE was mixed with FCV and infected the cells, a protective effect was observed, with the low dose (0.1%) showing a higher effect than the higher doses (Figures 4). This result suggests that contact inhibition may not be dose-dependent. In fact, dose ranges of 0.1, 0.2 and 1% did not show a
In conclusion, we discovered that GBE and its major component 5,7,3',4'-tetrarydroxyflavon-3-ol \( C_7-C_9 \) dimer, possess strong antiviral properties against norovirus (FCV as human norovirus surrogate) and HAV, which are two nonenveloped viruses causing acute symptoms without treatment. These results suggest that compounds derived from buckwheat family (\emph{Fagopyrum}, part of the flowering plant family Polygonaceae) could be used, either as a crude extract, or as purified phytochemicals to prevent and/or treat norovirus or hepatitis virus infections. The antiviral mechanisms, as well as whether they have a broad-spectrum antiviral activity require additional studies. Since these nutraceuticals have been widely used in human populations, this discovery may lead to new drug development against viral hepatitis and norovirus infection pending further work such as animal model safety and efficacy tests, and eventually leading to clinical trials.

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