Artemisia argyi potentially prevents the infections with SARS-CoV-2 variants

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Research Article

Keywords: Artemisia argyi, eriodictyol, umbelliferone, TMPRSS2, ACE2, SARS-CoV-2 variants

Posted Date: January 12th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2362385/v1

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Abstract

Background

Traditional Chinese medicine (TCM) has potential benefits to prevent multi-viral infection including by modulating the immune system or defending oxidative stress. *Artemisia argyi* (*A. argyi*) has been widely used for anti-microbial infection, anti-allergy, anti-diabetes, and anti-inflammation in Eastern Asia. However, it remains unclear whether *A. argyi* has the potential to reduce the infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Results

Through the docking simulation, eriodictyol and umbelliferone, two phytochemicals existed in *Artemisia argyi*, have showed their potential to bind to cellular proteins transmembrane serine protease 2 (TMPRSS2) and angiotensin-converting enzyme 2 (ACE2), which are required for the cellular entry of SARS-CoV-2. Our results further found that eriodictyol and umbelliferone suppressed the infection of ACE2-expressed HEK-293T cells with lentiviral-based pseudo-particles expressing wild type and variants of SARS-CoV-2 spike (S) protein via interrupting the interaction between S protein and cellular receptor ACE2 and via reducing ACE2 and TMPRSS2 expressions.

Conclusions

In summary, *Artemisia argyi* and its ingredients eriodictyol and umbelliferone are potential agents to reduce SARS-CoV-2 infection.

Introduction

The global pandemic of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been a serious public issue threatening people's health in most of countries [1]. The genomic evolution of SARS-CoV-2 creates the diverse mutant strains which become more transmittable and resistance to immune attack and anti-virus treatments [2]. In order to control the outbreak of COVID-19, multiple variants of SARS-CoV-2 were classified into variant of interests (VOIs) and variant of concerns (VOCs) by World Health Organization (WHO) according to their transmission rate, disease severity, therapeutic response, and healthy problem of mutant viruses [3]. For example, Omicron variant, identified as the lineage B.1.1.529 in South Africa in November 2021, has been viewed as one of circulating and high lethal VOIs associated with its rapid human-to-human transmission, high risk of reinfection, and resistance to vaccines [4–6].

SARS-CoV-2 belongs to the family of highly diverse coronaviruses (CoVs) constituted by positive-sense and single-strand RNA [7]. For cellular infection, SARS-CoV-2 utilizes diverse factors for the entry of host
cells, translation of RNA genome, and replication cycle, ultimately resulting in the release of viral progeny. The cellular entry of SARS-CoV-2 relies on its receptor engagement of spike (S) protein with the specific host receptor angiotensin-converting enzyme2 (ACE2) [8]. After S protein binding to ACE2, the cleavage and priming of S protein by different proteases, such as transmembrane serine protease 2 (TMPRSS2) and cathepsin L, are typically necessary to present fusion peptide (FP) for membrane fusion with host cells [9, 10]. Following cellular entry, viruses release, replicate, and transcribe their genomic RNA to form essential structural proteins that compose new and complete viral particles [11, 12]. Since SARS-CoV-2 evolves to diverse variants, the therapeutic effectiveness of antiviral treatments is limited and scarce. It is imperative to explore the potential protective treatments or integrated approaches against COVID-19.

*Artemisia argyi* (A. argyi) belonging to the family of *Asteraceae* is a renowned traditional Chinese medicine (TCM) and food ingredients in the Far East. In the broad-spectrum profile, *A. argyi* possesses several bioactive compounds, including flavonoids, terpenoids, and caffeoylquinic acids, and contributes to anti-microbial, anti-allergic, anti-diabetic, and anti-inflammatory activities by modulating the immune system or defending oxidative stress [13, 14]. In addition, this TCM was also shown to suppress the proliferation of tumor cells with the low cytotoxicity to normal cells [15, 16]. Recently, *A. argyi* has been implied as a potential TCM against severe acute respiratory syndrome (SARS), middle east respiratory syndrome (MERS), and COVID-19 [17, 18]. However, its activity against the infection with SARS-CoV2 has not been demonstrated yet and the active phytochemicals and underlying mechanisms also remain unclear.

**Results**

*Artemisia argyi* is a potential TCM against COVID-19

To identify the bioactive compounds of *A. argyi* with the potential against the infection of SARS-CoV-2, FRET-based enzymatic activity assay as illustrated in Fig. 1A was performed to examine the effects of 14 known ingredients of *A. argyi* on the activity of cellular proteins involved in the priming of S protein and viral replication. As shown in Table 1, the enzymatic activity of TMPRSS2 but not 3CLpro was dramatically attenuated by eriodictyol (100.0%±0.3%; Fig. 1B), umbelliferone (74.1%±3.5%), and 13-Oxo-9E,11E-octadecadienoic acid (13-Oxo-ODE) (76%±4.7%). 13-Oxo-ODE is a metabolite from dehydrogenation of 13-hydroxyoctadecadienoic acid (13-HODE), which is a oxidized product of linoleic acid by 12/15-lipoxygenase (LOX) [19, 20], and has been reported as an endogenous ligand for nuclear hormone receptor peroxisome proliferation-activated receptor gamma (PPARγ), resulting in amelioration of inflammatory bowel disease [21, 22]. Since activation of PPARγ has also been associated with cancer progression [23–25], these unfavorable effects of 13-Oxo-ODE lead us to focus on only eriodictyol and umbelliferone for their anti-corona virus activity. In addition, eriodictyol not only repressed the enzymatic activity of TMPRSS2 (Fig. 1B), but also abolished furin activity (Fig. 1C) in a dose-dependent manner. Following the priming of S protein by TMPRSS2 or furin, the cleaved S protein interacts with human cellular receptor ACE2 [26]. FRET assays as illustrated in Fig. 1D further showed the inhibitory effect of eriodictyol on the interaction between S protein and ACE2 in a dose-dependent manner (Fig. 1E). Taken
together, eriodictyol and umbelliferone in *A. argyi* potentially suppress the activity of TMPRSS2 and furin to reduce the S1/S2 priming of S protein and interferes the interaction between S protein and ACE2, thereby decreasing the binding activity of SARS-CoV2 to host cells.

**Molecular docking of eriodictyol and umbelliferone with SARS-CoV-2 proteins**

Since eriodictyol and umbelliferone suppressed the enzymatic activity of TMPRSS2 and S protein/ACE2 interaction, the binding mode of these two compounds in the catalytic pockets of TMPRSS2 and ACE2 were performed with Discovery Studio. The results indicated that eriodictyol (Fig. 2A and 2B) and umbelliferone (Fig. 3A and 3B) showed interactions with the catalytic pockets of TMPRSS2 and ACE2, and the changes in energy value were −13.05 kcal/mol (eriodictyol/TMPRSS2), -28.991 kcal/mol (eriodictyol/ACE2), -14.82 kcal/mol (umbelliferone/TMPRSS2), and −33.837 kcal/mol (umbelliferone/ACE2), respectively (Table 2). Furthermore, the interface between S protein and human receptor ACE2 was also interfered by these two compounds (Figs. 2C and 3C) with free energy decreases by -33.478 and −36.539 kcal/mol for eriodictyol/S protein-ACE2 and umbelliferone/S protein-ACE2 complexes, respectively (Table 2). Based on these results, eriodictyol and umbelliferone are phytochemicals of *A. argyi* directly targeting multiple proteins associated with the cellular entry of SARS-CoV-2.
Table 2
The binding energy and interaction sites of SARS-CoV-2-related proteins with eriodictyol and umbelliferone.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Proteins</th>
<th>Energy value (kcal/mol)</th>
<th>Amino acids</th>
<th>Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td>eriodictyol</td>
<td>TMPRSS2</td>
<td>-13.05</td>
<td>Cys 148</td>
<td>Hydrogen bond (2.60 Å)</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>Asp 482</td>
<td>Hydrogen bond (2.09 Å)</td>
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<td></td>
<td></td>
<td></td>
<td>Arg 489</td>
<td>Pi-Cation (3.73 Å)</td>
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<tr>
<td>ACE2</td>
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<td>-28.911</td>
<td>Ala 99</td>
<td>Hydrogen bond (2.98 Å)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Phe 40</td>
<td>Pi-Pi stacked (5.02 Å)</td>
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<td></td>
<td>Leu 391</td>
<td>Pi-Alkyl (4.74 Å)</td>
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<td>Asn 394</td>
<td>Hydrogen bond (2.49 Å)</td>
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<td></td>
<td></td>
<td>Arg 393</td>
<td>Hydrogen bond (2.37 Å, 2.63 Å)</td>
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<tr>
<td>S protein-ACE2</td>
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<td>-33.478</td>
<td>Arg 403*</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Glu 406*</td>
<td>Hydrogen bond (2.03 Å)</td>
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<td></td>
<td>Tyr 453*</td>
<td>Hydrogen bond (2.24 Å)</td>
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<td></td>
<td>Ser 494*</td>
<td>Pi-Lone pair (2.69 Å)</td>
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<td></td>
<td></td>
<td>Gly 496*</td>
<td>Hydrogen bond (2.02 Å)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>His 34**</td>
<td>Pi-Cation (3.47 Å); Hydrogen bond (2.55 Å); Amide-Pi Stacked (4.48 Å)</td>
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<td></td>
<td></td>
<td></td>
<td>Glu 35**</td>
<td>Hydrogen bond (2.03 Å)</td>
</tr>
</tbody>
</table>

* Compound interacts to S protein after receptor engagement.

** Compound interacts to ACE2 after receptor engagement.
<table>
<thead>
<tr>
<th>Compounds</th>
<th>Proteins</th>
<th>Energy value (kcal/mol)</th>
<th>Amino acids</th>
<th>Interactions</th>
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</thead>
<tbody>
<tr>
<td>umbelliferone</td>
<td>TMPRSS2</td>
<td>-14.82</td>
<td>Lys 353**</td>
<td>Hydrogen bond (1.83 Å, 1.93 Å)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Arg 150</td>
<td>Attractive charge (4.93 Å)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Pro 369</td>
<td>Hydrogen bond (2.41 Å); Pi-Alkyl (4.54 Å)</td>
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<td></td>
<td></td>
<td></td>
<td>Asp 482</td>
<td>Pi-Anion (3.99 Å)</td>
</tr>
<tr>
<td>ACE2</td>
<td>-33.837</td>
<td></td>
<td>Ala 99 Pi-Alkyl (4.57 Å, 4.30 Å)</td>
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<td></td>
<td></td>
<td></td>
<td>Lys 562</td>
<td>Salt bridge (1.61 Å)</td>
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<tr>
<td>S protein-ACE2</td>
<td>-36.539</td>
<td></td>
<td>Arg 403* Pi-Cation (4.03 Å)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gly 496*</td>
<td>Hydrogen bond (2.61 Å)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Tyr 505*</td>
<td>Pi-Pi T shaped (5.97 Å)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lys 353**</td>
<td>Salt bridge (1.65 Å)</td>
</tr>
</tbody>
</table>

* Compound interacts to S protein after receptor engagement.

** Compound interacts to ACE2 after receptor engagement.

A. argyi and its active compounds downregulate ACE2 and TMPRSS2 expressions in lung epithelial cells

The cytotoxic activities of *A. argyi*, eriodictyol, and umbelliferone were next examined in Beas 2B lung epithelial cell by using MTT assay. Cytotoxicity concentration 50% (CC₅₀) values of *A. argyi*, eriodictyol, and umbelliferone were 4471 µg/ml (Fig. 4A), 212.6 µM (Fig. 4B), and 302.4 µM (Fig. 4C), respectively. Base on the level of CC₅₀, we further addressed whether the expressions of ACE2 and TMPRSS2 are also affected by these potential anti-SARS-CoV agents in preventing the cellular entry of SARS-CoV-2. As shown in Fig. 4, they selectively suppressed the protein expressions of ACE2 and TMPRSS2 (Fig. 4D-4F), and significantly decreased the RNA levels of ACE2 and TMPRSS2 in a dose-depentment manner (Fig. 4G-4J), suggesting that *A. argyi* is able to inhibit SARS-CoV-2 infection via repressing the expressions of cellular ACE2 and TMPRSS2.

A. argyi and its phytochemicals broadly suppress the cell entry of Vpp of SARS-CoV-2 variants
The above data showed that A. argyi could potentially prevent the entry of SARS-CoV-2 to cells with multiple activities including inhibition of cellular ACE2 and TMPRSS2 activity and expressions and blockade of S protein/ACE2 interaction. Therefore, we further assessed the potential efficacy of A. argyi extracts and its phytochemicals in blocking SARS-CoV-2 infection by using SARS-CoV-2 S protein-pseudotyped lentiviral particles (SARS-CoV-2 S-Vpp). HEK-293T cells stably expressing ACE2 protein were employed to test the cellular entry of SARS-CoV-2 Vpp with S protein from different variants followed by the pretreatments with A. argyi extracts. The results displayed that the infection with most SARS-CoV-2 Vpp variants including wild type (WT), B.1.351 (Beta), Lineage P1 (Gamma), and B1.617.2 (Delta) was dramatically repressed by the treatments with A. argyi (Fig. 5A), eriodictyol (Fig. 5B), and umbelliferone (Fig. 5C) in a dose-dependent manner. However, the infections with B1.1.7 (Alpha) and B.1.429 (Epsilon) were not affected by A. argyi extracts (Fig. 5A-5C). The inhibitory concentration 50% (IC\textsubscript{50}) values of A. argyi, eriodictyol, and umbelliferone in antivirual activity were showed in Table 3. The IC\textsubscript{50} values of A. argyi, eriodictyol, and umbelliferone in the detected viruses, except B1.1.7 (Alpha) and B.1.429 (Epsilon), were around 84 ~ 110 µg/ml, 45 ~ 50 µM, and 47 ~ 53 µM, respectively. These findings suggested that A. argyi has the ability to inhibit the infections with SARS-CoV-2.

<table>
<thead>
<tr>
<th></th>
<th>A. argyi (µg/ml)</th>
<th>Eriodictyol (µM)</th>
<th>Umbelliferone (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>87.9028</td>
<td>49.1823</td>
<td>48.1518</td>
</tr>
<tr>
<td>B1.1.7 (Alpha)</td>
<td>275.0</td>
<td>_*</td>
<td>_*</td>
</tr>
<tr>
<td>B.1.351 (Beta)</td>
<td>87.6414</td>
<td>45.4977</td>
<td>51.6283</td>
</tr>
<tr>
<td>Lineage P1 (Gamma)</td>
<td>109.1042</td>
<td>48.9207</td>
<td>47.4213</td>
</tr>
<tr>
<td>B1.612.2 (Delta)</td>
<td>84.6332</td>
<td>45.1579</td>
<td>52.2901</td>
</tr>
<tr>
<td>B.1.429 (Epsilon)</td>
<td>174.0</td>
<td>_*</td>
<td>_*</td>
</tr>
</tbody>
</table>

* Not detection.

Omicrone (B.1.1.529) is a highly mutated variant of SARS-CoV-2 first reported in November 2021, and has become the predominant viral variant of concern (VOC) around the world. To validate the inhibition of Omicron entry, HEK-293T cell expressing ACE2 was infected with the Vpp of Omicron variants and the results showed that umbelliferone significantly and dose-dependently suppressed the cellular entry of Omicron variants BA.1 (Fig. 6A), BA.1.1 (Fig. 6B), and BA.2 (Fig. 6C). Unexpectedly, eriodictyol did not interfere the infection of Omicron variants (Fig. 6A-6C). Our findings suggeted that A. argyi and umbelliferone can be potential agents against the variants of SARS-CoV-2 Omicrone.
Eriodictyol decreases the replication of SARS-CoV-2 by enzymatic inhibition of RdRp

After the entry and uncoating of SARS-CoV-2, 3-chymotrypsin-like protease (3CL\textsuperscript{pro}) and papain-like protease (PL\textsuperscript{pro}) are the virus-encoded cysteine proteases for cleaving the viral polyprotein and then generating nonstructural proteins (nsps) during viral replication [27, 28]. RNA-dependent RNA polymerase (RdRp; also known as nsp12) is a viral replicase for the synthesis of the complementary RNA during the transcriptional cycle of SARS-CoV-2 [29]. Therefore, we further examined the inhibitory effects of tested compounds on the enzymatic activity of 3CL\textsuperscript{pro}, PL\textsuperscript{pro}, and RdRp by performing FRET-base enzymatic activity assays (Fig. 1A) and found that eriodictyol dramatically reduced the activities of these three viral enzymes (Fig. 7A-7C). However, the 50% inhibition concentrations of eriodictyol for 3CL\textsuperscript{pro} and PL\textsuperscript{pro} were over 240 µM (Fig. 7B and 7C). These results displayed that \textit{A. argyi} and its phytochemicals may interfere the cellular entry and RNA replication of SARS-CoV-2 at relative lower doses and showed inhibitory effects on the viral protease activities at higher doses.

Discussion

As of 6th December 2022, COVID-19 pandemic has caused more than 60 million confirmed cases and 6 million deaths worldwide, threatening people’s health and economy globally. The emergency of SARS-CoV-2 variants that facilitate viral replication, transmission, immune escape, and weaken the protective ability of recently developed vaccines [2, 30], creates great concerns on the prevention of SARS-CoV-2 infection. Traditional chinese medicines have been explored as potential therapeutics against COVID-19 and can improve the efficacy of routine treatments while diminishing disease deterioration [31, 32]. For example, Taiwan Chingguan Yihau (NRICM101) has been shown to disrupt virus invasion and host inflammation in patients with SARS-CoV2 infections [33, 34]. Our data showed that \textit{A. argyi}, a well-known herbal used in the Far East, can be a new strategy to prevent the infections with multiple variants of SARS-CoV2 by suppressing their cellular entry as well as viral replication via targeting cellular proteins TMPRSS2 and ACE2 and viral protein RdRp, respectively (Fig. 7D).

Although the binding affinity of jaceosidin and eupatilin to SARS-Cov-2 main protease (3CL\textsuperscript{pro}) has been predicted in molecular docking simulation [35]. In the present study, however, several flavonoids from \textit{A. argyi} including 5,7,3’-Trihydroxy-6,4’,5’-trimethoxyflavone, beta-Rhamnocitrin, eriodictyol, eupatilin, hispidulin, and jaceosidin containing the basic skeleton of bezno-γ-pyrene [36] did not showed the inhibitory effects on the activity of SARS-CoV-2 main protease as well as other related enzymes in the biochemical reaction assays (Table 1), suggesting that the flavonoids in \textit{A. argyi} provide an essential ability to decrease the COVID-19 deterioration by mainly targeting the activity of TMPRSS2.

SARS-CoV-2 lineage B.1.1.529, named Omicron strain, has been documented as a VOC on 26th November 2021 by WHO and becomes the major viral strain in COVID-19 pandemic. Unlike other SARS-CoV-2 variants, Omicron is marked by the large number of mutations across entire genome including its spike
glycoprotein gene [37], contributing to immune escape, a attenuating ability of vaccines, and a higher frequency of reinfections [5, 6]. Additionally, these high mutations of S protein are associated with its increased binding efficacy to host receptor ACE2 [38]. While other SARS-Cov-2 variants require the S protein priming by the host transmembrane protein TMPRSS2 for cellular entry, the infection of Omicron variants can be accomplished by an endocytic route in a TMPRSS2-independent manner [39, 40]. Our results found that both *A. argyi* and umbelliferone diminished the protein level of ACE2 (Fig. 4), accounting for their activity in blocking the cellular entry of Omicron variants (Fig. 6). Therefore, umbelliferone would be the critical phytochemical existed in *A. argyi* to reduce the infection with Omicron variants.

**Conclusions**

In general, this study explored the potent antiviral activity of *A. argyi* and its phytochemicals (eriodictyol and umbelliferone) against multiple SARS-CoV-2 variants with the IC₅₀ values ranging from 87 to 275 µg/ml and 45 to 52 µM, respectively. Mechanistically, all *A. argyi*, eriodictyol, and umbelliferone repress the enzymatic activity of TMPRSS2 and furin for priming S protein, impede the interaction between S protein and ACE2, and inhibit the RdRp-mediated viral replication (Fig. 7D). In addition, *A. argyi* and umbelliferone also showed specific activity against Omicron variants due to their inhibitory effect on ACE2 protein expression. Our findings suggest that *A. argyi* and its phytochemicals possess the potential to be developed as the inhibitors used for preventing or treating the infections with most SARS-CoV-2 variants.

**Methods**

**Cell lines culture**

Beas 2B epithelial cell line of normal human bronchus was grown in Dulbecco's Modified Eagle Medium (DMEM) with low glucose (1 g/L) and sodium pyruvate (Gibco), and ACE2-expressing HEK-293T cell line was cultured in DMEM/Nutrient Mixture (F-12) medium (Gibco). All cell lines were incubated at 37°C in a humidified 5% CO₂/95% incubator.

**Preparation of A. argyi extraction and pure compounds**

The powder of *A. argyi* was obtained from dry plants, and was dissolved and heated in sterilized H₂O at 37°C for 30 minutes. Eriodictyol (Cayman), umbelliferone (Sigma-Aldrich), and other ingredients of *A. argyi* listed in Table 1 were dissolved in DMSO.

**Infection with SARS-CoV-2 S protein-pseudotyped lentiviral particles**

The virus particle pseudotyped (Vpp) of SARS-CoV-2 S protein mutants and the control vesicular stomatitis-G (VSV-G) bearing luciferase were obtained from RNA Technology Platform and Gene
Manipulation Core, Academia Sinica in Taiwan. ACE2-expressing HEK-293T cells were pre-treated with *A. argyi* solution, eriodictyol, and umbelliferone at the indicated concentrations for 2 days and then was infected with the variants of SARS-CoV-2 pseudovirus. After 1 day, the infected cells were lysed with One-Glo™ Luciferase assay buffer (Promega), and the luciferase intensity was measured by Luminescence Plate Reader.

**Measurement of cell viability**

The cell viability was measured according to the manufacturer's protocols of MTT and cell counting kit-8 (CCK-8) assays. Beas 2B cell (5000 cells/well) in 96-well plates were treated with *A. argyi* solution, eriodictyol, or umbelliferone in a dose-dependent manner for 2 days and then subjected to MTT assay (Sigma-Aldrich). The viability of ACE2-expressing HEK-293T cells infected with the variants of SARS-CoV-2 pseudovirus was detected in CCK-8 assays (Sigma-Aldrich).

**Molecular docking simulation**

The structures of proteins and compounds in this study were retrieved from Protein Data Bank (PDB, https://www.rcsb.org/) and were applied to molecular docking calculation (BIOVIA Discovery Studio) to simulate the binding efficacy of tested compounds to S protein, TMPRSS, and ACE proteins.

**FRET-based enzymatic activity assay**

To examine the inhibitory effects of tested compounds on the protease activity in the cleavage of S protein and 3CLpro of SARS-CoV-2, the assay buffer (25 mM Tris 8.0, 150 mM NaCl) containing 15 µg/ml of recombinant proteins with/without eriodictyol was pre-incubated at room temperature for 30 mins. After adding 20 µM of fluorescent protein substrate, the reaction of substrate cleavage was monitored continuously for 6 hours by detecting mNeonGreen fluorescence (excitation: 506 nm/emission: 536 nm) using Synergy™ H1 hybrid multi-mode microplate reader (BioTek Instruments, Inc.). The first 1 hour of the reaction was used to calculate initial velocity ($V_0$). The initial velocity with each compound was calculated and normalized to DMSO control (illustrated in Fig. 1A). Additionally, the effects of tested compounds on the interaction between SARS-CoV-2 Spike S1 and human ACE2 proteins were measured by using TR-FRET assays according to manufacturer's protocol (BPS Bioscience) [41, 42]. Briefly, the recombinant proteins of ACE2 and SARS-CoV-2 Spike S1 were incubated with or without the tested compounds at the indicated concentrations at room temperature for 1 hour. TR-FRET signals were recorded by detecting the emission at a wavelength 620 or 665 nm with the excitation at a wavelength 340 nm (Fig. 1D).

**Western blotting**

Beas 2B lung cells were treated with *A. argyi* solution, eriodictyol, and umbelliferone in a dose-dependent manner for 2 days, and were subsequently lysed in RIPA buffer with protease and phosphatase inhibitors. The protein lysates were separated by SDS-PAGE and then transferred to PVDF membranes. The membranes were blocked in 5% milk in TBST buffer (TBS with 0.1% Tween 20), and were incubated with primary antibodies against TMPRSS2 (Santa Cruz), ACE2 (Genetex), or β-actin (Sigma-Aldrich), at 4 °C for
overnight followed by the incubation with HRP-conjugated second antibody at room temperature for 1 hour. After wash with TBST buffer, the immunoreactive signals were visualized by using enhanced chemiluminescence with ECL reagent.

**Statistical analysis**

Data are shown as the mean ± standard error of the mean (SEM). A two-tailed t-test was used for comparisons. A P value < 0.05 was considered statistically significant.

**Abbreviations**

*A. argyi*

*Artemisia argyi*

TCM

traditional Chinese medicine

SARS-CoV-2

severe acute respiratory syndrome coronavirus 2

TMPRSS2

proteins transmembrane serine protease 2

ACE2

angiotensin-converting enzyme 2

S protein

spike protein

COVID-19

coronavirus disease 2019

VOIs

variant of interests

VOCs

variant of concerns

WHO

World Health Organization

CoVs

coronaviruses

FP

fusion peptide

MERS

middle east respiratory syndrome

13-Oxo-ODE

13-Oxo-9E,11E-octadecadienoic acid

13-HODE

13-hydroxyoctadecadienoic acid
LOX
12/15-lipoxygenase
PPARγ
peroxisome proliferation-activated receptor gamma
CC₅₀
Cytotoxicity concentration 50%
SARS-CoV-2 S-Vpp
SARS-CoV-2 S protein-pseudotyped lentiviral particles
IC₅₀
inhibitory concentration 50%
3CLₚro
3-chymotrypsin-like protease
PLₚro
papain-like protease
nsps
nonstructural proteins
RdRp
RNA-dependent RNA polymerase.

**Declarations**

**Acknowledgments**

Parts of experiments and data analysis were performed with the use of the Medical Research Core Facilities, Office of Research & Development at China Medical University, Taichung, Taiwan, R.O.C.

**Authors’ contributions**

F.-J.C. conceived the study, designed and performed the experiments, analyzed data, interpreted results, and wrote the manuscript. T.K.H., H.-Y.K., C.-S.H., and Y.-L.Y. performed experiments. Y.-C.W., C.-H.T., C.-H.C., and C.-Y.T. revised the manuscript. W.-C.H. and C.-Y.H. conceived and supervised the entire project, experimental designs, interpreted results, and wrote the manuscript.

**Funding**

This study was supported in part by grants from the Ministry of Science Technology, Taiwan (MOST 110-2320-B-039-056-MY3, MOST 111-2320-B-039-016-MY3, MOST 111-2320-B-039-027-MY3, MOST 111-2811-B-039-028, and MOST 111-2320-B-039-036). This work was also financially supported by the “Drug Development Center, China Medical University” from The Featured Areas Research Center Program within the framework of the Higher Education Sprout Project by the Ministry of Education (MOE) in Taiwan.

**Availability of data and materials**
All of performed and analyzed results in this main article are available from the corresponding authors upon request.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interest**

The authors have declared no conflict of interest.

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Eriodictyol suppresses the activity of TMPRSS2 and furin in S protein priming and interferes the interaction between S protein and ACE2. FRET-based enzymatic activity assay (A) was performed to examine the activity of TMPRSS2 (B) and furin (C) in the presence of eriodictyol at the indicated concentrations. Schematics of FRET assay (D) was employed to test the inhibitory effect of eriodictyol on the protein interaction between S protein and ACE2 (E). Data are shown as mean ±SEM from three independent experiments with triplicates. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$. 
Figure 2

The interaction of eriodictyol with the cavity of SARS-CoV-2-related proteins. The predicted binding affinities of TMPRSS2 (PDB ID: 7MEQ; A), ACE2 (PDB ID: 6M0J; B), and S protein (PDB ID: 6M0J; C) with eriodictyol were investigated by using BIOVIA Discovery Studio. Black rings: the amino acid residues of S protein interacting with eriodictyol.
The interaction of umbelliferone with the cavity of SARS-CoV-2-related proteins. The predicted binding affinities of TMPRSS2 (PDB ID: 7MEQ; A), ACE2 (PDB ID: 6M0J; B), and S protein (PDB ID: 6M0J; C) with umbelliferone were investigated by using BIOVIA Discovery Studio. Black rings: the amino acid residues of S protein interacting with umbelliferone.
Figure 4

Eriodictyol and umbelliferone inhibit the expressions of ACE2 and TMPRSS2 in lung epithelial cell. Beas 2B cell line was treated with A. argyi, eriodictyol, and umbelliferone at the indicated concentrations for 2 days, and then the cell viability was detected in MTT assay (A-C). Cytotoxicity concentration 50% (CC\textsubscript{50}) value was calculated and indicated. The cell lysates and total RNA harvested from Beas 2B cells were subjected to Western blot analysis (A-C) and RT-qPCR (D-G) analysis to examine the effects of tested compounds on the protein and mRNA levels of ACE2 and TMPRSS2, respectively. Data are shown as mean ±SEM from three independent experiments with triplicates. * \( p < 0.05 \), ** \( p < 0.01 \), *** \( p <0.001 \), and **** \( p < 0.0001 \).
Figure 5

The inhibitory ability of *A. argyi* extracts on the infection with Vpp of SARS-CoV-2 variants. HEK-293T cells expressing ACE2 gene were infected with SARS-CoV-2 pseudoviral variants with luciferase, including wild type, B.1.1.7 (Alpha), B.1.351 (Beta), Lineage P1 (Gamma), B.1.617.2 (Delta), and B.1.429 (Epsilon) after treatments with *A. argyi* (A), eriodictyol (B) or umbelliferone (C) at the indicated concentrations for 2 days, and subsequently the luciferase activities were measured to determine the infection rate with the
pseudoviruses. Data are shown as mean ±SEM from three independent experiments with triplicates. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, and *** $p<0.0001$.

Figure 6

The inhibition ability of *A. argyi* extracts on the infections with SARS-CoV-2 Omicron variants. HEK-293T cells expressing ACE2 gene were infected with SARS-CoV-2 Omicron pseudoviral variants with luciferase, including BA.1 (A), BA.1.1 (B), and BA.2 (C) after treatments with *A. argyi*, eriodictyol, or umbelliferone at the indicated concentrations for 2 days, and subsequently the luciferase activities were measured to determine the infection rate with these pseudoviruses. Data are shown as mean ±SEM from three independent experiments with triplicates. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, and *** $p<0.0001$. 
Figure 7

**Eriodictyol suppresses the activity of viral replication enzymes.** FRET-based enzymatic activity assay was performed to address the activity of RdRp (A), 3CL\textsuperscript{pro} (B), PL\textsuperscript{pro} (C) under treatment with eriodictyol at the indicated concentrations. 

\( \text{D} \) Schematics of working model of *A. argyi*, eriodictyol, and umbelliferone in the inhibition of SARS-CoV-2 infection. Data are shown as mean ±SEM from three independent experiments with triplicates. \( * \) \( p < 0.05 \), \( ** \) \( p < 0.01 \), \( *** \) \( p < 0.001 \), and \( **** \) \( p < 0.0001 \).
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