Fostering the antiviral activity of green tea extract for sanitizing purposes through controlled storage conditions

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Abstract

Contamination of food-contact surfaces with enteric viruses, especially human norovirus (HuNoV) and hepatitis A virus (HAV), is considered an important vehicle for the indirect transmission of foodborne viral diseases. The aim of the present study was to evaluate the antiviral activity of green tea extract (GTE) at room temperature as a function of pH and storage time and to relate it with changes in composition as a consequence of degradation and epimerization reactions in the storage conditions. The obtained results revealed that freshly prepared GTE was very effective inactivating murine norovirus (MNV) and HAV at neutral and alkaline pH but was ineffective at pH 5.5. Additionally, storage of the solutions for 24 h at the various pH conditions significantly increased their antiviral activity. The reduction in MNV and HAV infectivity was related to the formation of catechin derivatives during storage, as demonstrated by HPLC/MS analysis.

In addition, the GTE prepared under the optimal conditions (24 h storage and pH 7.2) was applied at a concentration of 5 mg/mL for only 15 min on stainless steel and glass surfaces for sanitizing purposes, showing a reduction of more than 1.5 log of MNV and HAV infectivity. These findings indicate that GTE can be used as natural disinfectant for decontamination of food contact surfaces, thus preventing the indirect transfer of enteric viruses to food or persons.

1. Introduction

In the last decade an increased incidence of foodborne outbreaks have been attributed to viruses, most notably human norovirus (HuNoV) and hepatitis A virus (HAV). The World Health Organization (WHO) has recently estimated that norovirus causes approximately 120 million illnesses and 35,000 deaths, while HAV causes 14 million cases and 28,000 deaths attributed to foodborne illness each year (WHO, 2013).

HuNoV and HAV are transmitted through the fecal-oral route, either by direct contact with a virus-infected person, or through ingestion of contaminated water or food. Additionally, their low infectious dose, ranging from 10 to 100 (Teunis, et al., 2008), together with their prolonged stability in the environment, make HuNoV and HAV extremely infectious and highly transmissible through environmental surfaces (fomites).

All these factors justify the highest health risk associated to HuNoVs outbreaks in closed communities (e.g. nursing homes, hospitals, cruise ships, etc.) as exposure sites where common areas and facilities contribute to a rapid virus transmission (European Food Safety, European Centre for Disease, & Control, 2016; Hall, Wikswo, Pringle, Gould, & Parashar, 2014; Hedlund, Rubilar-Abreu, & Svensson, 2000).

In recent years, because of the great consumer awareness and concern regarding synthetic chemical additives or sanitizers, foods and food-contact surfaces treated with natural compounds have become very popular since they are consider safe, cheap, and pose no risk to both the environment and population (D. Li, Baert, & Uyttendaele, 2013). Many different natural compounds have been used as antimicrobials for food applications, either directly added into the product formulation, incorporated into the packaging material or used as natural sanitizers (Irkin & Esmer, 2015). Although bactericidal efficacy of most of these natural compounds has been well established,

current knowledge of the antiviral efficacy for food applications is limited and requires further investigation.

So far several natural compounds have already been characterized for their antiviral activity (reviewed by D. Li, et al. (2013), however limited information is available for their use in food applications. For example, grape seed extract (GSE) was effective reducing HuNoV surrogates, i.e. feline calicivirus (FCV) and murine norovirus (MNV), as well as HAV infectivity on lettuce, apple juice and milk (Joshi, Su, & D'Souza, 2015; X. Su & D'Souza, 2013). Likewise, carvacrol was effective in reducing MNV and FCV infectivity in lettuce and lettuce wash water (Sanchez, Aznar, & Sanchez, 2015). Recently, green tea extract (GTE) proved very effective as natural sanitizer to control virus contamination since 1.5 log reduction and complete inactivation were recorded for MNV and HAV on stainless steel and glass surfaces treated with 10 mg/mL GTE for 30 min (Randazzo, Falco, Aznar, & Sanchez, 2017). Similarly, GTE demonstrated good effectiveness to decrease contamination with human adenoviruses on lettuces, strawberries and green onions (Marti, Ferrary-Américo, & Barardi, 2017).

GTE is a complex mix of various components; it is a source of polyphenols, especially flavonoids, being catechins the mayor substances which make up approximately 30% of the dry weight of the tea leaf. Epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epicatechin (EC) are the major catechins in GTE (Abdel-Rahman, et al., 2011). Several studies confirmed that these catechins have antioxidative, thermogenic, anticarcinogenic, and anti-inflammatory effects depending on its concentration (Chacko, Thambi, Kuttan, & Nishigaki, 2010). Moreover, recent findings suggest that the antiviral activity of EGCG against norovirus surrogates and HAV is due to the activity of catechin derivatives rather than EGCG itself (Falcó, et al., 2017).

In this work, the antiviral activity of GTE over time was evaluated against MNV and HAV at 25 °C, using solutions freshly prepared at different pHs and after different times of storage. Moreover, HPLC analysis of the GTE solutions was performed to disclose if GTE derivatives would explain the differences in antiviral activity displayed. Finally, the optimum GTE solution was evaluated as a natural disinfectant for food-contact surfaces.

2. Materials and Methods

2.1. Virus propagation and cell lines

MNV-1 was propagated and assayed in RAW 264.7 cells, both were kindly provided by Prof. H. W. Virgin (Washington University School of Medicine, USA). HAV (strain HM-175/18f) was purchased from ATCC (VR-1402), which was propagated and assayed in confluent FRhK-4 cells (kindly provided by Prof. A. Bosch, University of Barcelona, Spain). Semi-purified viruses were harvested 2 days and 12 days post infection, MNV and HAV respectively, by three freeze-thaw cycles of infected cells followed by centrifugation at $660 \times g$ for 30 min to remove cell debris. Infectious viruses were enumerated by determining the 50% tissue culture infectious dose (TCID₅₀) with eight wells per dilution and 20 µL of inoculum per well using the Spearman-Kärber method (Pinto, Diez, & Bosch, 1994).

2.2. Antiviral activity of GTE solution depending on aging

GTE (Naturex SA, France), listed as GRAS (21CFR or AAFCO), with an EGCG content of approximately 40-50% was used in this study. It was dissolved in PBS (pH 7.2) to obtain concentrations of 1 mg/mL. Antiviral effect of GTE solutions was evaluated at time 0 (freshly prepared), after 24 h and seven days of storage. Each solution was mixed with an equal volume of HAV and MNV suspensions (ca. 5 log

TCID₅₀/mL) getting a final concentration of 0.5 mg/mL for GTE, followed by incubation at 25°C in a shaker for 2 h. Positive control was virus suspensions in PBS under the same experimental conditions. Each treatment was performed in triplicate. Confluent RAW 264.7 and FRhK-4 monolayers in 96-well plates were used to evaluate the effect of GTE as described above. Antiviral activity of GTE was estimated by comparing the number of infectious viruses on suspensions without GTE and on the GTE-treated virus suspensions. The decay of HAV and MNV titers was calculated as $\log_{10} (N_x/N_0)$, where N_0 is the infectious virus titer for untreated samples and N_x is the infectious virus titer for GTE-treated samples.

2.3. Effect of GTE depending on aging and pH

In order to elucidate the effect of pH and aging on the antiviral activity of GTE, MNV and HAV suspensions were ten-fold diluted in PBS at different pHs (5.5, 7.2 and 8.5) and immediately incubated with a freshly prepared GTE solution at 0.5 and 5 mg/mL prepared in PBS at pH 5.5, 7.2 and 8.5. Moreover, GTE solutions at different pHs were stored for 24 h and further incubated with virus suspensions (1:1). Samples were incubated during 2h at 25 °C in a shaker. Positive controls were virus suspensions in PBS under the same experimental conditions. Infectious viruses and effectiveness of the treatments were calculated as described above.

2.4. Time dependent effects of GTE

The effect of time over GTE solution was tested using a concentration of 10 mg/mL solution prepared by dissolving the supplied powder in PBS at pH 7.2 and stored for 24 h. This solution was then mixed with an equal volume of each virus to reach viral titers about 5 log TCID₅₀/mL and a final concentration of GTE of 5 mg/mL. Then samples

were incubated in a water-bath shaker at 150 rpm at 25°C for 15, 60, 120, 240 and 480 min. Positive controls were virus suspensions added with PBS at pH 7.2 under the same experimental conditions.

2.5. HPLC analysis

GTE solutions (5 mg/mL) in PBS at different pHs (i.e. 5.5, 7.2 and 8.5) were subjected to HPLC-MS analysis. Samples were analyzed after different storage periods: 0, 1 and 7 days. An Agilent 1290 HPLC system equipped with an Acquity UPLC BEH C18 column (Waters, 50 mm × 2.1 mm, 1.7 µm of particle size) was used, following the method described in Gómez-Mascaraque, Soler, and Lopez-Rubio (2016). The injection volume was 10 µL. Eluent A was water and eluent B methanol, both slightly acidified with 0.1% formic acid. The flow rate was 0.4 mL/min and the elution gradient started with 10% of eluent B during 2 min, followed by 100% eluent B for 13 min, and 10% eluent B for the last 7 min. A TripleTOF™ 5600 system with a DuoSpray™ source operating in the negative mode was used for detection (AB SCIEX). The parameter settings used were: ion spray voltage -4500 V, temperature 450 °C, curtain gas 30 psi, ion source gas 50 psi. Data were evaluated using the XIC manager in the PeakView™ software (version 2.2). The compounds detected were tentatively identified with the aid of the Phenol Explorer and Chemspider databases (<http://phenol-explorer.eu>, <http://www.chemspider.com>).

2.6. Surface disinfection tests on stainless steel and glass discs

Surface disinfection tests were performed adapting the ISO 13697:2001 standard (ISO/TS, 13697:2001) by adding 50 µL of MNV and HAV suspensions (around 5 log TCID₅₀/mL) on the stainless steel and glass discs (2x2 cm) and let dry completely at room temperature (RT). Subsequently, 100 µL of a solution of GTE (5 mg/mL, pH 7.2)

prepared on hard water, according to the ISO 13697:2001, and stored for 24h before the experiments were spotted on the inoculated discs, followed by incubation at RT for 5 or 15 minutes. Then the effect of GTE was neutralized by adding DMEM supplemented with 10% of fetal bovine serum (FBS). Positive controls were performed using discs treated with 100 μ L of hard water without GTE. HAV and MNV were recovered and titrated as described above. Infectious viruses and effectiveness of the treatments were calculated as described above.

2.7. Data analysis

Results from three replicates of the treatments and controls were statistically analyzed using ANOVA with STATISTICA software version 10 (StatSoft Inc., Tulsa, OK, USA) and Tukey's test on a completely randomized design. A P value <0.05 was deemed significant.

3. Results

3.1. Antiviral activity of GTE solutions as a function of storage conditions

Initially, evolution of antiviral activity of GTE upon the time was evaluated. Titers of MNV and HAV before and after treatment with a freshly prepared GTE solution (time 0), after 24 h storage (time 1) or after 7 days storage (time 7) are shown in Figure S1. GTE solution (0.5 mg/mL) decreased the titer of MNV by 0.23, 1.92 and 0.04 log while for HAV reductions were of 0.87, 2.02 and 0.69 with freshly prepared, after 24 h storage and 7 days storage, respectively.

Figures 1 and 2 clearly show that the antiviral activity of GTE solutions was dependent on the pH of the solutions as well as to the storage time. In particular, GTE at 0.5 and 5 mg/mL statistically reduced MNV titers by 0.7 and 1.25 log after 2 h exposure at 25°C

in slightly alkaline solutions (pH 8.5) (Fig. 1A). Similar reductions were reported after treatment with GTE stored for 24 h (Fig. 1B). On the opposite, freshly prepared GTE in neutral (pH 7.2) or acidic solutions (pH 5.5) showed no MNV inactivation while after 24 h storage, MNV titers were reduced by 1.19 and 1.21 log (at pH 5.5) and 0.85 and 2.17 log (at pH 7.2), when using 0.5 and 5 mg/mL of GTE solutions respectively (Fig 1A and 1B).

For HAV, freshly prepared GTE at 0.5 and 5 mg/mL statistically reduced HAV titers by 1.3 and 1.5 log in neutral solutions (pH 7.2) and by 1.19 and 1.90 log in slightly alkaline solutions (pH 8.5), respectively (Fig. 2A). Greater reductions were obtained when the GTE solution was stored for 24 h (Fig. 2B). For instance, GTE at 5 mg/mL reduced HAV infectivity by 2.58 log in acidic solutions (pH 5.5) while HAV infectivity was reduced below the detection limit in neutral (pH 7.2) and slightly alkaline solutions (pH 8.5) (Fig 2B).

3.2. HPLC analysis

Table 1 summarizes the main phenolic compounds found in the GTE solutions. These compounds were tentatively identified based on the values of m/z obtained from the mass spectra. All of them had been previously detected in tea leaves or tea-derived products (S. Li, Lo, Pan, Lai, & Ho, 2013; Liu, et al., 2016; Okello, Leylabi, & McDougall, 2012; Sang, Lee, Hou, Ho, & Yang, 2005; Suzuki, et al., 2003; Wang, Zhou, & Jiang, 2008; Yoshino, Suzuki, Sasaki, Miyase, & Sano, 1999). The peak areas ascribed to each phenolic compound in the extracted-ion chromatograms (XIC) and, thus, their concentration was different depending on the pH as previously observed for epigallocatechin gallate (EGCG) solutions (Falcó, et al., 2017) and on the incubation time. Figures S2-S4 of the Supplementary Material depict these changes, which were

the result of the degradation and epimerization of the tea-derived polyphenols. In general, a decrease in polyphenols concentration was observed upon storage, due to their degradation, with the greatest drops occurring within the first 24 h after preparation of the GTE solutions (Figures S2-S4). However, three exceptions to this general trend were observed, which are shown in Figure 3. The concentration of kaempferol 3-O-xylosyl-glucoside in the solutions slightly increased after 24h of storage, irrespective of the pH conditions assayed. On the other hand, the concentration of theasinensin A and theasinensin B dramatically changed both with pH and time of storage, being considerably higher in alkaline than in acidic conditions and increasing with time.

3.3. Reduction of MNV and HAV titers by GTE over time

As the antiviral activity of GTE improved after 24 h of storage and at neutral or slightly alkaline pH conditions, the following experiments were performed with GTE solutions (pH 7.2) stored at room temperature for 24 h. Figure 4 shows the reduction of MNV and HAV titers by GTE over the time. As it can be observed, after treatments with 5 mg/mL of GTE, MNV titers were statistically reduced by 0.25, 0.75, 1.04, 1.25 and 1.54 log after 15, 60, 120, 240 and 480 min of exposure, respectively. For HAV, under the same experimental conditions infectivity was reduced by 0.87, 0.92 and 1.88 log after 15, 60 and 120 minutes of exposure. Additionally, infectivity of HAV was reduced below the detection limit after 240 and 480 min of exposure.

3.4. Surface disinfection tests on stainless steel and glass discs

Figure 5 shows titers of recovered MNV and HAV on stainless steel and glass surfaces before, and after, 5 and 15 min treatment with 5 mg/mL of a GTE solution (pH 7.2)

stored during 24 hours. On stainless steel discs, titers of control samples were 5.45 ± 0.33 and 4.7 ± 0.13 log TCID₅₀/mL for MNV and HAV, respectively. GTE at 5 mg/mL reduced MNV infectivity by 0.75 and 1.75 log after 5 and 15 minutes of exposure, respectively, while for HAV reductions of 0.79 and 1.92 logs were recorded. On glass discs, control titers were 5.39 ± 0.09 and 4.87 ± 0.07 log TCID₅₀/mL for MNV and HAV, respectively. No significant reduction ($P > 0.05$) in MNV and HAV infectivity was observed after 5 minutes treatment, while GTE reduced MNV titers by 1.27 logs and HAV titers by 1.54 logs after 15 min treatment.

4. Discussion

Human enteric viruses may persist on surfaces for long periods of time (Mormann, Heißenberg, Pfannebecker, & Becker, 2015). Additionally, transfer of human enteric viruses between several types of environmental and food surfaces have been extensively reported (Cliver, 1997; D'Souza, et al., 2006; Escudero, Rawsthorne, Gensel, & Jaykus, 2012). In this context, prevention and hygiene measures should be taken to reduce the risk of cross-contamination and, thus, decrease the risk of viral pathogen transmission. Chemical compounds are commonly used for disinfecting surfaces in medical facilities. The efficacy of various chemical disinfectants and sanitizers (e.g. sodium hypochlorite, alcohols and quaternary ammonium compounds) against HAV, HuNoVs and HuNoV surrogates on different surface materials, primarily stainless steel, glass, and PVC, has been evaluated (Solomon, Fino, Wei, & Kniel, 2009). However, there are restrictions for their use for food-contact surfaces relevant to the food industry and restaurants, where other strategies such as the use of natural sanitizers are gaining attention. In this sense, GTE proved efficient in surface disinfection tests since 1.5 log reduction and complete inactivation were recorded for MNV and HAV on stainless steel and glass surfaces treated with 10 mg/mL GTE for 30 min at room temperature, analysed in

accordance with ISO 13697:2001 (Randazzo, et al., 2017). However, disinfection including 30 min contact time seems not feasible from a practical point of view. A possibility to improve the efficacy of GTE for surface disinfection is to increase the temperature to 37 °C (Randazzo, et al., 2017), but from a practical perspective neither seems to be feasible.

Therefore, the final aim of this study was to improve the antiviral activity of the GTE to be applied as natural sanitizer. As the antiviral activity of EGCG, which constitutes around the 40-50% of GTE, has been attributed to EGCG derivatives, and given that degradation and epimerization reactions are known to take place in aqueous solutions (Falcó, et al., 2017), the antiviral activity of GTE was evaluated after different solution preparation times and various pH conditions in order to promote degradation and epimerization reactions, thus increasing the amount of the potentially antiviral active compounds. The results of this study with freshly prepared GTE (0.5 mg/mL, pH 7.2 and 25 °C) showed comparable inactivation rates for MNV and HAV when compared to our earlier study (Randazzo, et al., 2017), with less than 1.2 log reduction (Fig S1, 1 and 2). The present study clearly demonstrates that GTE was effective in reducing the titers of MNV and HAV in a time-preparation dependent manner, since 24 h storage of GTE solutions significantly enhanced its antiviral activity on HAV and MNV at 25 °C. For HAV, 24 h storage of GTE solutions rendered similar inactivation rates than freshly-prepared GTE evaluated at 37 °C (Falcó, et al., 2017), thus highlighting the increase in effectivity upon storage of the solutions. Similar trends were observed regarding pHs. As for GTE and EGCG at 37 °C (Falcó, Randazzo et al. 2017; Randazzo, Falco et al. 2017), antiviral activity of freshly-prepared GTE at 25 °C was effective inactivating MNV and HAV at neutral and alkaline pHs but not at pH 5.5 (Fig 1A and 2A).

However, a marked improvement on the antiviral activity at pH 5.5, 7.2 and 8.5 was recorded after 24 h of storage of GTE solutions (Fig 1B and Fig 2B).

These results are in line with the compositional changes observed in the HPLC analysis as a consequence of the degradation and epimerization reactions of the tea-derived polyphenols in GTE, which are known to occur in aqueous solutions (Y. L. Su, Leung, Huang, & Chen, 2003) with pH-dependent kinetics (Zimeri & Tong, 1999). While a general decrease in polyphenols concentration was observed upon storage of the GTE solutions, due to their degradation, the concentration of kaempferol 3-O-xylosyl-glucoside increased after 24 h storage, but decreased again when the solutions were stored for 7 days. These differences in the concentration of kaempferol 3-O-xylosyl-glucoside could partially explain the observed increase in the antiviral activity of the GTE solutions after 24 h storage, and subsequent decrease after longer storage periods (Fig. S1). However, no substantial differences were observed with the pH for this compound. On the other hand, the concentration of theasinensins A and B dramatically changed both with pH and time of storage, being considerably higher in alkaline than in acidic conditions and increasing with time. Similar results were obtained in a previous work in which a purified tea catechin, EGCG, exhibited increased antiviral activity against MNV and HAV at higher pHs, which could be correlated with an increase in EGCG derivatives, including theasinensin A (Falcó, et al., 2017). Isaacs, et al. (2011) also reported stronger antiviral activity of EGCG against herpes simplex virus at high pH values, and observed a high antiviral activity of EGCG dimers regardless of the pH. This suggests that the presence of catechin dimers such as theasinensins A and B, in combination with kaempferol 3-O-xylosyl-glucoside, strongly contributed to the antiviral activity of the green tea extract used in this work against MNV and HAV. Results revealed that GTE has a pH dependent antiviral effect for both viruses at 25 °C,

showing greater inactivation rates at neutral and slightly basic pHs, greatly enhanced upon storage of GTE solution for 24 hours.

Studies evaluating natural compounds for virus inactivation within food service environments remain somewhat limited (D. Li, et al., 2013; Randazzo, et al., 2017). In the surface disinfection tests, GTE treatments were more effective as previously reported (Randazzo, et al., 2017) due to the fact that GTE solution was prepared 24 hours before its use, allowing degradation and epimerization of compounds. Randazzo, et al. (2017) reported only a marginal reduction (<1 log TCID₅₀/mL) for MNV and HAV in stainless steel surfaces treated with freshly-prepared GTE solution (5 mg/mL) for 15 min while on glass surfaces, infectivity was reduced by 1.73 and 0.02 log TCID₅₀/mL MNV and HAV, respectively. In the present study, a slight inactivation increase was recorded (1.75 and 1.92 log TCID₅₀/mL for MNV and HAV, respectively) on stainless steel treated with 5 mg/mL of GTE for 15 minutes (Fig 5). Moreover, 24-h storage of GTE solutions improved the antiviral efficacy of GTE on HAV on glass surfaces, while similar inactivation rates were reported for MNV.

In conclusion, this work demonstrates that by modulating the pH and storage conditions of GTE solutions, the antiviral activity of this natural compound can be fostered. These finding could be of high relevance, not only to formulate natural disinfectants for environmental surfaces, but also to be applied as natural washing of fresh produce. For example, Polyphenon 60 from green tea was effective against human adenovirus but not for MNV in organic fresh products (Marti, et al., 2017). Thus, aging of the solution may improve the efficacy of this natural sanitizer. However, as antiviral activity of GTE decreased after 7-days storage (Supplementary material Fig. S1), encapsulation of GTE could be explored as an alternative for the formulation of GTE sanitizers (Gomez-Mascaraque, Sanchez, & Lopez-Rubio, 2016).

The findings from this study suggest that the enhanced antiviral activity of GTE is probably related to the change in composition and, more specifically, in the amount of catechin derivatives, as a function of pH and storage time, as observed from the HPLC/MS results. Therefore GTE may be a powerful natural tool for decontamination of food contact surfaces, preventing the indirect transfer of enteric viruses to food or persons. Moreover, these results open other possibilities like wiping with cleaning cloths (Gibson, Crandall, & Ricke, 2012), applying other cleaning regimens or use other natural compounds with potential synergistic antiviral activity to improve the efficacy of GTE as natural disinfectant.

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Figure 1. Infectivity of murine norovirus (MNV) (log TCID₅₀/mL) after 2h at 25°C treatments with solutions of GTE freshly prepared (A) and after 24 h storage (B) at different pHs. Black: Untreated, grey: GTE 0.5 mg/mL, white: GTE 5 mg/mL.

Each column represents the average of triplicates. Within each column for each pH, different letters denote significant differences between treatments ($P < 0.05$).

Figure 2. Infectivity of hepatitis A virus (HAV) (log TCID₅₀/mL) after 2h at 25°C treatments with solutions of GTE freshly prepared (A) and after 24 h storage (B). Black: Untreated virus, grey: GTE 0.5 mg/mL, white: GTE 5 mg/mL.

Each column represents the average of triplicates. Within each column for each pH, different letters denote significant differences between treatments ($P < 0.05$).

527 **Figure 3.** Time- and pH-dependent changes in the concentration of kaempferol 3-O-xylosyl-glucoside, theasinensin
528 A and theasinensin B in buffered solutions of the green tea extract.

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Figure 4. Infectivity of murine norovirus (MNV) (A) and hepatitis A virus (HAV) (B) (log TCID₅₀/mL) after 0, 15, 60, 120 and 480 minutes treatment with GTE (5 mg/mL) at 25 °C.
 *Black lines: untreated virus; grey lines: GTE-treated virus.
 **Each point represents the average of triplicates. Different letters denote significant differences between treatments (P < 0.05).

Figure 5. Infectivity of murine norovirus (MNV) and hepatitis A virus (HAV) (log TCID₅₀/mL) before (black) and after 5 (grey) and 15 (white) minutes treatment with GTE (5mg/mL) on stainless steel (A) and glass (B) discs. Each column represents the average of triplicates. Within each column for each virus, different letters denote significant differences between treatments ($P < 0.05$).

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Table 1. Green tea derived phenolic compounds identified in the HPLC/MS spectra of GTE.

RT ^a	m/z	Tentative identification
1.6	761 [M-H] ⁻	Theasinensin B
2.4 / 4.8	305 [M-H] ⁻	Epigallocatechin / gallocatechin
2.6 / 5.3	289 [M-H] ⁻	Epicatechin / catechin
4.8 / 5.8	169 [M-H] ⁻	Gallic acid
4.9 / 5.9	913 [M-H] ⁻	Theasinensin A
4.8	457 [M-H] ⁻ ; 915 [2M-H] ⁻	Epigallocatechin gallate
5.3	579 [M-H] ⁻	Kaempferol 3-O-xylosyl-glucoside
5.8	457 [M-H] ⁻ ; 479 [M+Na-2H] ⁻ ; 915 [2M-H] ⁻	Gallocatechin gallate
6.2	471 [M-H] ⁻ ; 493 [M+Na-2H] ⁻	Methylated EGCG
6.6 / 7.0	441 [M-H] ⁻	Epicatechin gallate/ catechin gallate
6.6	883 [M-H] ⁻	P2
7.5	425 [M-H] ⁻	Epiafzechelin gallate
7.9 / 8.0	463 [M-H] ⁻	Quercetin galactoside
8.0	609 [M-H] ⁻	Theasinensin C / Prodelphinidin B4 / Quercetin rutinoside
8.5	317 [M-H] ⁻	Myricetin
8.5 / 8.7	447 [M-H] ⁻	Kaempferol hexose / quercetin rhamnose
8.7	593 [M-H] ⁻	Kaempferol rutinoside
9.0 / 9.5	301 [M-H] ⁻	Quercetin
9.8	563 [M-H] ⁻	Theaflavin
10.3	285 [M-H] ⁻	Kaempferol

RT^a = retention time (min)