Universally available herbal teas based on sage and perilla elicit potent antiviral activity against SARS-CoV-2 *in vitro*

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Abstract

The current SARS-CoV-2/COVID-19 pandemic represents an unprecedented medical and socioeconomic crisis. Highly efficient treatment options preventing morbidity and mortality are not broadly available and approved drugs are hardly affordable in developing countries. Even after vaccine approvals, it will take several months until the vaccinated and convalescent individuals establish herd immunity. Meanwhile, non-pharmaceutical interventions and antiviral treatments are indispensable to curb the death toll of the pandemic. To identify cost-effective and ubiquitously available options, we tested common herbs consumed worldwide as herbal teas. We found that aqueous infusions prepared by boiling leaves of the *Lamiaceae* plants perilla and sage elicit potent antiviral activity against SARS-CoV-2 in human cells. Sustained antiviral activity was evident even when cells were treated for only half an hour, and in therapeutic as well as prophylactic regimens. Given the urgency, such inexpensive and broadly available substances might provide help during the pandemic - especially in low-income regions.

Keywords:

COVID-19; SARS-CoV-2; Antiviral; Treatment; Herb; Perilla; Sage; Lamiaceae

Introduction

Fossil records suggest that humans may have applied plants as medicine at least from the Middle Palaeolithic age some 60,000 years ago [1,2]. Across cultures and spanning thousands of years, humans consumed aqueous plant infusions as teas. The first textual reference to tea consumption dates back to 59 years before the current era (BCE) and physical evidence even dates back to 255±80 years BCE [3]. In addition to reasons of enjoyment and taste, teas are frequently applied for disease prophylaxis, therapy, or symptom allevation. A major distinction is genuine teas based on *Camellia sinensis* infusions versus various types of herbal teas. For the latter, parts of other plants are boiled in water generating complex aqueous solutions. Especially members of the *Lamiaceae* family comprising plants such as sage (*Salvia officinalis)* and perilla (*Perilla frutescens)* are ubiquitously used to prepare herbal teas. Additionally, important aromatic spices such as basil, mint, rosemary, marjoram, oregano, thyme, and lavender also belong to the family of *Lamiaceae*. Across the world, the edible plant perilla and its variations have a variety of names such as *Tía tô* (Vietnam), *rattlesnake weed* (US), *silam* (India and Nepal), Korean perilla, *shiso* and *egoma* (Japan), *deulkkae* (Korea), *zǐsū* and *sūzǐ* (China). In parts of Japan, it is referred to as *jūnen* ('ten years') since it is believed to be able to add ten years to a person's lifespan. In addition to the consumption in herbal teas, certain perilla varieties are used either as spices or in traditional medicine in Southeast Asia. Members of the *Lamiaceae* family are well described for their medicinal effects against various diseases including pneumonia and cough [4]. While perilla is very popular in Asia, the related plant sage is more common in Europe and America. The name *Salvia officinalis* already indicates its medicinal potential since the *officinal* was a room in a monastery dedicated to herbs and medicine. Sage was also included in the so-called *four thieves vinegar* which was used in medieval times in attempts to protect users from the plaque. Intriguingly, perilla and sage extracts indeed possess antimicrobial activities (see, e.g. [5,6]).

In the era of modern medicine, some people have reservations concerning the use of traditional and herbal medicines. However, a highly relevant fraction of recently approved modern therapeutics directly or indirectly originate from natural products [7] - some of which known to and applied by our ancestors. In this respect, the antimalarial lactone Artemisinin derived from the sweet wormwood (*Artemisia annua*) is amongst the best-known examples [8,9].

Humans have been exposed to coronaviruses (CoV) for ages, given their broad prevalence in mammals (e.g. bats) and birds. At least seven CoVs are capable of autochthonous propagation

in the human population: human CoV (HCoV)-HKU1, HCoV-NL63, HCoV-229E, HCoV-OC43, HCoV-SARS1, HCoV-MERS, and the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The latter causes the current global pandemic of coronavirus disease 2019 (COVID-19). SARS-CoV-2 was first identified in Wuhan, China [10]. In various aspects, it shows similarities to SARS-CoV-1, however, it also exhibits certain specialties [11,12] such as the capacity of very efficient replication in the upper respiratory tract and the corresponding efficacy of human-to-human transmission. Given the broad coverage of this topic, we refer the reader to review articles concerning SARS-CoV-2 and COVID-19 (e.g., [13,14]). At the time of writing, more than 55.7 million individuals experienced laboratory-confirmed SARS-CoV-2 infections and over 1.34 million people succumbed in the context of COVID-19. According to the Johns Hopkins dashboard [15], 191 countries and regions are affected by SARS-CoV-2 cases - several of which are developing nations with very limited resources for the medical sector, especially when faced with overwhelming numbers of infected individuals [16].

A variety of SARS-CoV-like viruses can be found in bats, indicating an alarming reservoir of coronaviruses that could cause zoonotic animal-to-human spillover transmissions [17-19]. Based on the facts that CoVs are present in animals such as rodents, bats, and cats residing in utmost proximity of human settlements and civilization and seem to have caused human epidemics in the past [20], we speculated that human culture might provide certain behavioural adaptations to coronavirus infections. Such knowledge may be applicable to alleviate some of the hardship and suffering caused by SARS-CoV-2 in a process of 'cultural repurposing'. People with respiratory diseases often consume herbal products and teas in attempts of self-medication. Faced with the COVID-19 pandemic, people reported that they have changed their behaviour accordingly. In two studies comprising thousands of people, up to 57.6% of individuals reported having used nutritional supplements or herbal products, usually as teas, in attempts to protect themselves from COVID-19 [21,22]. Therefore, we wondered how effective herbal teas actually are against SARS-CoV-2.

Results

Perilla and sage contain water-soluble heat-stable components active against SARS-CoV-2 replication

Since efficient treatment options for COVID-19 patients are still not sufficiently available, we aimed to determine the therapeutic potential of herbal teas. To evaluate two universally available *Lamiaceae* plants commonly used in traditional medicine, perilla and sage, in terms

of their ability to elicit antiviral activity against SARS-CoV-2, we applied an experimental setup that reflects short-term incubation of infected cells with herbal teas. We infected highly SARS-CoV-2-permissive Vero E6 cells for 1 h before the virus suspension was removed and different dilutions of aqueous infusions of perilla and sage were added (Fig. 1a). As control, we included coriander, a herb that does not belong to the family of *Lamiaceae* and that is to our knowledge not commonly used as medicinal herb. We applied aqueous infusions that were prepared by boiling up the coriander, perilla, and sage leaves and subsequent simmering at 60°C for 2 h to ensure complete extraction of the water-soluble components of the herbs. Intriguingly, the short-term treatment with perilla and sage infusions was sufficient to significantly inhibit the replication of SARS-CoV-2 (Fig. 1b, upper panel; for details concerning the calculation of the infectivity see Methods section and Supplementary Fig. S1). This effect does not appear to constitute a general antiviral activity, since inhibition of HSV-1 replication in treated Vero E6 cells was not observed (Fig. 1b, lower panel). To visualize the impact of the herbal teas on the SARS-CoV-2 replication, we repeated the experiment with two different doses of virus and stained the infected cells for immunofluorescence microscopy. As depicted in Fig. 1c, the number of infected cells (stained in green) was clearly diminished after treatment with perilla and sage infusions. The antiviral activity was still evident even when a high amount of virus was used for infection (0.5 PFU/cell). In addition, cell viability was determined to exclude cytotoxicity as reason for diminished viral replication (Supplementary Fig. S2). When we evaluated different members of the family of *Perilla frutescens* (red perilla, green perilla, and bi-color perilla), we observed antiviral activity in all three cases (Fig. 1d). To confirm, that the experimental setup allows reporting of antiviral activity affecting post-entry steps, we included a SARS-CoV-2 convalescent serum sample with shown neutralizing capacity [23] in our analysis. We observed that the perilla and sage infusions outperformed the effect of the convalescent serum (Fig. 1e; NAbs, neutralizing antibodies) under these experimental conditions, showing that the herbs perilla and sage contain components active against SARS-CoV-2 replication by interfering with a post-entry step. Since the components were extracted by boiling the herbs in water, we concluded that the antiviral activity is elicited by water-soluble heat-stable compound(s).

Preserved sage and perilla leaves retain bioactive compounds

When we used a second clinical SARS-CoV-2 isolate to test the susceptibility towards the herbal components, we observed almost identical dose responses (Supplementary Fig. S3). The combined results of 8 independent experiments (using two distinct SARS-CoV-2 isolates for the infection of Vero E6 and α-S or α-N antibodies for staining) revealed highly

significant antiviral activity of all tested dilutions of the perilla infusion as well as of the 1/10 and 1/20 dilutions of the sage infusion (Fig. 2a). Considering that the herbal infusions were prepared from 150 g of fresh herbal material per liter, which correspond to 15-30 g of dried herbal material per liter (assuming a water content of 80-90% of fresh herbs), a 1/10 dilution of these infusions already meets the concentration of herbal teas prepared from standard tea bags (1.5-2.25 g per cup). This indicates that herbal teas prepared from commercially available tea bags or dried herb leaves might contain a sufficient concentration of the antiviral compound(s). To further adapt the experimental setup to realistic conditions of tea consumption, we shortened the treatment time from 1 h to 30 min. Even under this condition, significant reduction of viral replication was observed (Fig. 2b). Next, we compared the antiviral activity of infusions prepared from fresh or dried herb leaves. As shown in Fig. 2c, dried sage leaves retained most of the antiviral component(s) whereas dried perilla was less effective as compared to fresh perilla leaves although significant inhibition of viral replication was observed for the 1/10 dilutions. Since the herbal infusions were prepared by boiling, simmering, and over-night incubation (see Methods section), we also tested if the standard procedure of herbal tea preparation using dried sage leaves is sufficient to extract the antiviral component(s). To this end, dried sage leaves were boiled up in water and incubated for 10 min before the herb leaves were removed. When this '10 min infusion' of dried sage was compared to the 'over-night infusion', very similar dose response curves were observed (Fig. 2d). To assess whether conservation of perilla by freezing could be superior to drying in terms of preserving the antiviral component(s), we first tested if the antiviral activity of perilla infusions is reduced by freeze-thaw cycles. Since this did not seem to be the case (Fig. 2e), infusions prepared from fresh and frozen perilla leaves were compared. The comparison revealed that the preservation of the herbs by freezing was preferable to drying (Fig. 2f).

Perilla and sage elicit prophylactic antiviral activity *in vitro*

Having observed the potent antiviral activity of perilla and sage after only 1 h of treatment, we wondered whether the herbs might also elicit prophylactic effects. Therefore, Vero E6 cells were treated 1 h prior to infection with different dilutions of the herbal infusions before the supernatant including the herbal components was removed. Subsequently, SARS-CoV-2 infection was performed and the virus suspension was replaced by fresh medium at 1 h p.i. (Fig. 3a). By removing the herbal infusions before infection, we aimed to primarily assess antiviral effects based on cellular responses and not on direct virucidal elimination of infectious virus particles. The analysis of combined results of 6 independent experiments using two distinct SARS-CoV-2 isolates and α-S or α-N antibodies for staining showed highly

significant decrease of infectivity, especially upon pre-treatment with the perilla infusion (Fig. 3b). To compare the extent of prophylactic and therapeutic antiviral capacity, we conducted an experiment in which we treated and pre-treated the infected cells in parallel. As already indicated by the results of the independent experiments (Fig. 2a and 3b), therapeutic treatment elicits stronger antiviral activity for both perilla and sage (Fig. 3 c, d). Nonetheless, perilla and sage teas might not only be suitable for treatment of SARS-CoV-2 infections but also for prevention of infections.

Perilla and sage confer protection against SARS-CoV-2 infection in human cells

Since cells differ concerning the mode of entry of SARS-CoV-2 [24], a second independent cell line was tested. Caco-2 cells were used to analyse the antiviral activity elicited by perilla and sage infusions in human cells. We have observed that SARS-CoV-2 replication is more protracted in Caco-2 cells compared to Vero E6 cells [23]. Therefore, the experimental setup was adapted by increasing the time of treatment as well as the time of infection before analysis (Supplementary Fig. S4a). We observed a strong decrease in infectivity in perilla- and sage-treated Caco-2 cells (Supplementary Fig. S4b). Encouraged by this result, we applied the same treatment regimen of 1 h as was used for Vero E6 cells (Fig. 4a). This early short-term treatment was sufficient to potently inhibit SARS-CoV-2 replication, even in cells with a protracted viral replication cycle (Fig. 4b). To visualize the antiviral activity, Caco-2 cells were infected with and without treatment (as depicted in Fig. 4a) and were fixed for fluorescence microscopic analysis. This analysis showed clearly visible differences in the number of Spike-positive cells (Fig. 4c) validating the data obtained by in-cell-ELISA (icELISA).

Taken together, the *Lamiaceae* plants perilla and sage contain water-soluble heat-stable components eliciting potent therapeutic as well as prophylactic antiviral activity against SARS-CoV-2 in different cell lines.

Discussion

Using different assays such as visualization by fluorescence microscopy and measurements by icELISA, which provides objective quantification of either the SARS-CoV-2 N or S protein, we observed significant, potent, and dose-dependent antiviral activity of herbal teas produced by boiling leaves of the *Lamiaceae* plants perilla and sage. The effect was evident in different cellular models including highly permissive Vero E6 cells and human Caco-2 cells. In all assays, unspecific and general effects were excluded by a suitable negative control (coriander

infusion). As expected from the fact that these plants are edible, the treatment did not exert cytotoxicity. Although the FDA generally recognizes sage as safe [25], herbs and sage in particular should not be consumed by pregnant and breastfeeding women without consultation of their health-care provider [26] due to the potential to cause toxicity to the foetus or to induce premature labour. Sage is also discussed to reduce the milk supply when consumed during lactation. Additionally, sage and perilla can cause allergic reactions [27].

A typical cup of tea corresponds to ca. 250 ml of volume and commercial tea bags usually contain 1.5-2.25 g of plant material. Our initial extracts were produced using 15 g of herb leaves boiled in 100 ml water. In human cells, perilla and sage teas elicited significant antiviral effects at dilutions of 1:40 (3.75 g per litre or ca. 0.94 g per cup) and 1:30 (5 g per litre or ca. 1.25 g per cup) (Fig. 2a), indicating efficacy at concentrations usually consumed in such herbal teas. Intriguingly, as little as 30 min of treatment, after which the tea was removed, were sufficient to significantly diminish the SARS-CoV-2 replication (Fig. 2b). Through a series of experiments, we further showed that different perilla plant variations and all usual forms of preparation and preservation of perilla and sage such as teas prepared from fresh leaves, dried leaves as well as frozen and thawed leaves elicit significant inhibition of SARS-CoV-2 replication (Fig. 2).

Given that Vero cells are incapable to express type I interferons due to genetic aberrations [28], the antiviral activity observed in Vero E6 cells indicate that perilla and sage extracts elicit their effect independent of interferon induction. SARS-CoV-2 exploits different host-derived proteases. In TMPRSS2-negative cells such as Vero, the SARS-CoV-2 infection occurs mainly through an endosomal route facilitated by S cleavage by Cathepsins [24]. Accordingly, SARS-CoV-2 infection of these cells can be significantly inhibited by blocking the acidification of endosomes (e.g., by ammonium chloride) or Cathepsin inhibitors such as E-64d. Conversely, in TMPRSS2-positive cells such as Caco-2, the effect of E-64d is less pronounced while the priming of S is supported by TMPRSS2 as evident by Camostat mesylate treatment studies [24,29]. Our finding that *Lamiaceae* infusions exhibit antiviral activity in both cell types (Fig. 1 and 4) suggests that S cleavage and the endosomal entry route are at least not the only target of the antiviral mechanism. This conclusion is further supported by our observation that sage and perilla herbal teas diminished viral replication, especially when they were applied after infection (Fig. 1, 2, and 4) at a time at which neutralizing antibodies present in convalescent plasma (CP) had lost most of their antiviral activity. In such treatment regimens, the herbal teas significantly outperformed NAb-containing CP (Fig. 1e).

Plants of the *Lamiaceae* family have previously been shown to possess antiviral activity against the retrovirus HIV [30-32]. Similar to our findings with SARS-CoV-2, the anti-HIV activity seems to occur at a post-entry step [33]. It will be very interesting to identify the compounds responsible for the antiviral activity of aqueous perilla and sage infusions and to elucidate whether the antiviral activity against retroviruses and coronaviruses is mediated by the same or similar molecules.

The WHO SOLIDARITY trial comprising more than 400 hospitals and 11,000 patients in 30 countries showed that '*Remdesivir, Hydroxychloroquine, Lopinavir and Interferon regimens appeared to have little or no effect on hospitalized COVID-19, as indicated by overall mortality, initiation of ventilation and duration of hospital stay*' [34]. To a certain extent, this may be attributed to the practice of reserving such drugs for critically ill patients where they may be applied too late in terms of disease progression. Others and we hope that studies in which single therapies are administered earlier will be more successful. A widely discussed potential solution to the limitations of single therapies is obviously to combine substances that on their own have only limited effects. Such 'shotgun' treatment regimens have already shown some success [35]. Since herbal teas are ubiquitously available, almost free of charge, and exhibit excellent safety profiles given their consumption as spices, we propose to add perilla and sage infusions to such combinatorial treatment regimens. Additionally, these *Lamiaceae* teas may be applicable as an addition to the very important non-pharmaceutical interventions (NPI) such as wearing a face cover, proper hygiene, physical distance, and the restriction of social interactions.

Obviously, the consumption of herbal teas cannot and should not replace NPIs or clinically approved drugs. However, given their inexpensive and universal availability, they might contribute to prevent and/or relieve some of the hardness and suffering of the COVID-19 pandemic. We are convinced that our data argue in favour of future clinical studies addressing the question of whether herbal teas based on perilla and/or sage may either be able to prophylactically reduce infections or offer therapeutic benefits when administered concomitantly with the standard treatment - or both.

Methods

Materials and correspondence

Further information and requests for resources and reagents should be directed to and will be fulfilled by Mirko Trilling (Mirko.Trilling@uk-essen.de).

Cells, viruses, and infection

Vero E6 (ATCC CRL-1586) and Caco-2 (ATCC HTB-37) were cultivated in high glucose Dulbecco`s minimal essential medium (DMEM [Gibco 41966-029]) and Roswell Park Memorial Institute 1640 (RPMI-1640 [Gibco 21875-034]), respectively, supplemented with 10% (v/v) FCS, penicillin, and streptomycin at 37°C in an atmosphere of 5% CO_2. The SARS-CoV-2 strains were isolated from patient samples using Vero E6 and confirmed by SARS-CoV-2 diagnostic qRT-PCR. Viral titers were determined by TCID50 titration. The virus isolation has been approved by the ethics committee of the medical faculty of the University of Duisburg-Essen (20-9511-BO and 20-9512-BO). HSV-1-ΔgE-GFP was generated and described [36] by the laboratory of Prof. David C Johnson (Oregon Health & Science University, USA). With Prof. Johnson's written permission, we received the virus from Prof. Hartmut Hengel (University of Freiburg, Germany).

Generation of aqueous infusions of herbs

The herbal infusions were prepared by boiling up 15 g of fresh herbal leaves in 100 ml water and subsequent simmering at 60°C for 2 h. The infusions were stored over-night at 4°C before the leaves were removed and the aqueous solutions were sterile-filtered (200 µM filter, Whatman/GE Healthcare). Afterwards, the herbal infusions were stored in aliquots at -80°C. For the infusions based on dried herbs, 3 g of material per 100 ml were utilized. The '10 min infusion' was prepared by boiling up dried sage leaves in water (30 g per liter) and subsequent incubation for 10 min before the herb was removed. Sterile-filtered aliquots were then stored at -80°C. Infusions of frozen and rethawed herbal material were prepared in the same manner as those based on fresh herbs. The concentration of 150 g of material per liter was calculated based on the fresh weight before freezing. The sources of supply for the herbal leaves and plants are as follows: coriander and sage, farmer´s market (Essen, Germany); red and green perilla plants, online vendor Naturkraeutergarten (Kleinich, Germany); bi-color perilla plant, home-grown; dried red perilla, home-dried; dried green perilla, Keiko Shiso Finest Selection (Japan); dried sage, vom-Achterhof Bio-Salbei (Uplengen, Germany).

in-cell-ELISA (icELISA)

For the quantification of viral protein amounts in infected cells, an icELISA was applied. A detailed icELISA protocol is provided in [23]. Briefly, cells were infected with SARS-CoV-2 and fixed after 20 or 30 h of infection with 4% (w/v) paraformaldehyde/PBS. Cells were permeabilized with 1% (v/v) Triton-X-100/PBS and blocked with 3% (v/v) FCS/PBS. The primary antibody was added and incubated for 2 h at room temperature or over-night at 4°C.

Peroxidase-labelled secondary antibody was incubated for 1-2 h. Washing steps were performed with 0.05% (v/v) Tween-20/PBS. Tetramethylbenzidin (TMB) substrate was added to visualize the enzyme reaction. The reaction was stopped with 0.5 M HCl before the absorbance was determined using a microplate multireader and MicroWin software (Mithras2 LB 943; Berthold Technologies). The resulting data were analysed using Excel and GraphPad Prism software. The α-S mAb (kindly provided by Peter Miethe, fzmb, Bad Langensalza, Germany), α-N mAb (ABIN6952435), and POD-coupled secondary antibodies (Dianova) were used.

Dose-response curves of antiviral activity

To enable comparison among different icELISA measurements and experiments, we included on every plate a virus calibration curve. Residual infectivity after treatment was calculated using the formula computed from the calibration curve (see Supplementary Fig. S1 as an example). Dose-response curves were compiled based on the relative change in infectivity compared to the untreated control.

Immunofluorescence microscopy

Cells were infected with SARS-CoV-2 and fixed after 20 or 30 h of infection using 4% (w/v) paraformaldehyde/PBS for >2 h before they were discharged from the BSL-3 laboratory. Cells were permeabilized with 1% (v/v) Triton-X-100/PBS and blocked with 3% (v/v) FCS/PBS. SARS-CoV-2 infection was visualized by use of α-S mAb (kindly provided by Peter Miethe, fzmb, Bad Langensalza, Germany) and Cy2-conjugated goat anti-mouse IgG (Dianova). Nuclei were counterstained with 4'6-diamidino-2-phenylindole (DAPI; Sigma). Fluorescence was visualized using a THUNDER Imager 3D Cell Culture (Leica). Image analysis and processing were performed with LAS X Premium imaging software (Leica).

Statistical analysis

Statistical significance was determined using one-way ANOVA as described in the figure legends. A p value of <0.05 was considered statistically significant. *, p value <0.05. **, p value <0.01. ***, p value <0.001.

Data availability statement

All data generated or analysed during this study are included in this published article and its supplementary information files.

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Author contributions

VTKLT conceived the project, designed and performed experiments, analysed data, prepared figures, and wrote the manuscript. DM, CS, YFM, BK performed experiments. UD designed experiments. MT conceived the project, designed and performed experiments, analysed data, and wrote the manuscript. All authors reviewed and edited the manuscript.

Declaration of interest

The authors declare no competing interests.

Figure legends

Fig. 1: **Perilla and sage contain water-soluble heat-stable components active against SARS-CoV-2 *in vitro* replication.**

a Scheme of the experimental setup for the *in vitro* analysis of therapeutic effects against SARS-CoV-2.

b Representative dose-response curves of SARS-CoV-2-infected Vero E6 cells (2000 PFU per well) after treatment with aqueous infusions of coriander, perilla, or sage. Upper panel shows the effect on SARS-CoV-2 replication. Lower panel depicts the effect on HSV-1 replication. SARS-CoV-2 replication was analysed at 20 h p.i. by icELISA, HSV-1:GFP replication was determined at 48 h p.i. by quantification of fluorescence. Data are expressed as relative change in infectivity compared to the untreated control. Each condition was analysed in triplicate. See Methods section and Supplementary Fig. S1 for details. The comparison of the herb-treated samples of SARS-CoV-2 to the untreated controls by one-way ANOVA showed for all dilutions of coriander no significance and for all dilutions of perilla and sage significance. The perilla- and sage-treated conditions of SARS-CoV-2 were also compared to the corresponding coriander-treated condition (same dilution) and these results are depicted in the diagram. *, $p<0.05$. **, $p<0.01$. ***, $p<0.001$. The comparison of the herb-treated samples of HSV-1 to the untreated controls showed for all dilutions of all tested herbs no significance.

c, d Visualization of SARS-CoV-2 infection upon treatment with herbal infusions. Vero E6 cells were infected (MOI 0.1) and treated (1/10 dilution) as shown in **a**. α-S mAb and a Cy2-coupled secondary antibody were used for immunofluorescence staining (shown in green). Nuclei were counterstained with DAPI (shown in blue).

e Representative dose-response curves of SARS-CoV-2-infected Vero E6 cells (2000 PFU per well) after treatment (as shown in **a**) with aqueous infusions of coriander, perilla, sage, or SARS-CoV-2 convalescent serum (serum 6 from [23] with mid-high 50% neutralization titer of 256). Each condition was analysed in triplicate. The perilla- and sage-treated conditions were compared to the corresponding NAbs-treated condition (same dilution) by one-way ANOVA. **, $p<0.01$. ***, $p<0.001$.

Fig. 2: **Preserved sage and perilla leaves retain bioactive compounds.**

a Pooled icELISA data of 8 independent experiments of SARS-CoV-2-infected Vero E6 cells after treatment with herbal infusions using two distinct SARS-CoV-2 isolates for infection of

Vero E6 and α-S or α-N mAbs for staining. Data are expressed as relative change in optical density compared to the untreated control. The perilla- and sage-treated conditions were compared to the corresponding coriander-treated condition (same dilution) by one-way ANOVA. ***, $p<0.001$.

b Representative dose-response curves of SARS-CoV-2-infected Vero E6 cells (2000 PFU per well) after treatment with herbal infusions. Upper panel depicts the results for 1 h of treatment, lower panel the results for 30 min of treatment. Data are expressed as relative change in infectivity compared to the untreated control. Each condition was analysed in triplicate. The perilla- and sage-treated conditions were compared to the corresponding coriander-treated condition (same dilution) by one-way ANOVA. **, $p<0.01$. ***, $p<0.001$.

c Representative dose-response curves of SARS-CoV-2-infected Vero E6 cells after treatment with aqueous infusions of fresh or dried red perilla, green perilla, or sage for 1 h. Each condition was analysed in triplicate. All conditions were compared to the untreated controls by one-way ANOVA. *, $p<0.05$. **, $p<0.01$. ***, $p<0.001$.

d Representative dose-response curves of SARS-CoV-2-infected Vero E6 cells after treatment with herbal infusions generated by over-night or 10 min extraction of dried sage (see Methods section for details). Each condition was analysed in triplicate. All conditions were compared to the untreated controls by one-way ANOVA. ***, $p<0.001$.

e Representative dose-response curves of SARS-CoV-2-infected Vero E6 cells after treatment with aliquots of herbal infusions which were thawed once or twice. Each condition was analysed in triplicate. All conditions were compared to the untreated controls by one-way ANOVA. *, $p<0.05$. ***, $p<0.001$.

f Representative dose-response curves of SARS-CoV-2-infected Vero E6 cells after treatment with aqueous infusions of fresh or frozen red or green perilla. Each condition was analysed in triplicate. All conditions were compared to the untreated controls by one-way ANOVA. ***, $p<0.001$.

Fig. 3: **Perilla and sage elicit prophylactic antiviral activity *in vitro*.**

a Scheme of the experimental setup for the *in vitro* analysis of prophylactic effects against SARS-CoV-2.

b Pooled icELISA data of 6 independent experiments of SARS-CoV-2-infected Vero E6 cells after prophylactic treatment with herbal infusions using two distinct SARS-CoV-2 isolates for infection of Vero E6 and α-S or α-N mAbs for staining. Data are expressed as relative change in optical density compared to the untreated control. The perilla- and sage-treated conditions

were compared to the corresponding coriander-treated condition (same dilution) by one-way ANOVA. *, $p<0.05$. **, $p<0.01$. ***, $p<0.001$.

c, d Comparison of prophylactic and therapeutic treatment (-1 to 0 h p.i. versus 1 to 2 h p.i.) of SARS-CoV-2-infected Vero E6 cells (2000 PFU per well). Each condition was analysed in triplicate. All conditions were compared to the untreated controls by one-way ANOVA. **, $p<0.01$. ***, $p<0.001$.

Fig. 4: **Perilla and sage confer protection against SARS-CoV-2 infection in human cells.**

a Scheme of the experimental setup for the *in vitro* analysis of antiviral activity against SARS-CoV-2 in human Caco-2 cells.

b Pooled icELISA data of 4 independent experiments using two distinct SARS-CoV-2 isolates for infection of Caco-2 cells and α-S or α-N mAbs for staining. Data are expressed as relative change in optical density compared to the untreated control. The perilla- and sage-treated conditions were compared to the corresponding coriander-treated condition (same dilution) by one-way ANOVA. *, $p<0.05$. **, $p<0.01$. ***, $p<0.001$.

c Visualization of SARS-CoV-2 infection upon treatment with herbal infusions. Human Caco-2 cells were infected and treated as shown in **a**. α-S mAb and a Cy2-coupled secondary antibody were used for immunofluorescence staining (shown in green). Nuclei were counterstained with DAPI (shown in blue).

Supplementary information

Fig. S1: **Representative example of the virus calibration curves (included on every plate) applied to calculate the residual infectivity after treatment.**

a icELISA data of SARS-CoV-2-infected Vero E6 cells after treatment with herbal infusions using α-S for staining. Each condition was analysed in triplicate.

b icELISA data of the virus calibration curve. Each condition was analysed in duplicate.

c The formula of the calibration curve of the same plate was applied to calculate the residual PFU after treatment.

Fig. S2: **The one-hour treatment with the herbal infusions is not cytotoxic.**

Vero E6 cells were treated for 1 h with indicated dilutions of herbal infusions in parallel to an infection experiment. At 18 h post treatment, cell viability was analysed by Orangu cell counting solution (Cell Guidance Systems).

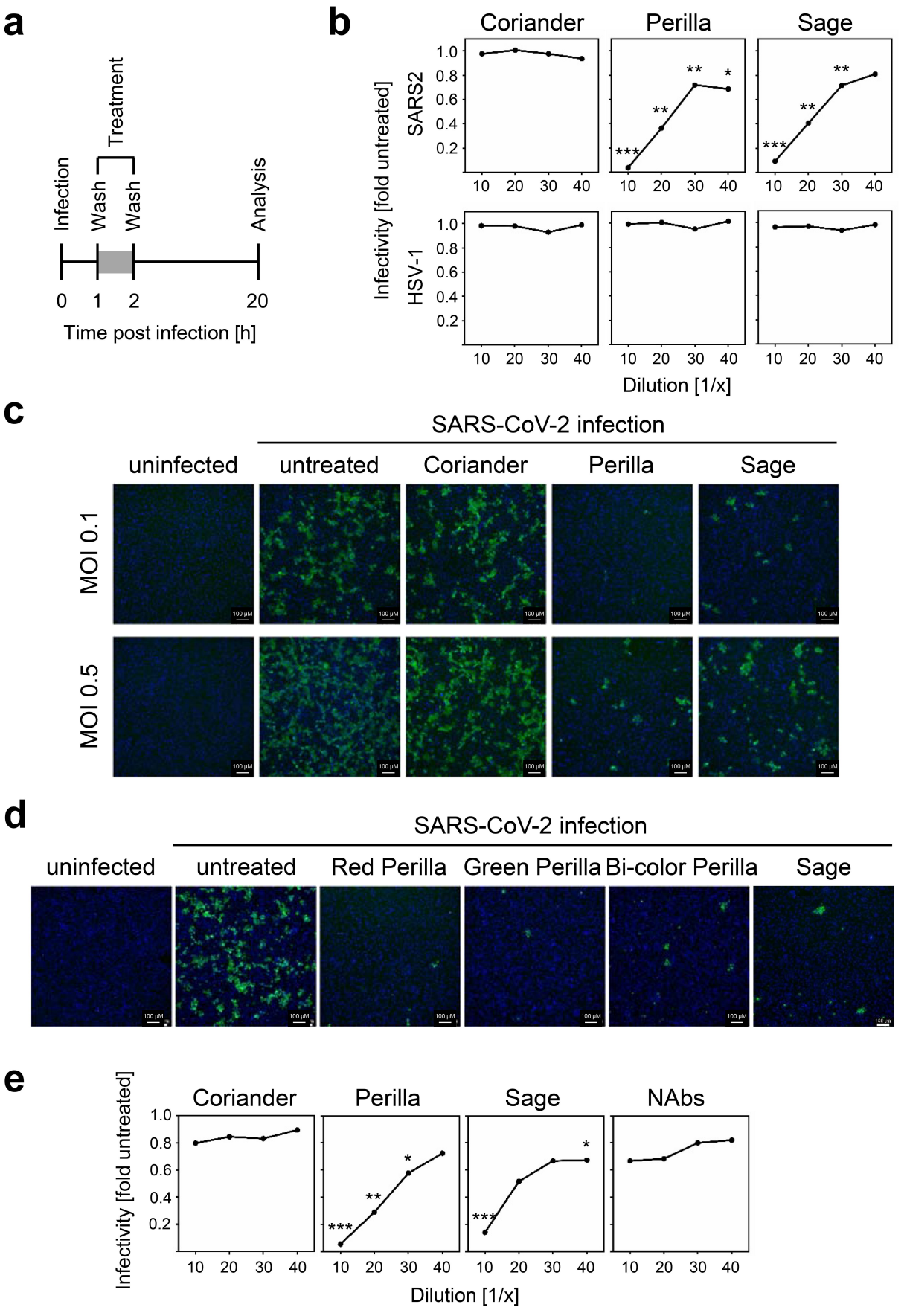
Fig. S3: **Two different clinical SARS-CoV-2 isolates exhibit almost identical susceptibilities towards perilla and sage.**

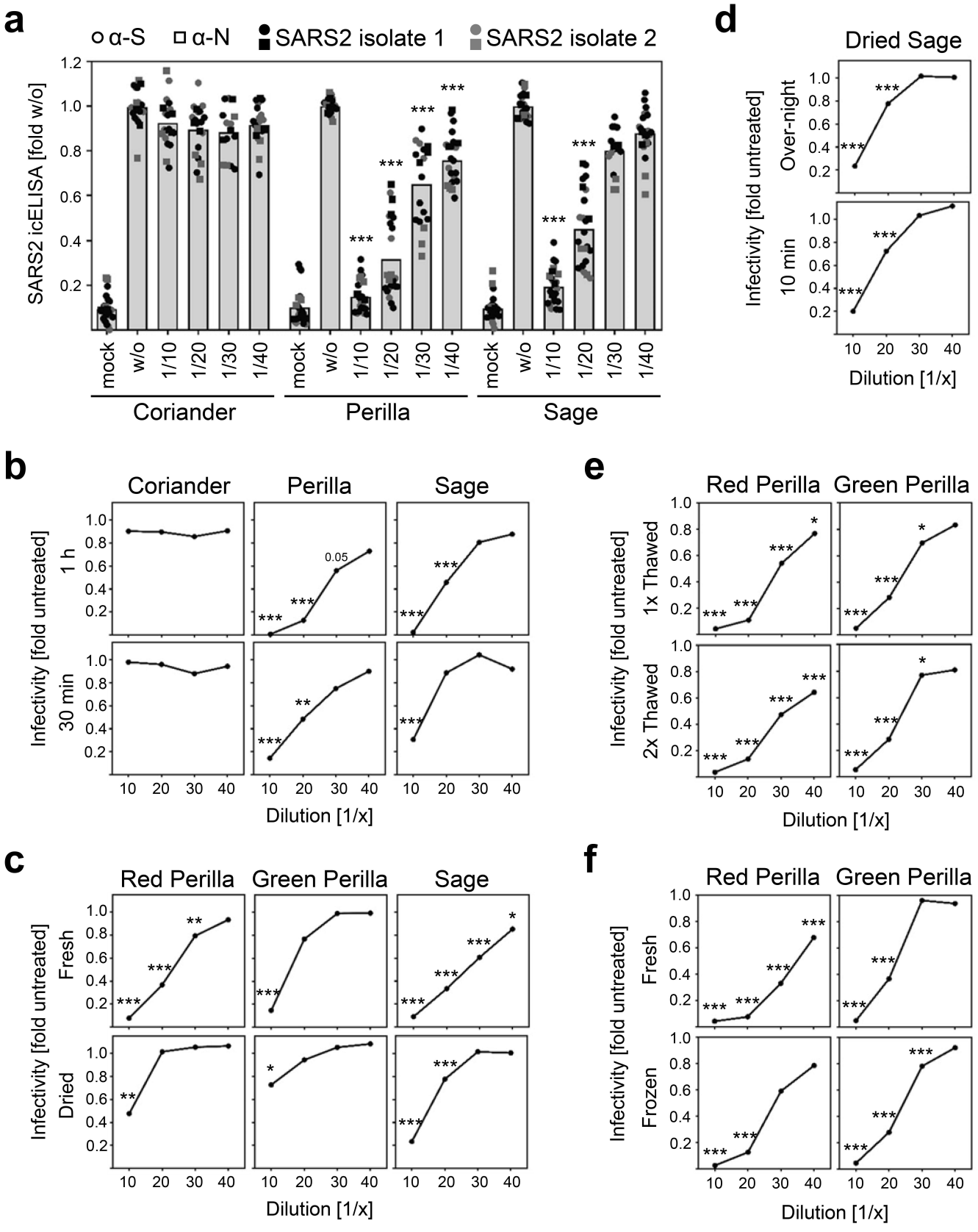
a, b Pooled icELISA data of 5 (**a**, isolate 1) and 3 (**b**, isolate 2) independent experiments of SARS-CoV-2-infected Vero E6 cells after treatment with herbal infusions using two distinct SARS-CoV-2 isolates for infection of Vero E6 and α-S or α-N mAbs for staining. Data are expressed as relative change in optical density compared to the untreated control. The perilla- and sage-treated conditions were compared to the corresponding coriander-treated condition (same dilution) by one-way ANOVA. **, $p<0.01$. ***, $p<0.001$.

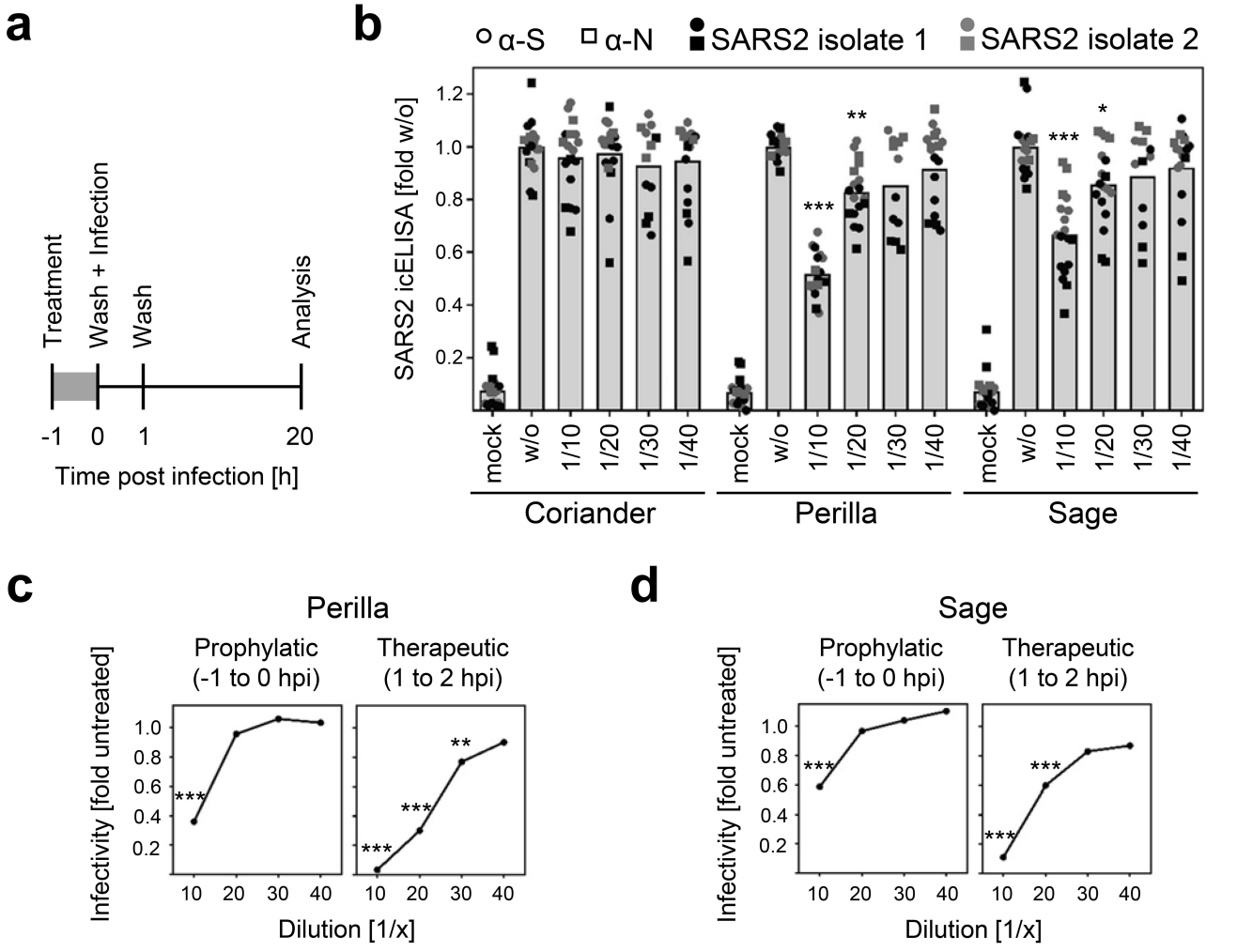
Fig. S4: **Perilla and sage confer protection against SARS-CoV-2 infection in human cells.**

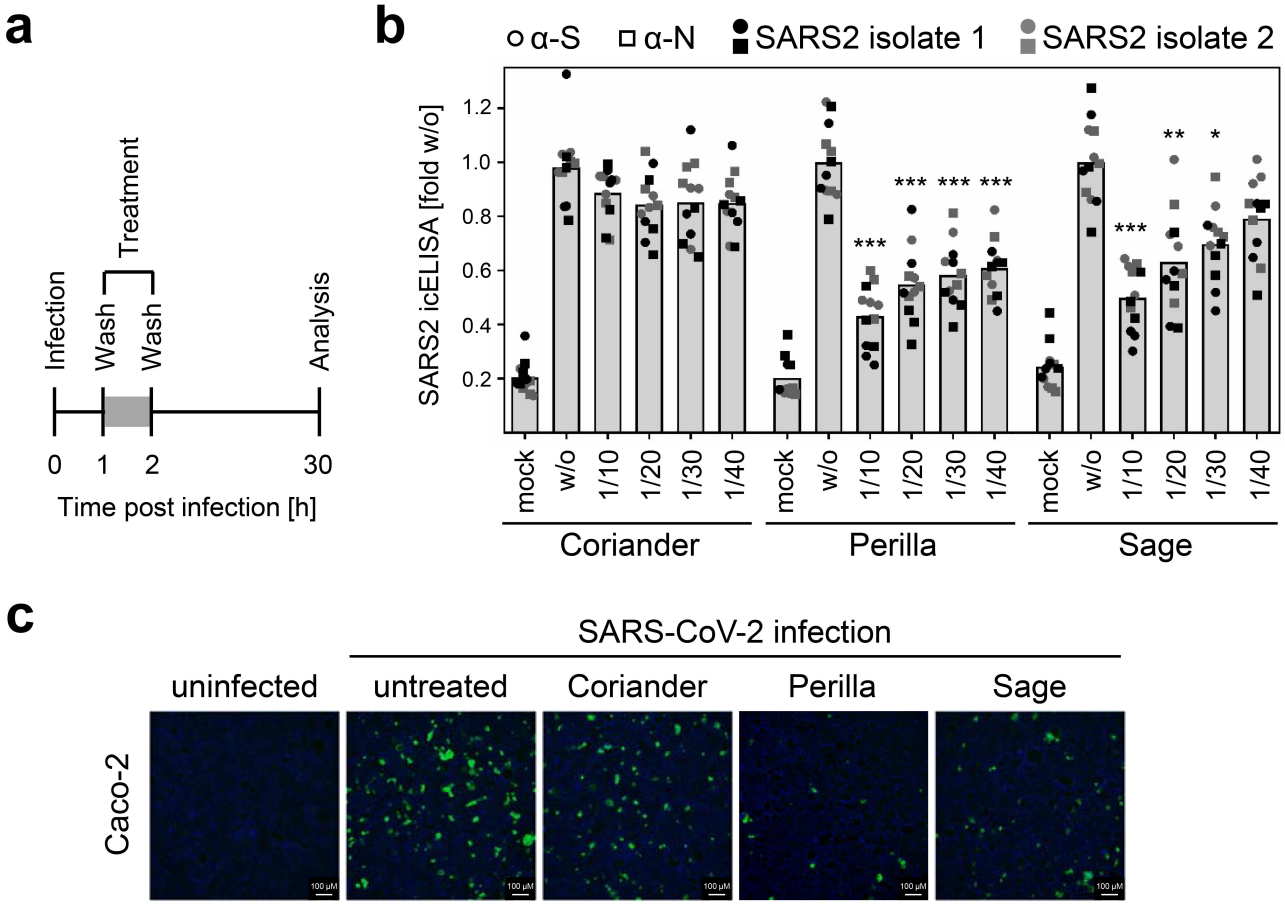
a Scheme of the applied experimental setup.

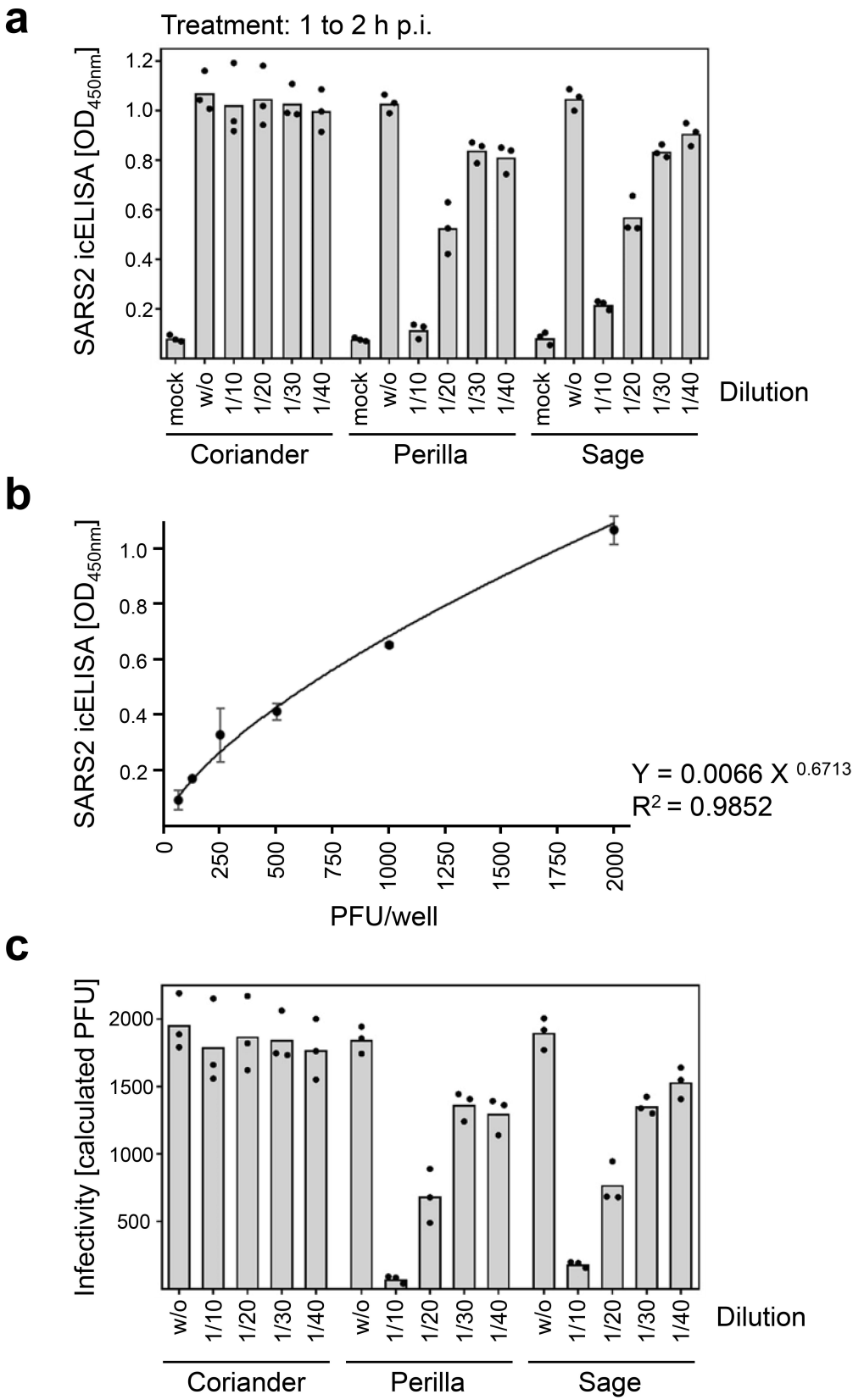
b icELISA data of SARS-CoV-2-infected Caco-2 cells using α-S for staining. Data are expressed as relative change in optical density compared to the untreated control. Each condition was analysed in triplicate.

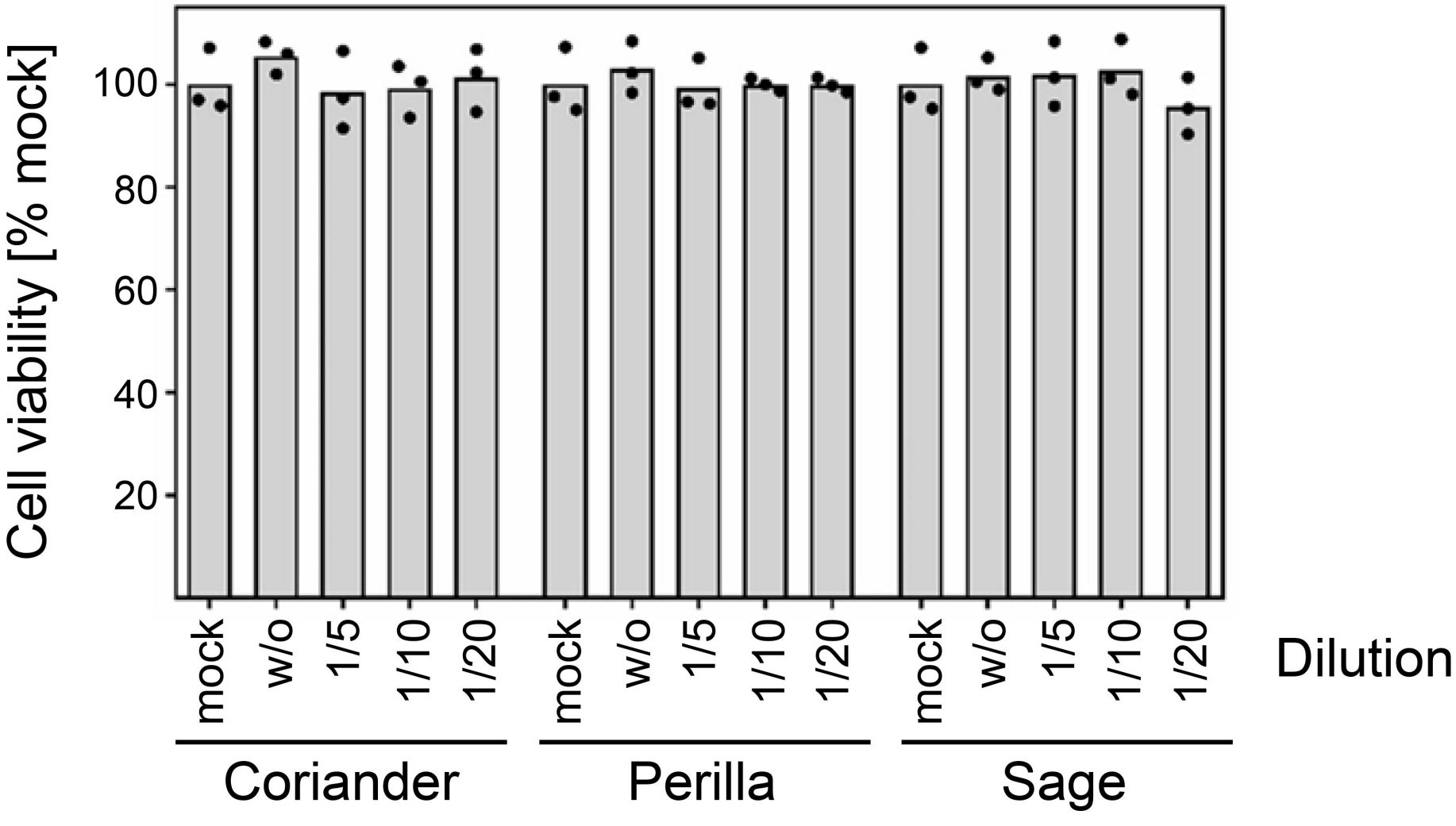

a
Treatment
Infection
Wash
Wash
Analysis
0
1
2
20
Time post infection [h]
b
Coriander
Perilla
Sage
SARS2
HSV-1
Infectivity [fold untreated]
Dilution [1/x]
c
SARS-CoV-2 infection
uninfected
untreated
Coriander
Perilla
Sage
MOI 0.1
MOI 0.5
d
SARS-CoV-2 infection
uninfected
untreated
Red Perilla
Green Perilla
Bi-color Perilla
Sage
e
Coriander
Perilla
Sage
NAbs
Infectivity [fold untreated]
Dilution [1/x]

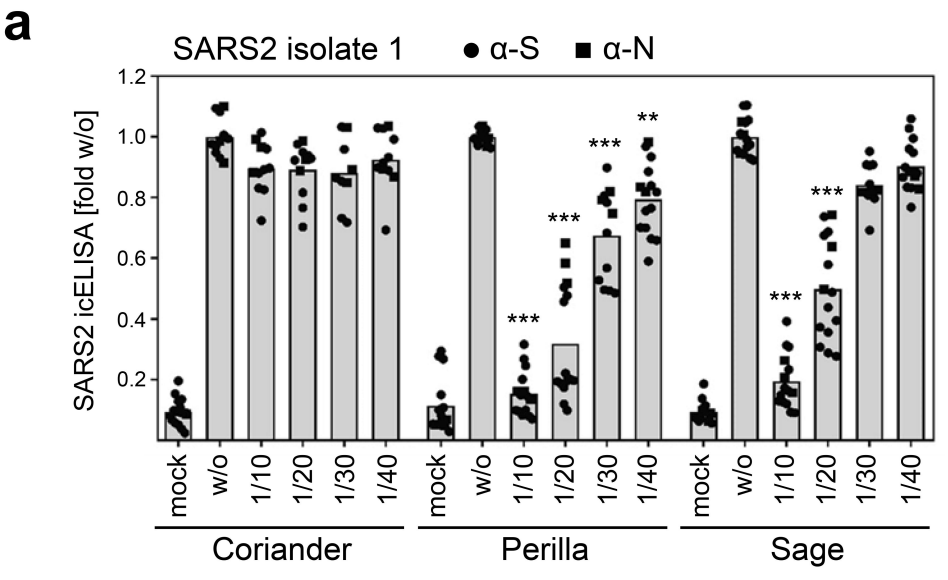

a
α-S
α-N
SARS2 isolate 1
SARS2 isolate 2
SARS2 icELISA [fold w/o]
mock
w/o
1/10
1/20
1/30
1/40
Coriander
Perilla
Sage
d
Dried Sage
Over-night
10 min
Infectivity [fold untreated]
Dilution [1/x]
b
Coriander
Perilla
Sage
1 h
30 min
0.05
e
Red Perilla
Green Perilla
1x Thawed
2x Thawed
c
Fresh
Dried
f
Frozen

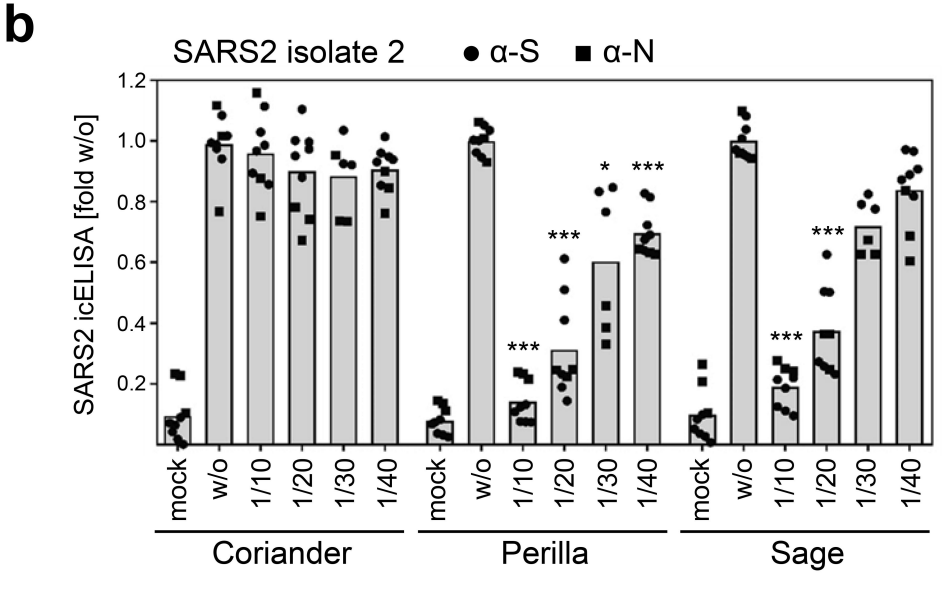

a
Treatment
Wash + Infection
Wash
Analysis
-1
0
1
20
Time post infection [h]
b
α-S
α-N
SARS2 isolate 1
SARS2 isolate 2
SARS2 icELISA [fold w/o]
1.2
1.0
0.8
0.6
0.4
0.2
***
**
***
*
mock
w/o
1/10
1/20
1/30
1/40
Coriander
Perilla
Sage
c
Perilla
Prophylatic
(-1 to 0 hpi)
Therapeutic
(1 to 2 hpi)
Infectivity [fold untreated]
1.0
0.8
0.6
0.4
0.2
***
**
10
20
30
40
Dilution [1/x]
d
Sage
Prophylatic
(-1 to 0 hpi)
Therapeutic
(1 to 2 hpi)
Infectivity [fold untreated]
1.0
0.8
0.6
0.4
0.2
***
10
20
30
40
Dilution [1/x]

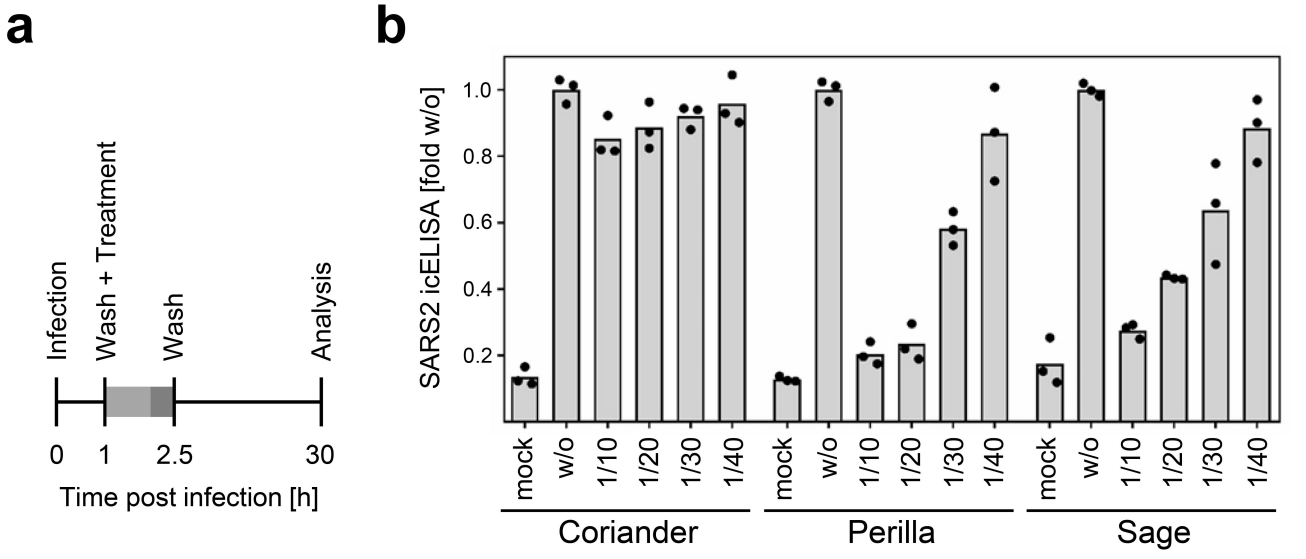

a
Treatment
Infection
Wash
Wash
Analysis
0
1
2
30
Time post infection [h]
b
α-S
α-N
SARS2 isolate 1
SARS2 isolate 2
SARS2 icELISA [fold w/o]
1.2
1.0
0.8
0.6
0.4
0.2
***
***
***
***
**
*
***
mock
w/o
1/10
1/20
1/30
1/40
Coriander
Perilla
Sage
c
SARS-CoV-2 infection
uninfected
untreated
Coriander
Perilla
Sage
Caco-2
100 µM


a
Treatment: 1 to 2 h p.i.
SARS2 icELISA [OD450nm]
1.2
1.0
0.8
0.6
0.4
0.2
mock
w/o
1/10
1/20
1/30
1/40
Dilution
Coriander
Perilla
Sage
b
SARS2 icELISA [OD450nm]
1.0
0.8
0.6
0.4
0.2
Y = 0.0066 X 0.6713
R2 = 0.9852
0
250
500
750
1000
1250
1500
1750
2000
PFU/well
c
Infectivity [calculated PFU]
2000
1500
1000
500
w/o
1/10
1/20
1/30
1/40
Dilution
Coriander
Perilla
Sage


Cell viability [% mock]
100
80
60
40
20
mock
w/o
1/5
1/10
1/20
mock
w/o
1/5
1/10
1/20
mock
w/o
1/5
1/10
1/20
Dilution
Coriander
Perilla
Sage


a
SARS2 isolate 1
● α-S
■ α-N
SARS2 icELISA [fold w/o]
1.2
1.0
0.8
0.6
0.4
0.2
***
***
***
**
***
***
mock
w/o
1/10
1/20
1/30
1/40
Coriander
Perilla
Sage


b
SARS2 isolate 2
● α-S
■ α-N
SARS2 icELISA [fold w/o]
1.2
1.0
0.8
0.6
0.4
0.2
***
***
*
***
***
***
mock
w/o
1/10
1/20
1/30
1/40
Coriander
Perilla
Sage


a
Infection
Wash + Treatment
Wash
Analysis
0
1
2.5
30
Time post infection [h]
b
SARS2 icELISA [fold w/o]
1.0
0.8
0.6
0.4
0.2
mock
w/o
1/10
1/20
1/30
1/40
Coriander
Perilla
Sage