Phytocompounds for the control of human enteric viruses
Doris H D'Souza

Plant extracts and associated polyphenols are known for their varied health benefits that include antioxidant effects and antimicrobial properties. The increasing consumer demand for cost-effective and natural alternatives to chemically-synthesized antimicrobials and therapeutics that are also sustainable makes the field of phytochemical research rather intriguing and challenging. Human enteric viruses are increasingly recognized worldwide as significant causes of human disease in adults and children, alike. In the absence of available vaccines for the human noroviruses, plant extracts are gaining popularity for the prevention and treatment of viral diseases. Research on plant extracts (particularly polyphenols derived from fruits) for human enteric virus control will be briefly summarized in this article.

Addresses
Department of Food Science and Technology, University of Tennessee-Knoxville, 2600 River Drive, Room 102 FSPB, Knoxville, TN 37996, United States

Corresponding author: D'Souza, Doris H. (ddsouza@utk.edu)

Introduction

Plant-derived extracts that contain abundant chemically diverse secondary metabolites have been extensively researched by the scientific community for their varied antimicrobial properties [1,2,3,4•,5,6•,7]. Numerous plant extracts have been utilized in traditional medicine since centuries, for raw and processed food preservation, in pharmaceuticals, in alternative medicine and natural therapies, and as potential sources of new drugs [4•,8]. The rise in demand for plant-derived antimicrobials has been attributed to several reasons including decreased microbial susceptibility (increased resistance) to traditionally-used antibiotics, increased consumer demand for plant-derived antimicrobials, potentially-decreased cost, ease of availability in developing countries, and sustainability. This trend is also suggested to be promoted by the World Health Day 2011 theme of 'Antimicrobial resistance: no action today, no cure tomorrow' [6•]. However, among the estimated 250,000–500,000 plant species present on earth, only a small fraction are reportedly screened for com-pounds with antimicrobial activity and only 1–10% of plants are reported to be used as food by animals and humans [4•,6•].

Of recent interest has been the application of plant-derived antimicrobials to control and prevent the transmission of human enteric viruses. Human enteric viruses of public health concern include human noroviruses (HNoVs), hepatitis A virus (HAV), rotaviruses, sapoviruses, Aichi virus, adenoviruses, hepatitis E virus, astroviruses, and various human enteroviruses [8]. It has been recognized worldwide that HNoVs are the leading cause of non-bacterial gastroenteritis, being responsible for an estimated 5.5 million foodborne illnesses, 27% hospitalizations, and 11% of food-related deaths in the United States, annually [9•]. When taking into account all modes of transmission, HNoVs cause an average of 570–800 deaths, including 400,000 Emergency Department visits, and 19–21 million total illnesses annually in the United States alone [10••]. This results in medically associated financial losses as well as loss in time at work.

In the absence of available animal cell-culture systems to propagate HNoVs, cultivable surrogates such as feline calicivirus (FCV-F9), murine norovirus (MNV-1), bacteriophages (MS2 or phiX174) and more recently Tulane virus (TV) and porcine enteric sapovirus are used to evaluate virus inactivation by processing or chemical technologies. As currently there are no vaccines available to prevent HNoV-related illness, adequate control measures are needed, to prevent contamination, inactivate the viruses, and/or treat diseases. Much research continues toward investigating the role of fruit-derived natural phenolics and other plant extracts against human enteric viruses. This article will provide a glimpse/preview of plant phenolics with a focus on the fruit-derived compounds that have been screened for activity against human enteric viruses.

Cranberry bioactives

Cranberries (*Vaccinium macrocarpon*) are native to North America and were used by Native Americans to treat bacterial infections [11•]. The phytochemicals associated with cranberry bioactivity include flavonoids, phenolic acid derivatives, hydroxycinnamic acid derivatives, organic acids, and isoprenoids (including ursolic acid and lutein) [12]. Cranberries have a high content of oligomeric and polymeric pigments, also referred to as condensed non-hydrolyzable tannins or proanthocyanidins [13]. Cranberry proanthocyanidins (CPAC) consist primarily of epicatechin tetramers and pentamers with at least one A-type linkage [20]. The proanthocyanidin

content of raw cranberries can average 410 mg/100 g fresh fruit weight [3]. In addition, 100% cranberry juice contains tannins at concentrations as high as 0.13 g/L [14]. Cranberry phenolics possess antioxidant and free radical-scavenging activities, with health benefits that include anticarcinogenic, anti-inflammatory, antibacterial, and antiviral properties [3].

Effects of cranberry bioactives against human enteric viruses

Commercial cranberry juice (CJ) and cranberry proanthocyanidins (CPAC) at 0.15, 0.3, and 0.6 mg/ml were reported to reduce HNoV surrogates including FCV-F9 at ∼5 log plaque forming units (PFU)/ml to undetectable levels after 1 h at room temperature (RT) [15]. On the other hand, the hardier surrogate virus, MNV-1 was reported to be reduced by 2.06 log PFU/ml with CJ, while CPAC at 0.15, 0.3 and 0.6 mg/ml resulted in reductions of 2.63, 2.75, and 2.95 log PFU/ml after 1 h at RT, respectively (Table 1). Bacteriophage MS2 (single-stranded RNA) titers were reported to be reduced by 1.14 log PFU/ml with CJ, and 0.55, 0.80, and 0.96 log PFU/ml with 0.15, 0.30, and 0.60 mg/ml CPAC, respectively, while bacteriophage phi-X174 (double-stranded RNA) titers were reduced by 1.79 log PFU/ml with CJ, and 1.95, 3.67, and 4.98 log PFU/ml with CPAC at 0.15, 0.30, and 0.60 mg/ml, respectively [15].

In a further study to determine time-dependent effects, CJ at pH 2.6 and pH 7, and CPAC at 0.15 and 0.3 mg/ml was shown to reduce FCV-F9 (5 log PFU/ml) to undetectable levels within 30 min, while the other three viruses required increased time for higher/incremental reduction [16]. These researchers also attempted to understand the mechanism of action of CPAC, using transmission electron microscopy (TEM) and showed slight structural changes for FCV-F9 treated with CJ and CPAC. To understand effects of CJ and CPAC on virus binding to host cells, cell monolayers treated for 1 h with CJ (pH 7) or 0.3 mg/ml CPAC, followed by FCV-F9 or MNV-1 infection, were not reported to show any reduction in FCV-F9 titers, while MNV-1 titers were reported to be reduced by 0.51 and 0.91 log PFU/ml with 50% CJ and 0.3 mg/ml CPAC, respectively [16]. When these researchers first infected CRFK and RAW cells with FCV-F9 and MNV-1 to determine effects on replication, followed by treatment with CJ at 50% and 0.3 mg/ml CPAC for 1 h, FCV-F9 titers were reduced by 0.43 and 0.44 log PFU/ml, respectively, while MNV-1 titers were reduced by 0.80 and 0.42 log PFU/ml, respectively. Thus, they concluded that CJ and CPAC showed some modest effects on both MNV-1 adsorption and replication. These data along with the infectivity data, confirmed that CJ and CPAC show some antiviral effects against the tested HNoV surrogates.

Other studies showed that cranberry juice (CJ) at concentrations of 50%, 30%, and 10%, could reduce bacteriophages

T2 and T4 of *Escherichia coli* strains C and B, respectively, and simian rotavirus SA-11 from 9 log PFU/ml to undetectable levels after 30 min at 37 °C, 23 °C, and 4 °C [5]. However, concentrations lower than 10% produced no significant reduction. Scanning electron microscopy (SEM) studies revealed that CJ-treated phage T4 was unable to attach to bacterial host cells. CJ at 20% concentration was also shown to completely inhibit hemagglutination of simian rotavirus [17]. These researchers proposed that CJ led to alterations in glycoprotein moieties, resulting in failure of virus binding to the host-cell receptors. They also showed that CJ and CPAC had antiviral activity against waterborne rotavirus SA-11 and bovine reovirus [17]. Rotavirus SA-11 treated with cranberry extracts had single-shelled or anomalous virus-like particles, while the control samples contained the typical double-shelled, icosahedral 'wheel-like' particles by TEM. Additionally, they reported that pre-treatment of MA-104 host cell monolayers with a concentration of 5% NC-90 (NutriCran-90^{TM}) and NC-100 (NutriCran-100^{TM}) extracts (cranberry cocktail) reduced reovirus infectivity titers to ∼50% of control, while commercial CJ cocktail caused only 10% reduction [17]. Earlier, *in vitro* studies using polio virus type 1 treated with a cranberry drink at pH 2.6 (natural pH) and 7.0 demonstrated maintenance of 21% and less than 1% poliovirus infectivity, respectively [18].

Using cranberry and grape juices and their proanthocyanidins, loss of rotavirus capsid integrity in cell-free suspension was determined using enzyme-linked-immunosorbent assay (ELISA) [19]. These researchers suggested that an alteration or modification of type A CPAC structural integrity at or near physiologic pH could potentially impact anti-rotavirus activity. They also used electron microscopy (EM) to visualize rotavirus binding to PACs, suggesting blockage of viral-antigenic binding determinants. Other researchers showed that 1 and 10 mg/ml commercial analytical grade proanthocyanidin could achieve ≥2-log reductions in FCV-F9 and coxsackievirus A7 strain (Cox.A7) titers after 10 s, respectively [13]. Although, the antiviral mechanism of proanthocyanidin was not deduced, they suggested that proanthocyanidin could be an effective disinfectant against HNoVs [13].

Pomegranate bioactives

Pomegranate (*Punica granatum*) is a fruit native to Iran, China and the Indian subcontinent, and its peel, rind, and seeds are known for their medicinal properties since biblical times [20•]. Pomegranate extract is rich in polyphenolic compounds that include flavonoids (anthocyanin such as delphinidin, cyanidins, pelargonidin; and their glycosides; catechins and other complex flavonoids) and hydrolyzable tannins (punicalin, pedunculagin, punicalagin, gallic and ellagic acid), accounting for 92% of their antioxidant activity [21]. Pomegranate juice (PJ) and

Table 1

Brief summary of fruit juices and/or their associated polyphenol extracts against human enteric viruses and viral surrogates.

Type of bioactive	Concentration	Virus	Reduction	Reference
American Cranberry extracts (*Vaccinium macrocarpon*)	0.6 mg/ml CPAC at RT for 1 h	FCV-F9 MNV-1 MS2	≥5 log PFU (nondetectable) ~3 log PFU ~1 log PFU	[15,16]
Cranberry juice (*Vaccinium macrocarpon*)	50% juice after 30 min	Simian rotavirus	Undetectable from 9 log PFU/ml	[5,17]
Pomegranate (*Punica granatum*)	32 mg/ml Polyphenol at RT for 1 h	FCV-F9 MNV-1 at 5 log PFU MS2 at 5 log PFU	≥5 log PFU (nondetectable) ~3.6 log PFU ~1 log PFU	[25,26]
Black raspberry (*Rubus coreanus*)	Juice	FCV-F9, MNV-1	PFU reduction after pretreatment of hostcells or co-treatment	[35]
Grapeseed extract (GSE; *Vitis vinifera*)	0.2 mg/ml GSE at 37 °C for 1 h	MNV-1	3 log PFU/ml	[27]
GSE	1 mg/ml GSE at 37 °C for 2 h	FCV-F9	4.61 log PFU/ml	[7]
		MNV-1 HAV	1.73 log PFU/ml 3.2 log PFU/ml	
Analytical grade PAC	1 and 10 mg/ml PAC for 10 s	FCV-F9, coxsackievirus A7, reovirus	>2 log	[13]

pomegranate polyphenols (PP) are known to exhibit a range of antioxidant, antimicrobial, anticancer, and anti-inflammatory activities [1,22–24].

Effects of pomegranate bioactives against human enteric viruses

Commercial pomegranate juice was reported to decrease low titers (~5 log PFU/ml) of HNoV surrogates, FCV-F9, MNV-1, and bacteriophage MS2 by 2.56, 1.32, and 0.32 log PFU/ml, respectively and high titers (~7 $\log_{10}$ PFU/ml) by 1.20, 0.06 and 0.63 log PFU/ml, respectively after 1 h at RT [25]. FCV-F9 at low (~5 log PFU/ml) and high (~7 log PFU/ml) titers were found to be reduced to non-detectable levels with 8, 16, and 32 mg/ml of pomegranate polyphenol (PP; polyphenolic extract from fresh pomegranate fruit-POM^{TM} obtained from POM Wonderful$^{®}$) after 1 h at RT by these researchers. Furthermore, MNV-1 at low titers was reported to be reduced by 1.30, 2.11, and 3.61 log PFU/ml and high initial titers by 1.56, 1.48 and 1.54 log PFU/ml with 4, 8, and 16 mg/ml PP, respectively after 1 h at RT. Low titers of bacteriophage MS2 were reduced by 0.41, 0.45, and 0.93 log PFU/ml and high titers by 0.32, 0.41 and 0.72 log PFU/ml with 4, 8, and 16 mg/ml PP, respectively [25]. When these researchers changed the pomegranate juice pH from 3.4 to 7.0, they found no significant decrease in the antiviral effects, attributing the antiviral effects to the bioactive compounds of pomegranate juice and not the pH alone. The antiviral effects of PJ and PP were also reported to be dependent on virus type and virus titer, storage of the pomegranate juice (shelf-life), but not on the commercial brand of pomegranate juice [26].

In further time-dependent studies with three viral surrogates, ≥50% of the total reduction was reported to be achieved in 20 min using pomegranate bioactives [26]. FCV-F9, MNV-1, and MS2 titers were reported to be decreased by 3.12, 0.79, and 0.23 log PFU/ml respectively, after 20 min with commercial PJ. After 20 min, FCV-F9, MNV-1, and MS2 were reduced by 4.02, 0.68, and 0.18 log PFU/ml with 2 mg/ml PP and by 5.09, 1.14, and 0.19 log PFU/ml with 4 mg/ml PP, respectively [26]. MS2 was quite resistant to PJ and PP treatment.

In order to understand the mode of action and the effect of PJ or PP on virus replication, these researchers treated confluent host cells with PJ or PP for 45 min after FCV-F9 or MNV-1 infection, and showed that FCV-F9 titers were reduced by 0.75 and 0.40 log PFU/ml with 20% PJ and 0.4 mg/ml PP, respectively (at concentrations not cytotoxic to the host cells), while MNV-1 titers were reduced by 0.41 log PFU/ml with 30% PJ, with no significant reduction using 0.8 mg/ml PP [26]. They also showed that pretreatment of host cell monolayers with PJ or PP for 45 min, followed by FCV-F9 or MNV-1 infection, resulted in 1.05 and 0.40 log PFU/ml reduction of FCV-F9 with 20% PJ and 0.4 mg/ml PP, respectively, and 0.55 and 0.29 log PFU/ml reduction of MNV-1 with 30% PJ and 0.8 mg/ml PP, respectively. These slightly higher reductions with pretreatment of cells suggested that PJ bioactives and PP may block viral binding to host-cells to some degree. Using ELISA, cranberry and pomegranate juices were shown to reduce the specific binding ability of HNoV P particles of GII.4 [27]. This is in agreement with earlier reported work using cranberry and pomegranate juices and their extracts against HNoV surrogates [25,26].

Konowalchuk and Speirs (1978) found <1% survival of 3 log PFU poliovirus/0.05 ml after storage at 4 °C for 24 h in PJ, though the mechanism of action was not reported [18]. Punicalagin was shown to inhibit human enterovirus

71(EV71) infection (single-stranded RNA virus that causes hand, food and mouth disease of children under 6 yr) of human rhabdomyosarcoma (RD) cells in a dose-dependent manner with an inhibitory concentration (IC_{50} value) of 15 μg/ml after 2 h [28]. These researchers also found that punicalagin treatment of mice challenged with a lethal dose of EV71 reduced mortality and relieved clinical symptoms.

Among the 50 Chinese medicinal herbs tested for blocking of HNoV protusion protein (P) binding to histo-blood group antigens (HBGAs), tannic acid-rich Chinese gall was reported to block NoV P dimer binding to type A saliva at IC_{50} = 5.35 μg/ml and to B saliva at IC_{50} = 21.7 μg/ml, while tannic-acid rich pomegranate blocked binding of NoV P dimer to type A saliva at IC_{50} = 15.59 μg/ml and B saliva at IC_{50} = 66.67 μg/ml [29]. These researchers also confirmed that commercially available, highly purified tannic acid was a strong inhibitor of HNoV P protein binding to both A and B saliva ($IC_{50} \approx 0.1$ μM), suggesting that tannic acid is a promising antiviral against HNoVs [29].

Blueberry bioactives
Blueberries (genus *Vaccinium*), perennial flowering plants native to North America, are rich in polyphenolic flavonoids, predominantly anthocyanins (anthocyanidin glycosides) and phenolic acids, catechins (flavanols), and proanthocyanidins (condensed tannins) that reportedly play a role in their antioxidant effects [30]. Blueberries are also associated with anticarcinogenic, neuroprotective, cardioprotective, anti-inflammatory, and antimicrobial properties [31•].

Effects of blueberry bioactives against human enteric viruses
In a survival study, FCV-F9 was shown to be completely reduced in blueberry juice (BJ) at 4 °C after 1 day, while MNV-1 showed minimal reduction after 14 d, and bacteriophage MS2 showed complete reduction after 7 d [32]. Recent studies with FCV-F9 and MNV-1 using commercial blueberry juice (BJ) and blueberry proanthocyanidins (B-PAC) at 37 °C showed that FCV-F9 titers were reduced to undetectable levels after 5 min with 1 and 2 mg/ml B-PAC, and after 3 h with BJ [33]. MNV-1 titers were reduced to undetectable levels after 3 h exposure to 1 mg/ml of B-PAC, but with no effect with BJ even after 6 h. These findings suggest that blueberry bioactives may have antiviral activity. Methanolic extracts of blueberry leaves (epicatechin) was also shown to inhibit hepatitis C virus (not typically spread by the enteric viral route) [34].

Black raspberry bioactives
Rubus coreanus is a species of black raspberry that is native to Korea, Japan and China, and is rich in polyphenols (gallic acid and quercetin), with reported anti-inflammatory, antibacterial, and antiviral properties [35].

Effects of black raspberry against human enteric viruses
There is only a single report on black raspberry (BRB) juice showing reduction in plaques formed by HNoV surrogates, FCV-F9 and MNV-1 [35]. Simultaneous addition of BRB with MNV-1 to the host cells was reported to result in maximal antiviral effects, while for FCV-F9 pre-treatment of host cells or simultaneous addition of FCV-F9 with BRB, both resulted in antiviral activity. These researchers postulated that BRB inhibited MNV-1 and FCV-F9 surface protein attachment to host-cell receptors. However, gallic acid and quercetin were not reported to cause reduction of FCV-F9 or MNV-1 [35].

Grape seed extract (GSE) bioactives
Grape-(genus *Vitis*)-seed extract is a by-product of the grape juice and wine industry that contains bioactives including flavonoids, polyphenols, anthocyanins, proanthocyanidins, procyanidins, and the stilbene derivative, resveratrol [36]. Their pharmacological and therapeutic benefits include antioxidative, anti-inflammatory, cardioprotective, hepatoprotective, neuroprotective, and antimicrobial effects [37•].

Effects of GSE against human enteric viruses
GSE at 0.25, 0.5, and 1 mg/ml was found to cause FCV-F9 reductions of 3.64, 4.10, and 4.61 log PFU/ml; MNV-1 reductions of 0.82, 1.35, and 1.73 log PFU/ml; and HAV reductions of 1.81, 2.66, and 3.20 log PFU/ml at 37 °C after 2 h, respectively [7]. Other researchers showed that GSE at 0.2 mg/ml after 1 h at 37 °C decreased MNV-1 titers (>3 log PFU/ml), decreased the specific binding ability of NoV GII.4 to Caco-2 cells, and the binding of virus-like P particles (VLPs) to salivary human histo-blood group antigen receptors [27]. TEM analysis revealed inflation and deformation of GSE-treated human NoV GII.4 VLPs suggesting that by causing virus capsid structural damage, GSE potentially prevents virus binding to host-cells [27].

GSE at 0.25, 0.50, and 1 mg/ml was shown to reduce high titers (~7 log PFU/ml) of FCV-F9 by 2.33, 2.58, and 2.71 log PFU when used as a surface wash on lettuce after 1 min; and by 2.20, 2.74, and 3.05 log PFU on peppers, respectively, whereas low FCV-F9 titers were reduced to undetectable levels after 1 min [38]. Reduction of 0.2–0.3 log PFU on lettuce and 0.8 log PFU on peppers for low MNV-1 titers (~5 log PFU/ml) were also reported. Reductions of 0.71–1.1 and 1–1.3 log PFU were obtained for high and low HAV titers, respectively on both produce items by washing with GSE at 0.25–1 mg/ml [38]. Other researchers showed that GSE effects decreased in the presence of increasing concentrations of dried milk (0.02 and 0.2%) or lettuce extract, where

increased concentrations of 2 mg/ml GSE were needed to have similar effects on MNV-1 to that obtained without the addition of milk or extracts [39]. These investigators showed that under simulated food industry conditions, GSE at 2 mg/ml was required to decrease MNV-1 titers by 1.5–2-log PFU/ml for sanitizing washing-bath water of fresh-cut lettuce, irrespective of its chemical oxygen demand (0–1500 mg/ml) [39]. Instrumental color analysis demonstrated that there were no significant differences between GSE-treated and untreated produce, suggesting that GSE can potentially be used as part of hurdle technologies for foodborne viral reduction on produce [38].

Other plant flavonoids against human enteric viruses

In a study that evaluated four flavonoids associated with citric and other fruits (myricetin, L-epicatechin, tangeretin, and naringenin) against HNoV surrogates, FCV-F9 at low titers was found to be reduced to undetectable levels by 0.5 mM and 1 mM myricetin, and by 1.4 log PFU/ml with 0.5 mM L-epicatechin after 2 h at 37 °C [40]. It was also shown that FCV-F9 at high titers was decreased by 3.17 and 0.72 log PFU/ml with myricetin and L-epicatechin at 1 mM, respectively and by 1.73 log PFU/ml with 0.5 mM myricetin. However, MNV-1 was not reported to be reduced by these flavonoids at the tested concentrations. These results suggest that the antiviral effects of the tested flavonoids were dependent on the flavonoid concentration, virus type, and virus titer [40]. However, since these phytochemicals at the concentrations studied did not show any significant effect on MNV-1, caution is advised when extrapolating these data for HNoV inactivation.

Tannins and catechins against human enteric viruses

Tannins from persimmon (*Diospyros kaki*) native to China, which contains *ca.* 22% tannin was found to reduce poliovirus, coxsackie virus, adenovirus, rotavirus, FCV-F9 and MNV-1 by >4 logs [41]. Other tannins derived from green tea, acacia and gallnuts were also reported to be effective against some of these viruses, while coffee extracts were not effective against any of the tested viruses. These investigators reported that protein aggregation played a role in decreasing viral infectivity [41]. Crude theaflavin extracts from U.S. black tea when tested against bovine coronavirus (ATCC P2 strain) were shown to have a mean effective concentration that inhibited 50% infection (EC_{50}) at 34.7 µg/ml (standard error 9 µg/ml) using tissue culture infectious dose (TCID50) assays. For bovine rotavirus strain, NCDV-Lincoln, the reported EC_{50} values were 0.353 µg/ml against 200–400 immunofluorescent focus-forming units (IFFU) [2].

Quillaja saponaria aqueous extracts against human enteric viruses

Quillaja aqueous extracts obtained from the Chilean soapbark tree are rich in tannins, saponins, and polyphenols, with strong anti-rotaviral activity. A dosage of 0.015 mg saponin extract/mouse could significantly reduce rhesus rotavirus (RRV) induced diarrhea from 79% to 11% when mice were exposed to 500 RRV plaque-forming-units (PFU) for five consecutive days [42]. These researchers suggested that *Quillaja* extracts are able to 'block' rotavirus infection by inhibiting virus-host attachment through disruption of cellular membrane proteins and/or virus receptors showing promise as antivirals to reduce rotavirus infection and disease severity [42].

Conclusions and future directions

The increasing consumer demand for effective, safe, sustainable, and inexpensive natural products provides a good basis to study phytochemicals for use as antiviral agents [4•,6•]. Plant polyphenols derived from tree barks or that are by-products of the food industry such as GSE show promise for industrial applications due to their decreased cost and availability. Overall, it appears that the tested plant extracts have antiviral activity through destruction of the viral structure and prevention of host-cell binding and/or entry into the host. Though most studies with plant extracts have been conducted *in vitro* focus should be placed toward application in food systems and environments to control the spread of viral disease.

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