Tea crude extracts effectively inactivate severe acute respiratory syndrome coronavirus 2

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Significance and Impact of the Study: Here, we show that black tea extract and tea bag infusion could reduce the viral titre of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in vitro by 5 logs TCID50 per ml within 10 s. Black and green teas are widely available and are relatively cheap to purchase in both developed and developing countries. In translational terms, drinking tea might contribute to the in vivo reduction in numbers of SARS-CoV-2 virus in saliva of infected persons, as well as lowering the viral burden in the mouth and upper gastrointestinal and respiratory tracts. A clinical trial is urgently needed to establish if drinking tea results in the prevention of transmission of SARS-CoV-2 virus between infected and non-infected persons.

Keywords
antiviral activity, black and green tea extracts, COVID-19, SARS-CoV-2, tea bag infusion (black tea), tea-derived polyphenol.

Abstract
It is well known that black and green tea extracts, particularly polyphenols, have antimicrobial activity against various pathogenic microbes including viruses. However, there is limited data on the antiviral activity against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which emerged rapidly in China in late 2019 and which has been responsible for coronavirus disease 2019 (COVID-19) pandemic globally. In this study, 20 compounds and three extracts were obtained from black and green tea and found that three tea extracts showed significant antiviral activity against SARS-CoV-2, whereby the viral titre decreased about 5 logs TCID50 per ml by 1-375 mg ml−1 black tea extract and two-fold diluted tea bag infusion obtained from black tea when incubated at 25°C for 10 s. However, when concentrations of black and green tea extracts were equally adjusted to 344 µg ml−1, green tea extracts showed more antiviral activity against SARS-CoV-2. This simple and highly respected beverage may be a cheap and widely acceptable means to reduce SARS-CoV-2 viral burden in the mouth and upper gastrointestinal and respiratory tracts in developed as well as developing countries.
Introduction

A novel coronavirus termed as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) suddenly emerged in China in late 2019 and has subsequently spread all over the world accounting for 190,671,330 infected persons and 4,098,758 deaths in the world, as of July 20 (World Health Organization 2021). Recently, new variant strains of this virus have emerged in the United Kingdom (α), Brazil (β), South Africa (γ) and India (δ), and have quickly spread to various parts of the world (Centers for Disease Control and Prevention 2021). This is a worrying development in the epidemiology of this disease globally, as these new variants appear to be more infectious and virulent.

Since late 2019, SARS-CoV-2 has emerged as the aetiologic agent of coronavirus disease 2019 (COVID-19) disease, where its pathogenic action, treatment pathways and suitable candidates for vaccination are now beginning to emerge. Several vaccines have been rapidly developed in several nations, which include innovative approaches to vaccination through mRNA approaches and introduced in the global community through mass immunization campaigns (Huang et al. 2021). As a result, vaccination has appeared to reduce the numbers of infected people, deaths, as well as attenuating disease severity and symptoms. However, it remains too early to compare the effectiveness of these vaccine candidates and also how each will respond to the variant beginning to emerge in several countries. Therefore, it is important to provide parallel platforms of interventions to vaccines that will allow concurrent antiviral approaches for preventing and controlling the COVID-19 pandemic, as there remain several questions regarding the immunogenicity and the effectiveness of vaccinated individuals mounting an immune response, as well as the rate of antibody waning.

Unlike influenza virus, SARS-CoV-2 is being shed not only from symptomatic patients but also during the asymptomatic period prior to the onset of illness (Zhao et al. 2020). Most of the infected persons are asymptomatic or are showing mild symptoms (Hu et al. 2021). However, morbidity and mortality increase significantly for those >60 years old and/or in those with a comorbidity such as hypertension and diabetes (Hu et al. 2021). Therefore, it is important to develop a simple, cheap, pragmatic and easily available interventions to complement the vaccination approach and further support measures to prevent the transmission of viral spread infected persons to non-infected person.

Tea is widely consumed all over the world. About 20% of the tea produced in the world is green tea, about 2% is oolong tea, and the rest is black tea (Kuroda and Hara 1999). All of these teas are made from the Theaceae tea plant called *Camellia sinensis*. Various types of tea can be made from the fresh leaves of this plant. First, the leaves are harvested at maturation withered and then fermented to various degrees, which produces different teas. Green tea, which is mainly consumed in Japan and China, contains polyphenols such as epigallocatechin gallate (EGCg), epigallocatechin, epicatechin gallate (ECg) and epicatechin. These molecules have antioxidant effects and are effective in alleviating diabetes, preventing dementia and reducing the risk of cerebral infarction (Yan et al. 2020). This tea type also exhibits antiviral activity against a wide range of human viruses including influenza, hepatitis B, herpes simplex, Zika virus, etc. (Ghosh et al. 2020). Black teas, popular all over the world, contain theaflavins, which have been reported to suppress cancer and inflammation through antioxidant activity (Peluso and Serafini 2017). Theaflavins also have antiviral effects against bovine coronavirus, bovine rotavirus, HIV-1, influenza virus and HSV-1 (De Oliveira et al. 2015).

Although vaccines have found clinical application in several countries globally, however, each country varies in its vaccine roleout ability, as well as its vaccine uptake rate, particularly as the vaccine is not available in many countries, as yet. Thus, alternative simple and cheap measures which are easily available for everyone to help mitigate the COVID-19 pandemic are urgently needed. Recently, it has been reported that catechin mixture reagent from green tea inactivated SARS-CoV-2 virus (Nishimura et al. 2021). However, it remains unclear if a particular compound or tea extract mixture can inactivate SARS-CoV-2. Thus, in this study, we attempted to evaluate if any of compounds present in the tea extract or tea extract mixture display antiviral activity against SARS-CoV-2.

Results and discussion

In this study, we focused on tea which is very popular among people all over the world, particularly in countries which have suffered high numbers of COVID-19 infections, along with high levels of related mortality, including India, Iran, USA and the United Kingdom.

Twenty types of tea-derived polyphenols, green and black tea extracts and tea bag infusion (black tea) were prepared to evaluate the antiviral activity against SARS-CoV-2. First, we examined the maximum concentrations at which these samples did not show any cytotoxicity to VeroE6/TMPRSS2 cells used for culture of SARS-CoV-2 and set the test concentrations as shown in Table S1. When concentrated virus suspended in phosphate-buffered saline (PBS) was incubated with these samples at 25°C for a few min, SARS-CoV-2 titre decreased in the order of tea bag infusion (black tea), black tea extract and green tea extract, as compared with the control (data...
not shown). Polyphenols tested in this study showed less antiviral activity against SARS-CoV-2 in comparison to tea extracts (data not shown). It should be noted that immediately after addition of the medium (Dulbecco’s modified eagle medium (DMEM) supplemented with 2% fetal bovine serum (FBS) and 1 mg ml⁻¹ G418) to each test substance, antiviral activity of any test substances was neutralized (data not shown). A number of studies showed that polyphenols have antiviral activity against various viruses including SARS-CoV-2 (Xu et al. 2017; Mhatre et al. 2021). However, here we show that crude tea extract rather than polyphenol itself has potent antiviral activity against SARS-CoV-2.

Therefore, we selected these three samples, namely green tea extract, black tea extract and tea bag infusion for further studies. When each sample was incubated with SARS-CoV-2 for 1, 5, 10 and 30 min, 344 µg ml⁻¹ green tea extract decreased SARS-CoV-2 titre more than 5 logs TCID₅₀ per ml in 30 min incubation (Fig. 1). When incubation time decreased, less antiviral activity was observed in a time-dependent manner (Fig. 1). In contrast, two-fold diluted tea bag infusion and 1375 µg ml⁻¹ black tea extract decreased SARS-CoV-2 titre more than 5 logs TCID₅₀ per ml even in 1 min incubation (Fig. 1). Therefore, we further examined if 2-fold diluted tea bag infusion and 1375 µg ml⁻¹ black tea extract showed antiviral activity within 1 min incubation. As shown in Fig. 1, a twofold diluted tea bag infusion and 1,375 µg ml⁻¹ black tea extract decreased SARS-CoV-2 titre about 5 logs TCID₅₀ per ml and more than 5 logs TCID₅₀ per ml after only 10-s incubation respectively. It should be noted that antiviral activity of tea extract was lost when 2% FBS as a final concentration, which is same as that of culture medium, was added into the mixture after 10-s incubation, indicating that SARS-CoV-2 can be inactivated by the tea bag infusion and black tea extract very quickly.

Next, we examined if lower concentrations of black tea extract could inactivate SARS-CoV-2. When the concentrations of black tea extract decreased to 687 µg ml⁻¹, 5 min was needed to decrease to the level of detection limit. Furthermore, the concentrations of black tea extract decreased to 344 µg ml⁻¹, which is same as that of green tea, the SARS-CoV-2 titre decreased only about 3 logs TCID₅₀ per ml, indicating that the green tea extract was more effective in inactivating SARS-CoV-2 in comparison to black tea extract when the same concentration was applied. Nishimura et al. (2021) also recently reported that green tea extract inactivated SARS-CoV-2. However, 10 mg ml⁻¹ catechin reagent reduced the viral titre by 4-2 logs and a 1-0 mg ml⁻¹ solution reduced the viral titre by only one log in following 5 min incubation. In our study, 344 µg ml⁻¹ green tea extract could reduce

![Figure 1](https://example.com/figure1.png)

**Figure 1** Time-dependent evaluation of antiviral activity against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by tea extracts. Viral titres were examined when each tea-derived extract was incubated with SARS-CoV-2 for each time (n = 3). PBS was used as a negative control. The detection limit was confirmed to be ≤1-8 logs TCID₅₀ per ml ([---] detection limit).
the viral titre by 4 log in the same incubation time (Fig. 1). Furthermore, 1,375 mg ml\(^{-1}\) black tea extract could reduce the viral titre by more than 5 logs TCID\(_{50}\) per ml after just 10-s incubation (Fig. 1). These data suggest that not all tea leaves or tea extracts contain the same compounds or catechins, which can show antiviral activity against SARS-CoV-2.

Upadhyay et al. (2020) reported that aqueous extracts of green tea and black tea inhibit 3C-like protease activity important for virus replication and propagation in SARS-CoV-2. In addition, docking simulations for 3C-like proteases and studies of inhibitory activity against 3C-like proteases of SARS-CoV-2 have shown the effectiveness of polyphenols, such as catechin gallate (Cg), ECg, and gallo-catechin gallate (GCg) (Zhu and Xie 2020). These polyphenols are also present in the green tea extract used in the current study (Table S2). Previously, 10–30 \(\mu\)mol l\(^{-1}\) of these polyphenols were required to inhibit 90% of 3C-like proteases activity required 10–30 \(\mu\)mol l\(^{-1}\) of these polyphenols. However, in our present study, about 740 \(\mu\)mol l\(^{-1}\) were required to reduce the viral titre of SARS-CoV-2 to the same level, which is 37 times higher than what is required to inhibit 3C-like proteases activity (data not shown). Although there is no direct evidence showing that tea extract or tea polyphenols affect surface proteins of SARS-CoV-2 or its viral particle, an in silico analysis indicated that tea polyphenolos such as EGCG and theaflavins can interact with SARS-CoV-2 spike protein and may inhibit SARS-CoV-2 infection in humans (Maiti and Banerjee 2021). In addition, it has been reported that EGCG had a destructive effect on the viral particles of HIV-1 (Yamaguchi et al. 2002). Taken together, these data suggest that spike proteins or viral particles of SARS-CoV-2 viruses might be affected by crude tea extracts by reducing the viral titre. Crude tea extract (i.e. mixtures of polyphenols) rather than polyphenols alone might effectively inactivate SARS-CoV-2. Nevertheless, inactivation of SARS-CoV-2 through synergistic effects, including inhibition of 3C-like protease and interaction with S-protein in SARS-CoV-2 should also be considered. Further studies are now required to understand the specific mechanisms on how SARS-CoV-2 was inactivated through its interaction with tea.

Tsuchiya et al. (1997) reported that catechins were retained in human saliva up to 60 min after rinsing with green tea extract. Lee et al. (2004) also reported that half-life of theaflavin in human saliva was 49–76 min after holding freshly brewed black tea in the mouth for 2–5 min. These reports suggest that even when the tea extract is consumed, a sufficient amount of catechins and theaflavins, which are major components of tea extracts, may remain active in saliva for the necessary time to inactivate SARS-CoV-2. The green tea extract, black tea extract and tea bag infusion (black tea) used in this study showed antiviral activity at lower concentrations than which are usually present in common consuming tea drink (green tea extract and black tea extract are usually prepared at 2 mg ml\(^{-1}\) for consumption). Although we were unable to test a higher concentration of green tea extract due to tea’s cytotoxic intrinsic effect on Vero cells, it is anticipated that a higher concentration of green tea extract can remain in mouth when we drink the green tea. These data suggest that drinking tea, such as black tea and green tea can be expected to decrease the SARS-CoV-2 titre in the mouth and this reduce in viral burden in the mouth may in turn reduce the risk of spreading viruses by droplet transmission from oral sources. Further studies are now needed to investigate if crude tea extracts can also reduce the viral titre of SARS-CoV-2 in the presence of saliva. Nonetheless, tea is the second most popular drink globally after water and given its popularity worldwide, this simple and highly respected beverage may be a cheap and widely acceptable means to reduce the risk of SARS-CoV-2 viral burden in the mouth and upper gastrointestinal and respiratory tracts in developed as well as developing countries.

Materials and methods

Materials

All polyphenols, green tea extract (Polyphenon\(^{\text{®}}\) G), black tea extract (Polyphenon\(^{\text{®}}\) PF) and Nittoh Daily Club\(^{\text{®}}\) tea bags were provided by Mitsui Norin Co., Ltd (Tokyo, Japan). Green tea extract and black tea extract were produced from fresh tea leaves, which had been processed according to good manufacturing processes and spray drying.

Sample preparation

All polyphenols were dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO) to a final concentration of 6-6 mg ml\(^{-1}\). The solution was then diluted with modified PBS (pH 6.0) to 330 \(\mu\)g ml\(^{-1}\). Green tea extract and black tea extract were dissolved in ultrapure water to a final concentration of 2.75 mg ml\(^{-1}\), and the solution was filtered through a 0.2 \(\mu\)m filter (Merck Millipore, Billerica, MA). For tea bag infusion (black tea), Nittoh Daily Club\(^{\text{®}}\) tea bag (2.2 g black tea leaves) was extracted into 140 ml of hot ultrapure water for 1.5 min and the solution was filtered through a 0.2 \(\mu\)m filter (Merck Millipore). All the samples were prepared by diluting with modified PBS to reach a concentration with no observed cytotoxicity. Modified PBS was used as the control.


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Cell culture

VeroE6/TMPRSS2 cells were used for the cultivation of SARS-CoV-2 and the cells were incubated at 37°C in a 5% CO₂ humidified incubator (ESPEC CORP., Osaka, Japan). VeroE6/TMPRSS2 cell line was purchased from the Japanese Collection of Research Bioresources (Osaka, Japan). Cells were cultured in DMEM, low glucose, pyruvate (Thermo Fisher Scientific Inc., Waltham, MA) supplemented with 5% (v/v) heat-inactivated FBS (Thermo Fisher Scientific Inc.) and 1 mg ml⁻¹ of G418, which is geneticin commonly used as a selective agent for eukaryotic cells (Nacalai Tesque, Inc., Kyoto, Japan).

Virus preparation

VeroE6/TMPRSS2 cells (n = 140,000) were cultured in a 25 cm²-cell culture flask at 37°C for 16 h in a 5% CO₂ humidified incubator. The cells were infected with MOI = 0.001 of SARS-CoV-2 JPN/TY/WK-521 strain and incubated at 37°C in DMEM supplemented with 2% (v/v) heat-inactivated FBS (Thermo Fisher Scientific Inc.) and 1 mg ml⁻¹ of G418 (Nacalai Tesque Inc.) for 48 h. After a cytopathic effect was observed, the spent culture medium was harvested and centrifuged at 1600 × g for 5 min, and the supernatant fraction containing virus particles was collected. Then, 1 g of polyethylene glycol 6000 and 233 mg of NaCl (Nacalai Tesque Inc.) were added to 10 ml of collected virus solution and incubated at 4°C for 16 h. After that, the virus solution was centrifuged at 20,000 g at 4°C for 10 min, the supernatant was discarded and the pellet was suspended in 1 ml of PBS at pH 7-4. Experiments with live SARS-CoV-2 virus were conducted at containment Level 3, in Osaka Prefecture University after obtaining the permission from the Biological Safety Committee of Osaka Prefecture University.

Antiviral activity evaluation

Twenty-five microlitres of concentrated SARS-CoV-2 in PBS was mixed with 475 μl of 344 μg ml⁻¹ of green tea extract, 1375, 687 or 344 μg ml⁻¹ of black tea extract or two times diluted black tea infusion. Then, the mixture was incubated at room temperature (25°C) for 10 or 30 s, and 1, 5, 10 or 30 min. After incubation, 55.5 μl of 10× DMEM (Nissui Pharmaceutical Co. Ltd, Tokyo, Japan), 11.1 μl of PBS and 11.1 μl of 50 mg ml⁻¹ G418 disulfate aqueous solution were added. Subsequently, 10-fold dilution was carried out with DMEM supplemented with 2% (v/v) FBS and 1 mg ml⁻¹ of G418 and titration was done as described below.

About 2.5 × 10⁴ cells/100 μl of VeroE6/TMPRSS2 cells were seeded in a 96-well plate and cultured at 37°C for 16 h in the medium. The culture medium was removed and 100 μl of 10-fold serially diluted viral solution in DMEM supplemented with 2% (v/v) FBS and 1 mg ml⁻¹ of G418 was added. The infected VeroE6/TMPRSS2 cells were cultured at 37°C for 72 h. Cells were fixed with methanol (Nacalai Tesque Inc.), and stained with 0.5% (w/v) crystal violet stain. Then, 50% tissue culture infective dose (TCID₅₀) was calculated by employing the Behrens-Kärber method (Kärber 1931). The detection limit was confirmed to be ≤1.8 logs TCID₅₀ per ml.

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Conflict of Interest

S. Otani, S. Maeda, and M. Suzuki are employees of Mitsubishi Norin Co., Ltd. All other authors declare no competing interests.

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**Supporting Information**

Additional Supporting Information may be found in the online version of this article:

Table S1. Concentrations of tea-derived polyphenols and extracts used in this study.

Table S2. Major polyphenols content in each prepared tea extract.