Suppression of SARS-CoV-2 Infection by Existing Kampo Formulas and Crude Constituent Drugs Used for Treatment of Common Cold Respiratory Symptoms

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Several traditional Japanese Kampo formulas are known to have inhibitory effects on infections with viruses that cause respiratory symptoms. Although some herbs and their components have been reported to suppress SARS-CoV-2 replication in vitro, it is difficult to compare effective Kampo formulas because of the different methods used in studies. Thus, we carried out in vitro experiments on the suppression of SARS-CoV-2 infection by Kampo formulas and crude drugs used for the common cold to compare their suppressive effects on virus infection. After infecting VeroE6/TMPRSS2 cells with SARS-CoV-2, lysates of the Kampo formulas and crude drugs were added, and after 24 h, the infectious titer in the medium was measured by the TCID50 method. Maoto was the most effective among the Kampo formulas, and Ephedrae herba was the most effective among the constituent crude drugs. However, a comparison of the suppressive effects of Ephedrae herba and Kampo formulas containing Ephedrae herba showed that the suppressive effect on virus infection did not depend on the content of Ephedrae herba. Based on the results, we believe that the use of Maoto among Kampo formulas is suitable as a countermeasure against COVID-19.

Keywords: anti-viral, COVID-19, SARS-CoV-2, herbal medicine, kampo medicine, crude drug

INTRODUCTION

In September 2021, the cumulative number of SARS-CoV-2 infection cases worldwide was more than 200 million and the number of deaths from SARS-CoV-2 infection was more than 4.5 million. There has been no apparent reduction in the number of new infections or deaths (World Health Organization, 2021). Although the efficacy of vaccines has been recognized and vaccination has been accelerated in many countries worldwide, there are still many unclear issues about vaccines as the efficacy of vaccines against emerging mutant strains (Liu et al., 2021). On the other hand, there are few drugs that can be expected to have antiviral effects and most of the drugs are expensive (Hsu, 2020). Therefore, there is an urgent need to find new anti-viral drugs or alternative treatments with drug repositioning.
Kampo medicine is a systematized medical system based on traditional East-Asian medicine and unique drugs. Kampo medicine originated in traditional Chinese medicine, which was introduced to Japan around the 5th century and was refined from the 17th century to the currently used Kampo medicine in Japan. Kampo formulas are mixtures of crude drugs, which are extracts of herbs, insects, minerals, fungi, and other substances (Tsumura and Co., 2016). All Kampo formulas were approved as drugs for humans by Japan’s Ministry of Health, Labour and Welfare in 1986 and are regulated by the National Institute of Health and Welfare (STORK, 2020). Among the medicines certified by the Ministry of Health, Labour and Welfare, medical Kampo formulas are comparatively inexpensive, and many of them have versatility in use. On the other hand, herbal medicines themselves are also used as folk remedies not only in Japan but also in many other countries.

Influenza and respiratory syncytial viruses are RNA viruses and the primary symptoms of infections with these viruses are respiratory symptoms that are similar to the symptoms of SARS-CoV-2 infection. In Japan, two Kampo prescriptions, Maoto and Saikokeishito, have been approved for treatment of influenza infection. There have been in vitro studies showing suppression of infection with these viruses by Kampo formulas and crude drug extracts (Mantani et al., 1999; Nomura et al., 2019; Hou et al., 2020). There seems to be a relationship between the clinical effects of Kampo formulas and inhibition of influenza virus growth in cultured cells.

In this study, we examined the suppressive effects of Kampo formulas on SARS-CoV-2 infection using VeroE6/TMPRSS2 cells, which are highly susceptible to SARS-CoV-2 infection (Matsuyama et al., 2020). We selected eight Kampo formulas that have been shown to be effective against influenza virus infections and common cold symptoms and examined their inhibitory effects on SARS-CoV-2 infection. We also investigated the inhibitory effects of six crude constituent drugs constituting those Kampo formulas on SARS-CoV-2 infection.

MATERIALS AND METHODS

Cells and Viruses
VeroE6/TMPRSS2 cells [African green monkey kidney-derived cells expressing human TMPRSS2, purchased from Japanese Collection of Research Bioresources (JCRB) Cell Bank, JCRB 1819] were propagated in Dulbecco’s modified Eagle’s minimum essential medium (DMEM, Invitrogen) supplemented with 10% fetal calf serum (FCS; Biosera, Kansas City, MO, United States), penicillin G (100 units/ml, Meiji Seika Pharma, Tokyo, Japan), and streptomycin (100 μg/ml, Meiji Seika Pharma). The cells were cultured at 37°C in 5% CO₂. SARS-CoV-2/JP/Hiroshima-46059T/2020 (Yamamotoya et al., 2021; B.1.1.1, GISAID accession ID: EPI_ISL_6289932, GenBank/DDBJ/EMBL accession number: MZ853926) was used as the test virus.

To prepare virus suspensions, VeroE6/TMPRSS2 cells were infected with the virus and incubated in DMEM. When cytopathic effects were fully developed, the culture supernatant was harvested and filtered through a 0.45-μm filter after low-speed centrifugation. The virus titer was determined by the standard 50% tissue culture infectious dose (TCID₅₀) method. Briefly, a 10-fold serial dilution of the virus was inoculated into cells in a 96-well plate in tetraplicate or octuplicate and incubated for 7 days to check for CPE. Based on this result, infectivity was calculated and expressed as TCID₅₀/ml, as described previously (Nomura et al., 2021).

Reagents
Extract powders of Kampo formulas including Maoto, Saikokeishito, Shomakakkonto, Kakkonto, Shoseiryuto, Senkyuchachosan, Bakumondo, and Hochuekkito were kindly provided by Tsumura & Co. (Tokyo, Japan) (Table 1). Crude drugs including *Glycyrrhiza uralensis* Fisch. ex DC [Fabaceae] (*Glycyrrhiza* radix), *Ephedra sinica* Stapf [Ephedraceae] (*Ephedra* herba), *Paonia lactiflora* Pall [Paeoniaceae] (*Paonia* radix), *Ligusticum officinale* Makino Kitag [Apiaceae] (Cnidii rhizoma), *Scutellaria baicalensis* Georgii [Lamiaceae] (*Scutellaria* radix), and *Bupleurum falcatum* L. [Apiaceae] (*Bupleuri radix*) were purchased from Tsumura & Co. The Kampo formulas used in this study and their constituent crude drugs (generic name, scientific name, and percentage included) are shown in detail in Table 1.

Solutions of the Kampo formulas for testing were prepared as described previously (Nomura et al., 2019). The powder of each Kampo formula was mixed with DMEM to a concentration of 20 mg/ml. The powder was dissolved at 50°C for 1 h and the mixture was centrifuged at a low speed and then the supernatant was filter-sterilized through a 0.22-μm filter. The crude drugs were prepared in a similar way. For both the Kampo formulas and the crude drugs, little insoluble material was found after low-speed centrifugation, and the weight of the initial powder was therefore used as the weight of the solute.

Cytotoxicity Assay
VeroE6/TMPRSS2 cells were cultured in DMEM with the specified concentrations of reagents for 24 h, and lactate dehydrogenase (LDH), which was released from the cells into the medium, was assayed with a colorimetric method using the Cytotoxicity LDH Assay Kit-WST (Dojindo Laboratories, Kumamoto, Japan) by measuring absorbance at 490 nm in the TriStar LB 941 plate reader (Bertohold Technologies, Wildbad, Germany). The cytotoxicity of the reagents was calculated from the absorbance measurements as 100% for the high control (cell lysis by surfactant) and 0% for the low control (culture medium only).

Replication of SARS-CoV-2 in vitro
Confluent monolayers of VeroE6/TMPRSS2 cells in a 96-well plate were infected with 50 μl/well of the virus at an input multiplicity of infection (m.o.i.) of 0.05 or 10. After adsorption for 2 h, the inoculated viruses were removed, and the cells were further cultured in 100 μl/well of DMEM containing different concentrations of Kampo formulas or crude drugs. The conditions of m.o.i. and virus adsorption time were based on the conditions used in a previous study for effective infection of
| TABLE 1 | The Kampo medicines used in this study and their constituent crude drugs. |
|---------|--------------------------------------------------------------------------------|
| **Maoto** | **Indications** | Common cold, Influenza (Acute), Rheumatoid arthritis |
| **Crude drugs** | **(Latin name)** | Armeniacae semen | Ephedrae herba* | Cinnamomi cortex | Glycyrrhizae radix* |
| **Scientific name** | **Armeniacae semen** | **Ephedrae herba** | **Cinnamomi cortex** | **Glycyrrhizae radix** |
| **Rate of crude drugs configuring Kampo extracts (excluding additives)** | 32.3% |

| **Saikokeishito** | **Indications** | Common cold, Influenza, Feverish diseases such as pneumonia and pulmonary tuberculosis, Gastric ulcer, Duodenal ulcer, Cholecystitis, Gallstone, Pain in liver dysfunction and pancreatitis |
| **Crude drugs** | **(Latin name)** | Bupleuri radix* | Pinelliae Tuber | Scutellariae radix* | Glycyrrhizae radix* | Cinnamomi cortex | Paeoniae radix* | Ziziphi fructus | Ginseng radix | Zingiberis rhizoma |
| **Scientific name** | **Bupleuri radix** | **Pinelliae Tuber** | **Scutellariae radix** | **Glycyrrhizae radix** | **Cinnamomi cortex** | **Paeoniae radix** | **Ziziphi fructus** | **Ginseng radix** | **Zingiberis rhizoma** |
| **Rate of crude drugs configuring Kampo extracts (excluding additives)** | 22.7% |

| **Kakkonto** | **Indications** | Common cold, Early stage of febrile disease, Inflammatory diseases (conjunctivitis, keratitis, otitis media, tonsillitis, mastitis, lymphadenitis), Stiff shoulders, Neuralgia in the upper body, Urticaria |
| **Crude drugs** | **(Latin name)** | Puerrariae Radix | Ziziphi fructus | Glycyrrhizae radix* | Cinnamomi cortex | Paeoniae radix* | Zingiberis rhizoma |
| **Scientific name** | **Puerrariae Radix** | **Ziziphi fructus** | **Glycyrrhizae radix** | **Cinnamomi cortex** | **Paeoniae radix** | **Zingiberis rhizoma** |
| **Rate of crude drugs configuring Kampo extracts (excluding additives)** | 22.2% |

| **Shomakakkonto** | **Indications** | Common cold (early stage), Dermatitis |
| **Crude drugs** | **(Latin name)** | Puerrariae Radix | Paeoniae radix* | Cimicifugae Rhizoma | Glycyrrhizae radix* | Zingiberis rhizoma |
| **Rate of crude drugs configuring Kampo extracts (excluding additives)** | — |

(Continued on following page)
TABLE 1 | (Continued) The Kampo medicines used in this study and their constituent crude drugs.

| Scientific name | Rate of crude drugs configuring Kampo extracts (excluding additives) | Crude drugs (Latian name) |
|-----------------|---------------------------------------------------------------|--------------------------|
| **Shoseiryuto** |
| Indications    | Common cold, Bronchitis, Bronchial asthma, Rhinitis, Allergic Rhinitis, Allergic conjunctivitis |
| Scientific name| Pinelliae tuber | Paeoniae radix* |
|                | Zingiber rhizoma processum | Glycyrrhizae radix* |
| Rate of crude drugs configuring Kampo extracts (excluding additives) | 22.2% | 11.1% |

| **Senkyuchachosan** |
| Indications | Common cold, automatic imbalance syndrome peculiar to women resembling climacteric disturbance, and headache |
| Scientific name | Cyperi rhizoma |
|                | Cnidii rhizoma |
|                | Liganum officinale (Makino) Kt. (Apiaceae) |
|                | Hansenia weberbaueriana (Fedde ex H.Wolff) |
|                | Nepeta tenella (Benth. [Lamiaceae]) |
| Rate of crude drugs configuring Kampo extracts (excluding additives) | 20.0% | 15.0% |

| **Bakumondoto** |
| Indications | Coughing with a hard, Obstructive sputum, Bronchitis, and bronchial asthma |
| Scientific name | Ophiopogonis radix |
|                | Oryzae fructus |
|                | Pinelliae tuber |
|                | Zizphi fructus |

(Continued on following page)
The Kampo medicines used in this study and their constituent crude drugs are shown with indications of the Kampo medicines. The ratio of constituents included in Kampo drugs of extract powder and crude drugs referred to the website of Tsumura & Co.

### Table 1 (Continued)

| Scientific name                   | Rate of crude drugs configuring Kampo extracts (excluding additives) |
|-----------------------------------|---------------------------------------------------------------------|
| Ophiopogon japonicus (Thunb.) Ker Gawl. [Asparagaceae] | 37.0%                                                               |
| Oryza sativa L. [Poaceae]         | 18.5%                                                               |
| Pinellia ternata (Thunb.) Makino [Araceae] | 18.5%                                                               |
| Ziziphus jujuba Mill. [Rhamnaceae] | 11.1%                                                               |
| Glycyrrhiza uralensis Fisch. ex DC. [Fabaceae] | 7.4%                                                               |
| Panax ginseng C.A.Mey. [Araliaceae] | 7.4%                                                               |

| Rate of crude drugs configuring Kampo extracts (excluding additives) |
|---------------------------------------------------------------------|
| 16.7%                                                               |

Kampo medicines used in this study and their constituent crude drugs are shown with indications of the Kampo medicines. The ratio of constituents included in Kampo drugs of extract powder and crude drugs referred to the website of Tsumura & Co.

*The crude drugs investigated are marked.*
cells (Wang et al., 2020). The medium was harvested after 24 h, and viral infectivity was assayed by the TCID_{50} method. The logarithm of infectivity titer and reagent concentration in the medium were plotted and an approximation straight-line was drawn to calculate the 50% inhibitory concentration (IC_{50}), as described previously (Nomura et al., 2021).

We conducted infection experiments under two conditions: m.o.i. of 0.05 and m.o.i. of 10. At m.o.i. of 10, all cells are infected at once, allowing us to observe the process of virus entry and replication (one-step replication). When m.o.i. of 0.05, on the other hand, 20 cells are infected with a single virus. In addition to the entry and replication of the virus into the cell, the progeny virus is released from the cell and further infects the surrounding cells (multi-step replication).

**Assay for Inactivation of Viral Particles**

For the Kampo formulas, the solution was mixed with 90 µl of the drug at a concentration of 20 mg/ml and 10 µl of the virus solution at 2.0 × 10^{9} TCID_{50}/ml and incubated for 3 min at room temperature. The mixture was then serially diluted 10-fold in DMEM, and adsorbed on VeroE6/TMPRSS2 cells for 1 h. The inoculum was removed, and cell culture medium was added and incubated for 7 days. The infectivity of the solution was determined by the TCID_{50} method. For crude drugs, reagents at 1.25–10 mg/ml concentrations were used considering their cytotoxicity. Phosphate-buffered saline (PBS) was used as an untreated control, and 70% (w/w) ethanol was used as an inactivation control as described previously (Nomura et al., 2021).

**RESULTS**

**Suppressive Effects of Kampo Formulas on SARS-CoV-2 Infection**

Eight Kampo formulas used to treat respiratory symptoms, including respiratory symptoms of influenza and common cold infections, were investigated for their inhibitory effects on SARS-CoV-2 infection in vitro. Table 1 shows the clinical indications of each Kampo formula, the crude drugs included, and the proportions of the crude drugs. Initially, the cytotoxicity of each Kampo formula was examined by an LDH assay, and the values were plotted on a graph (Figure 1, △ marker, right Y-axis). Twenty mg/ml of each Kampo formula of Maoto (Figure 1A), Saikokeishito (Figure 1B), Kakkonto (Figure 1C), and Shomakakkonto (Figure 1D) showed more than...
Each Kampo formula on the virus particles (titer. This suggests that there was no direct inactivating effect of measured, none of the Kampo formulas decreased the infectious formula was mixed with the virus and the infectious titer was infection. Still, it can be considered that they have clear suppressive effects on virus infection at that concentration. Almost completely inhibited at a concentration of 10 mg/ml replication was inhibited as the concentration was increased and was all of the cells would be infected (Figure 3). Ephedrae herba showed suppressive effects on virus infection even at an m.o.i. of 10 so that all of the cells would be infected (Figure 3). Ephedrae herba showed a strong suppressive effect on virus infection, while Paeoniae radix, Scutellariae radix, and Glycyrrhizae radix showed weaker inhibitory effects (Figure 3). To investigate the mechanisms by which the crude drugs suppress virus infection, we conducted infection experiments using crude drugs (Ephedrae herba, Scutellariae radix, Paeoniae radix, Glycyrrhizae radix) at an m.o.i. of 10 so that all of the cells would be infected. From the results of the experiment described above (Figure 2), it is unlikely that the viral particles are directly inactivated, and intracellular proliferation or release from the cell may therefore be impaired.

Suppressive Effects of Crude Drugs on SARS-CoV-2 Infection

We investigated the suppressive effects on virus infection of the available crude drugs with high percentages of composition among the crude drugs constituting Maoto, Senkyuchachosan, and Saikokeishito, which had strong inhibitory effects on SARS-CoV-2 infection. The crude drugs tested were Ephedrae herba, Glycyrrhizae radix, Scutellariae radix, Paeoniae radix, Bupleuri radix, and Cinclid rhizoma (Figure 3). Ephedrae herba showed cytotoxicity at concentration of 2.5 mg/ml and above, Bupleuri radix showed cytotoxicity at concentrations of 10 mg/ml and above, and Scutellariae radix showed cytotoxicity at concentrations of 5 mg/ml and above. Therefore, the effects of these crude drugs at lower concentrations were evaluated.

The cells were infected with the virus at an m.o.i. of 0.05, and the infectious titer was measured by the TCID₅₀ method (Figure 3). Ephedrae herba showed a strong inhibitory effect on virus infection, while Paeoniae radix, Scutellariae radix, and Glycyrrhizae radix showed weaker inhibitory effects (Figure 3).

Contribution of Ephedrae Herba in Kampo Formulas

In this study, we found that Ephedrae herba has a strong inhibitory effect on SARS-CoV-2 infection. Ephedrae herba is found in several
Kampo formulas, and to verify the role of Ephedrae herba in inactivation of SARS-CoV-2 by the herbal medicines, we recalculated the effect of Ephedrae herba on SARS-CoV-2 based on the amount of Ephedrae herba in each Kampo formula.

The IC\textsubscript{50} value of Ephedrae herba alone was 61.1 µg/ml (Figures 3A, 5A). Maoto contains 32.3% of Ephedrae herba (Table 1), and the IC\textsubscript{50} value was calculated by plotting the amount of Ephedrae herba in Maoto on the horizontal axis of the graph (Figure 5B) to be 163.6 µg/ml. Thus, although it should be the same amount of Ephedrae herba, Ephedrae herba in the form of Maoto was weakened, suggesting that other components of Maoto may be inhibiting the effect Ephedrae herba. The IC\textsubscript{50} value of Maoto itself was calculated to be 0.5 mg/ml (Figure 1A), being consistent with this hypothesis.

Similarly, in the case of Kakkonto containing 16.7% of Ephedrae herba (Table 1), the IC\textsubscript{50} value was calculated to be as high as 517.3 µg/ml (Figure 5C). The IC\textsubscript{50} value of Kakkonto itself was 2.7 mg/ml (Figure 1C). Shoseiryuto contained 11.1% of Ephedrae herba (Table 1), and the IC\textsubscript{50} value of Ephedrae herba was as high as 450.4 µg/ml based on Ephedrae herba (Figure 5D). The IC\textsubscript{50} value of Shoseiryuto itself was 4.9 mg/ml (Figure 1E). These results suggest that the inhibitory effect of Ephedrae herba in the Kampo formulas on SARS-CoV-2 infection is weakened by other components.

**DISCUSSION**

Kampo formulas consist of various combinations of crude drugs, traditionally regarded as units, and are often used on the base of ancient experience (Tanaka et al., 1995; Odaguchi et al., 2019). In Japan, Kampo formulas are covered by the national health...
Kampo formulas are also available as over-the-counter drugs that can be purchased at pharmacies without a doctor’s prescription. The extensive experience of using Kampo formulas in Japan has led to their safe and easy use in medical practice. In recent years, scientific analysis of the effects of Kampo formulas has provided evidence of their clinical benefits, and their clinical usefulness for the treatment of COVID-19 has also been suggested (Motoo et al., 2014; Takayama et al., 2021).

Some advantages of Kampo formulas are that they can be taken orally, are relatively inexpensive, and can be taken at an early stage. For example, Maoto, one of the Kampo formulas examined in this study, can be used to treat common colds in infants even before the onset of cold symptoms such as fever and nasal discharge (Kubo and Nishimura, 2007; Nagai et al., 2014). On the other hand, antivirals, as shown in clinical trials for influenza (Muthuri et al., 2014; Dobson et al., 2015), need to be taken after the onset of illness and before the peak of viral replication. The clinical course of COVID-19 is longer than that of influenza, and viral replication in patients with COVID-19 likely to continue for a long time. Although existing antivirals such as remdesivir may be effective if treatment with the antivirals started after the diagnosis of COVID-19, consideration should be given to the use of Kampo formulas, which can be administered earlier.

Kampo formulas that are used for the treatment of influenza have been reported to inhibit the growth of influenza viruses in cultured cells (Mantani et al., 1999; Nomura et al., 2019; Hou et al., 2020). Although the relationship between inhibition of influenza virus replication in cultured cells and clinical efficacy is not entirely clear, the therapeutic effect of Kampo formulas may be elicited by suppression of the ability of the virus of replicate in the human respiratory tract. In addition, Lian hua qing wen, which contains Ephedrae herba and is one of the therapeutic agents used for influenza viruses in traditional Chinese medicine, has been shown in vitro experiments to have an inhibitory effect on SARS-CoV-2 infection and has also been shown to be effective for COVID-19 in a clinical setting, (Su et al., 2020; Hu et al., 2021). Therefore, we investigated the effects of Kampo formulas on SARS-CoV-2 infection in vitro experiments.

Since commercial Kampo formulas contain additives and the types and amounts of additives are not uniform, we used the same concentrations of Kampo formulas without additives in this study. We found that virus infection was inhibited by...
The Kampo formulas Maoto, Saikokeishito, and Senkyuchachosan but not by the Kampo formulas Bakumondoto and Hochuekkito. We also examined the viral inhibitory effects of crude drugs as constituents of the Kampo formulas that had strong inhibitory effects on SARS-CoV-2 infection. Ephedrae herba had the strongest inhibitory effect on SARS-CoV-2 infection. Among the six crude drugs examined, Ephedrae herba, Paeoniae radix, Scutellariae radix, and Glycyrrhizae radix had the strongest inhibitory effects in that order.

Ephedrae herba showed direct inactivating effects on virus particles as well as on infected cells. Previous studies have shown that tannins, which are components of Ephedrae herba, inhibit influenza virus infection (Mantani et al., 1999) and that tannins extracted from plants such as persimmon have inactivating effects on various viruses (Ueda et al., 2013). In the present study, it was also thought that tannins in Ephedrae herba may have direct inhibitory effects on virus infection. However, in the infection experiments to investigate the suppressive effects of crude drugs, on virus infection, it was found that Ephedra herba had a suppressive effect even under the condition of an m.o.i. of 10, in which all cells were infected at once, and no virus particle formation process was involved. Therefore, Ephedrae herba may have both a direct inactivation effect on virus particles and a suppressive effect on infection of cells, suggesting a combined virus suppression mechanism.

However, it was found that Kampo formulas containing Ephedrae herba had little direct inactivation effect on virus particles. The contents of Ephedrae herba in the Kampo formulas used in the virus direct inactivation test (20 mg/ml) were 6.45 mg/ml in Maoto, 3.33 mg/ml in Kakkonto, and 2.22 mg/ml in Shoseiryuto. These concentrations were higher than the concentration of Ephedrae herba tested as a crude drug (1.25 mg/ml), which was found to be effective in the virus particle direct inactivation test. Furthermore, when the IC50 values of Kampo formulas containing Ephedrae herba and Ephedrae herba alone were compared on the basis of the content of Ephedrae herba, the effects of all Kampo formulas were weaker than the effect of Ephedrae herba alone. These results suggest that the combination of Ephedrae herba with other crude drugs in Kampo formulas has a weaker antiviral effect than that of Ephedrae herba. Ephedra herba has various side effects, and Kampo formulas are shown to contain crude drugs that alleviate the side effects of the strong Ephedra herba (Odaguchi et al., 2019). The reduced suppressive effect of Ephedra herba on virus infection may mean that its side effects on the human body are reduced.

Among the Kampo formulas that do not contain Ephedrae herba, Saikokeishito and Senkyuchachosan showed efficacy. These Kampo formulas have been reported to inhibit influenza virus in vitro (Shirayama et al., 2016; Nomura et al., 2019), and the Ministry of Health, Labour, and Welfare has approved Saikokeishito for treatment of influenza. Therefore, it would not be surprising if these Kampo formulas also have inhibitory effects on SARS-CoV-2 infection. Furthermore, viral inhibitory effects of Glycyrrhizae radix, Paeoniae radix, and Scutellariae radix as crude drugs contained in these Ephedrae herba-free Kampo formulas were confirmed. It was shown in an in vitro study that rhizoma had an inhibitory effect on influenza virus infection (Nomura et al., 2019), but it had no inhibitory effect on SARS-CoV-2 infection. Glycyrrhizae radix is a crude drug in many Kampo formulas. All of the Kampo formulas used in this study contained Glycyrrhizae radix. Kampo formulas contained Glycyrrhizae radix alone on SARS-CoV-2 infection was shown in a previous study (van de Sand et al., 2021), not all of the Kampo formulas containing Glycyrrhizae radix showed suppressive effects on virus infection, and some Kampo formulas showed no effect at all. Therefore, other crude drug components in the Kampo formulas may weaken the effect of Glycyrrhizae radix. Alternatively, the amount of Glycyrrhizae radix in the Kampo formulas may have been too small to reach the threshold for suppression of virus infection.

Paoniae radix was suggested to be effective against SARS-CoV-2 infection by molecular modeling predictions (Ma et al., 2020). It was also shown to be effective against influenza virus infection in cell infection experiments (Ho et al., 2014). Scutellariae radix is one of the crude drugs contained in Saikokeishito, which was effective against SARS-CoV-2 infection. In addition, Baicalin, a component of Scutellariae radix, has been shown to have an inhibitory effect on SARS-CoV-2 infection in cell and animal experiments (Song et al., 2021). In the present study, Glycyrrhizae radix, Paeoniae radix, and Scutellariae radix showed inhibitory effects on SARS-CoV-2 infection, but their effects were inferior to the effect of Ephedrae herba.

**CONCLUSION**

We performed in vitro experiments to determine the inhibitory effects on SARS-CoV-2 infection of Kampo formulas and their constituent crude drugs that are used to treat respiratory symptoms including influenza and common cold symptoms. We found that Maoto among the Kampo formulas and Ephedrae herba among the crude drugs had the strongest inhibitory effects on SARS-CoV-2 infection. Some other Kampo formulas and crude drugs also showed suppressive effects on virus infection. Although further analysis and evidence are needed, Kampo formulas might contribute to the treatment of COVID-19.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.
AUTHOR CONTRIBUTIONS

MK, TNo and TNa. developed the study design, performed the experiments, and analyzed the data. HK, KO-O, HO and TS provided assistance in developing the study design and experiments. MK, TNo, TNa and TS wrote the manuscript. KK, KO-O, HO and MI provided advice on interpretation of data and revised the manuscript. All authors read and approved the manuscript.

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