

Phytochemicals Characterization of Lianqiao (*Forsythia suspensa*) extract and evaluation of its antiviral and antioxidant activities

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Abstract

Forsythia suspensa (Lianqiao) has long been touted for its anti-viral effects in folklore. Although promising, limited research were conducted to evaluate its potential in defending the severe acute respiratory syndrome coronavirus (SARS-CoV-2). This study evaluated the fruits of *Forsythia suspensa* (Lianqiao), an important economic crop, for the chemical components of its water and ethanol extracts, along with their inhibitory effects on SARS-CoV-2 spike protein binding to angiotensin-converting enzyme 2 (ACE2), inhibitions of ACE2 activity, and capacities to scavenge the selected free radicals. A total of 42 compounds were tentatively identified in the extracts by HPLC-MS/MS analysis. Both water and ethanol extracts of Lianqiao showed great inhibition of ACE2 activity and the binding affinity between SARS CoV-2 spike protein and ACE2. Furthermore, the water extract had greater scavenging capacities against HO[•], DPPH[•] and ABTS^{•+} than the ethanol extract. Findings of this research contribute to the knowledge of different extraction solvent systems and support the potential of Lianqiao in reducing the risk of coronavirus infection and alleviating the symptoms after infection.

INTRODUCTION

Forsythia suspensa, an important economic crop primarily grown in China, is well-known for its fruit Lianqiao (*Forsythiae fructus*) as an essential component of anti-bacterial remedies for decades. It contains many bioactive components including lignans, phenylethanoid glycosides, flavonoids, terpenoids, cyclohexylethanol derivatives, alkaloids and steroids [1], which endow Lianqiao with antioxidant [2], antibacterial [3] and antiviral activities [4]. Law et al reported that Lianqiao extracts prolonged the life span and increased the survival of mice infected with the A/PR/8/34 (H1N1) virus in a dose-dependent manner [5]. Lin et al collected clinical data of 77 patients of severe acute respiratory syndrome (SARS) treated with integrated regular and alternative medicines including botanical foods, and the results showed that the administration of Lianqiao was able to prevent severe SARS cases [6].

Considering the similarity between severe acute respiratory syndrome coronavirus (SARS-CoV-2) spike protein and SARS-CoV spike protein (around 76–78%) [7], the anti-viral and anti-inflammatory activity of Lianqiao-containing formula were examined *in vitro*, and the results showed that Lianqiao had significant inhibitory effects against the SARS-CoV-2 replication, and markedly reduced the production of pro-inflammatory cytokines [8]. This evidence suggests that Lianqiao could have potential effects in reducing the SARS-CoV-2 infection and the progression of severe symptoms of coronavirus infection.

According to its pathogenic mechanism, SARS-CoV-2 enters host cells in a similar mechanism as the SARS-CoV does via the angiotensin-converting enzyme 2 (ACE2) receptor. After the binding of SARS-CoV-2 spike (S) protein to the ACE2, the membrane of the virus and the host cell fuses, and the viral RNA is subsequently released into the cytoplasm of the host cell to establish infection [9]. The sites involved in this process might be focused as potential targets for the development of coronavirus prevention and/or therapeutics. For example, recombinant human (rh) ACE2 is an ACE2 blocker binding to virus S protein, thus preventing host cells from infection [10].

Although some preliminary studies suggested that Lianqiao might be one of the possible candidates for reducing the risk of coronavirus infection and its severe symptoms [8, 11], its potential active components, and the action site and mechanism(s) have never been investigated. Therefore, this study was conducted to investigate the chemical compositions of Lianqiao water and ethanol extracts, and their potential effects on the binding of SARS-CoV-2 spike protein to ACE2, inhibition of ACE2 activity (altering the ACE2 availability), and their antioxidant capacities.

MATERIALS AND METHODS

Materials

Lianqiao samples (*Forsythia suspensa* fruit) at Chinese Pharmacopeia grade were obtained from a local Pharmacy (Rockville, MD, USA). Antioxidant assays-related chemical reagents, including the Folin-Ciocalteu reagent (FC), sodium carbonate (Na₂CO₃), gallic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), fluorescein (FL), disodium hydrogen phosphate dodecahydrate (Na₂HPO₄·12H₂O), sodium dihydrogen phosphate dehydrate (NaH₂PO₄), ferric chloride (FeCl₃), hydrogen peroxide (H₂O₂) (30%), 2,2-diphenyl-1-picrylhydrazyl (DPPH[•]) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma-Aldrich (St. Louis, MO). Formic acid and acetonitrile (LC-MS grade) were obtained from Merck (Darmstadt, Germany). ACE2 Inhibitor Screening Assay Kit (No. 502100) and SARS-CoV-2 spike ACE2 interaction Inhibitor Screening Assay Kit (No. 502050) were purchased from Cayman (Ann Arbor, Michigan USA). All other chemicals used in this study were at analytical grade purity and purchased from Fisher Scientific (Hampton, NH, USA) without further processing.

Sample preparation and extraction

Lianqiao fruit samples were ground using a micromill grinder (Bel Art Products, Pequannock, NJ, USA) to a particle size of 0.42 mm powder. For water extraction, Lianqiao powder was extracted with ultrapure water in a water bath at 85 °C for an initial 2 h (1:10, w/v) and then was extracted for 22 h at ambient temperature. For ethanol extraction, the powder was extracted with pure ethanol for an entire 24 h at ambient temperature (1:10, w/v). The water and ethanol extracts were centrifuged, and the supernatants were stored at -20 °C before analyses.

Chemical compositions of Lianqiao (*Forsythia suspensa* fruit)

Chemical compositions of Lianqiao extracts were identified and characterized by Vanquish UHPLC (Thermo Fisher Scientific, Norristown, PA, USA) coupled with an Orbitrap Fusion ID-X Tribrid mass spectrometer (Thermo Fisher Scientific, Norristown, PA, USA). Chemical components in Lianqiao extract samples were separated using an Agilent Eclipse Plus-C₁₈ UHPLC column (150 mm × 2.1 mm, 1.8 μm) (Santa Clara, CA, USA) connected after an UltraShield pre-column (Santa Clara, CA, USA), with an injection volume of 1 μL. And 0.1% formic acid in water (v/v) and 0.1% formic acid in acetonitrile (v/v) were used as mobile phases A and B, respectively. The column was pre-equilibrated with 2% B for 5 min, then the linear gradient was programmed as follows: 0 min, 2% B;

15 min, 10% B; 35 min, 40% B; 55 min, 95% B, lasting until 60 min, and then re-equilibrated the column with 2% B for 10 min. The flow rate was 0.3 mL/min throughout the injection. For the high-resolution mass spectrometry analyses, the mass scanning process took 39 min in the scan range of m/z 120–1200. The spray voltages in positive and negative ion modes were set to 3900 and 2500 V, respectively. The temperature of the ion transport tube was kept at 300 °C, and the temperature of the vaporizer was 275 °C.

Evaluation on the binding affinity between SARS-CoV-2 spike protein and ACE2

Briefly, 100 μ L of buffer (Immunoassay Buffer C (1X)) and 50 μ L of ACE2 inhibitor screening reagent were added to a 96-well plate as the background. For the control sample, 50 μ L of buffer, 50 μ L of ACE2 inhibitor screening reagent and 50 μ L of spike inhibitor screening reagent were added to the 100% initial activity wells. For the tested sample, 50 μ L of Lianqiao extract sample solution, 50 μ L ACE2 inhibitor screening reagent and 50 μ L of spike inhibitor screening reagent were added to the sample wells. The 96-well plate was covered and incubated for 60 min at ambient temperature with shaking. Then the plate was emptied and rinsed five times with wash buffer, and then 150 μ L of anti-His HRP conjugate was added. The plate was incubated at room temperature with shaking for 30 min, and the washing procedure was repeated. A volume of 175 μ L of TMB substrate solution was added to each well and the plate was incubated again at ambient temperature with shaking for 15 min. A volume of 75 μ L of HRP stop solution was added to each well to terminate the reaction, and absorbance was read at 450 nm.

Evaluation on the ACE2 enzyme activity

A volume of 5 μ L solvent and 85 μ L ACE2 assay buffer were added to the background wells. For the control reaction, 5 μ L solvent, 75 μ L ACE2 assay buffer and 10 μ L of ACE2 enzyme were added to the 100% initial activity wells. And 5 μ L of Lianqiao extract, 75 μ L of ACE2 assay buffer and 10 μ L diluted ACE2 enzyme were added to the sample reaction wells. A volume of 10 μ L of ACE2 substrate was added to all wells to start the reactions. The 96-well plate was covered and incubated for 30 min at ambient temperature in dark. After the incubation, the fluorescence (AF) (λ_{ex} = 320 nm, λ_{em} = 405 nm) of the plate was recorded.

Determination of phenolic contents

The phenolic contents of Lianqiao water and ethanol extracts were examined according to the method described in a published paper with minor modifications [12]. One aliquot of 3 mL of ultrapure water was mixed with 50 μ L of Lianqiao extracts or gallic acid standards, followed by an addition of 250 μ L of FC. After waiting 5 min, 750 μ L of 20% (w/v) Na_2CO_3 was added to activate the reaction. The tubes were covered with parafilm and aluminum foil for 2 h at ambient temperature in the dark. The absorbances were measured at a wavelength of 765 nm and the phenolic contents were expressed as milligram gallic acid equivalents per gram of Lianqiao sample (mg GAE/g).

Evaluation of free radical scavenging capacities

The hydroxy (HO), DPPH and ABTS⁺ radicals scavenging capacities of the Lianqiao water and ethanol extracts were evaluated following the method described in our previously published article [13]. Trolox was selected as a standard when determining all these three radical's scavenging capacities. Briefly, for the HO[•] scavenging capacity assay, 170 μ L fluorescein was mixed with 30 μ L of Lianqiao water and ethanol extracts or standard (Trolox), followed by the addition of 40 μ L H_2O_2 and 60 μ L FeCl_3 . The fluorescence intensities of the reaction mixture were recorded every five min over an 8 h duration with an excitation wavelength of 485 nm and emission wavelength of 535 nm. For the DPPH[•] scavenging capacity assay, 100 μ L Lianqiao water and ethanol extracts or Trolox standard was mixed with 100 μ L of freshly prepared DPPH solution. The absorbances of the reaction mixture were recorded every min over a 90 min duration at a wavelength of 515 nm. For the ABTS[•] scavenging capacity assay, 160 μ L Lianqiao water and ethanol extracts or Trolox standard was mixed with 2 mL of freshly prepared ABTS[•] working solution to initiate the reaction. After allowing a 90 sec reaction duration, the absorbances of the mixture was recorded at the wavelength of 734 nm. The determined HO[•], DPPH[•] and ABTS[•] values were expressed in μ mol of Trolox equivalents per gram of dry Lianqiao sample (μ mol TE/g).

Statistical analysis

The t-test was conducted to determine the differences in the inhibitory, radical scavenging activities and the phenolic content between Lianqiao water and ethanol extracts using IBM SPSS Statistics (version 25.0, SPSS, Inc., USA), and $P < 0.05$ was considered statistically significant. The mass data and spectrums were obtained and analyzed by Xcalibur™ (Version 4.2, Thermo Fisher Scientific, USA).

RESULTS AND DISCUSSION

Chemical compositions of the Lianqiao (*Forsythia suspensa* fruit) Extracts.

Forty-two compounds were tentatively identified with the molecular weights referring to high-resolution MS, MS² fragment information and literature review. Among these compounds, there were 8 lignans (**30**, **32–35**, **37**, **38**, **40**), 9 phenylethanoid glycosides (**4**, **9**, **13**, **18**, **20**, **26–29**), 5 terpenoids (**16**, **36**, **39**, **41**, **42**), 11 phenolics (**1**, **3**, **5**, **8**, **11**, **12**, **14**, **19**, **21**, **23**, **25**) and 9 other compounds (Fig. 1). As shown in Table 1, Lianqiao samples with different extraction solvents were observed with varied identified compounds and different relative contents. Generally, 37 compounds were detected and identified in Lianqiao water extracts, and 25 compounds were identified in ethanol extracts. For the relative contents, halleridone (**7**), forsythalian B (**35**), (+)-1-hydroxylpinoresinol (**33**), were the three major compounds identified in water extracts, while forsythalian B (**35**), (+)-1-hydroxylpinoresinol (**33**), azelaic acid (**31**), epipinoresinol-4'- β -D-glucopyranoside (**32**) and forsythoside H (**26**) were the top five in ethanol extracts. Forsythalian B (**35**), (+)-1-hydroxylpinoresinol (**33**) and forsythoside H (**26**) could be recognized as the three primary compounds in both Lianqiao water and ethanol extracts. Compounds 28, 35 and 18 were selected as examples to elucidate the identification progress. The rest major chemical components in the Lianqiao extracts were identified consistently using the approaches described below.

For compound **28**, the molecular ion detected in the positive ion mode ($[M + H]^+$) is m/z 625.21246, which is consistent with $C_{29}H_{37}O_{15}$ (mass precision, -0.379 ppm), indicating that the molecular formula of compound **28** is $C_{29}H_{36}O_{15}$. The main fragment ions in the normal mode are 479.1540 $[M + H - Ara]^+$, 471.1494 $[M + H - C_8H_{10}O_3]^+$, 325.0915 $[M + H - Ara - C_8H_{10}O_3]^+$. According to these results and literature, compound **28** was identified as forsythoside A, a type of phenylethanoid glycosides. The content of forsythoside has been designated as a marker for Lianqiao's quality control according to Chinese Pharmacopoeia [14]. Forsythoside A has been proven to exhibit antioxidant, anti-inflammatory and antiviral activities, and is known as one of the representative active substances in Lianqiao [15–17]. The viral infections can induce the production of superoxide molecules, leading to oxidative stress within the host [18]. Meanwhile, SARS-CoV-2 was found to trigger the inflammatory storm, which is a significant factor leading to mortality in numerous patients throughout the ongoing pandemic [19]. The antioxidant and anti-inflammatory properties of forsythoside A may help quench reactive oxygen species and regulate the immune function, and thereby ameliorate the symptoms of COVID-19. In addition, forsythoside A was found to directly act on SARS-CoV-2 main protease through CYS145 and HIS41 sites, which may inhibit the SARS-CoV-2 replication and transcription [20].

For compound **35**, in the positive ion mode ($[M + H]^+$), the molecular ion is m/z 389.15921, which is consistent with $C_{21}H_{25}O_7$ (mass accuracy, -0.693 ppm). And in the negative ion mode ($[M - H]^-$) the molecular ion is m/z 387.14408, which is consistent with $C_{21}H_{23}O_7$ (mass accuracy, 0.647 ppm). These results indicate that the molecular formula of compound **35** is $C_{21}H_{24}O_7$. The fragment ions detected in the positive mode are at m/z 371.1485 $[M + H - H_2O]^+$, 247.0963 $[M + H - H_2O - 124]^+$, 217.0858 $[M + H - H_2O - 124 - CH_2O]^+$. The fragment ions of $[M + H - H_2O - 124]^+$ are formed by the reduction of methoxyphenol group. The major fragment ion in the negative mode is 372.1202 $[M - H - CH_3]^-$. Based on these results and literature review, compound **35** was identified as forsythialan B. Forsythialan B is a tetrahydrofuran, one of the representative lignans in Lianqiao, which has been proven to be protective against peroxynitrite-induced oxidative stress [21].

For compound **18** (Fig. 2), the molecular ion is m/z 487.14523 in the negative mode ($[M - H]^-$), which is consistent with $C_{21}H_{27}O_{13}$ (mass accuracy, 1.258 ppm), indicating its molecular formula is $C_{21}H_{28}O_{13}$. The fragment ions detected in the negative ion mode are at m/z 469.1335 $[M - H - H_2O]^-$, 427.1229 $[M - H - H_2O - C_2H_2O]^-$, 397.1122 $[M - H - H_2O - C_2H_2O - CH_2O]^-$, 233.0445 $[M - H - H_2O - C_2H_2O - CH_2O - O-Ara]^-$, 203.0340 $[M - H - H_2O - C_2H_2O - CH_2O - O-Ara - CH_2O]^-$, 179.0341 $[M - H - H_2O - C_2H_2O - CH_2O - O-Ara - CH_2O - C_2]^-$. Based on the data we collected and the existing evidence from the literature, compound **18** was tentatively identified as cistanoside F, which has been found in other botanicals [22, 23].

Inhibition on the binding affinity between SARS-CoV-2 spike protein and ACE2

Significant differences were observed in the inhibitory effects of SARS-CoV-2 spike protein and ACE2 interaction between Lianqiao water and ethanol extracts (Fig. 3). Notably, the water extract at 3.3 mg dry botanical equivalents/mL in the testing mixture (WE3.3) inhibited only 1.43% of the interaction activity, while the initial ethanol extracts (EE3.3) inhibited 45.57% of the interaction activity at the same concentration. At a concentration of 1.7 mg dried botanical equivalent/mL, the ethanol extracts (EE1.7) were observed with a 7.45% inhibition on SARS CoV-2 spike protein binding to ACE2. However, the inhibitory effects were negligible with a similar concentration of the water extracts (WE1.7, 1.7 mg dry botanical equivalents/mL in the testing mixture). This is possible because the ethanol extracts of Lianqiao exhibit greater activity in inactivating and denaturing the SARS-CoV-2 virus [24]. Given that SARS-CoV-2 enters host cells by binding ACE2 as the receptor, intervention and inhibition of this process may be one of the key points for preventing the infection and relieving the symptoms of COVID-19.

Inhibitor on ACE2 enzyme activity

Both Lianqiao water and ethanol extracts exhibited inhibitory effects on ACE2 (Fig. 4). Specifically, the initial water extracts (33.3 mg dry botanical equivalents/mL in the testing mixture, WE33.3) inhibited 77.82% of ACE2 activity, and an inhibition of 14.93% was observed with the initial water extracts (WE3.3) at a concentration of 3.3 mg dry botanical equivalents/mL. In contrast, the initial ethanol extracts (33.3 mg dry botanical equivalents/mL, EE33.3) inhibited 68.33% of ACE2 activity, which was about 12.2% lower than the water extracts at the same concentration. Current evidence suggests that ACE2 is the receptor for SARS-CoV-2 to enter host cells, and coronavirus aggravates infection by upregulating ACE2 expression via interferon [25, 26]. Therefore, the inhibition of its own activity may be one approach to interfering with the infection process. However, it is worth noting that ACE2 is also a critical component of the renin-angiotensin system (RAS), which regulates pathological responses such as blood pressure, inflammation, and oxidative stress [9, 27]. And further research is still needed to determine possible inhibition effects of ACE2 activity by Lianqiao extracts in simultaneously inducing some other side effects.

Phenolic contents and free radical scavenging capacities

Phenolics are capable of scavenging free radicals or chelating metals, which competitively inhibit the oxidation process [28]. The TPC values of Lianqiao water and ethanol extracts were 45.19 and 6.89 mg GAE/g dry botanical, respectively (Fig. 5). The TPC values of the Lianqiao water extracts in this present study were more remarkable than that of a previous study, which was reported to be 13.2–13.9 mg GAE/g dry botanical from Lianqiao methanol (70%, v/v) extracts [2]. These results indicated that different extraction methods might lead to substantial differences in the TPC of Lianqiao extracts, which was aligned with our chemical profile findings.

Free radicals in biological systems can damage a variety of cell components and cause adverse effects. For instance, peroxidation of membrane phospholipids changes the fluidity and permeability of cell membranes, leading to cell dysfunction or even death, which will thereby result in metabolic disorders and a variety of diseases [29]. Meanwhile, the reactive oxygen species (ROS) have also been discovered to be involved in the pathogenesis of neurodegenerative diseases [30], atherosclerosis [31], asthma [32], and chronic kidney diseases [33]. In COVID-19, SARS-CoV-2 unbalances oxidative homeostasis and stimulates the production of ROS, triggers inflammatory reactions and then causes organ failure [34]. Cytokine storm, a severe inflammatory reaction mediated by oxidative stress, leads to even severe symptoms [25, 34]. Therefore, evaluating the antioxidant abilities of certain components is

essential when developing potential nutraceuticals for COVID-19 treatment. Considering the difference in mechanisms of action and the targeted free radicals, three free radical scavenging assays, including HOSC, RDSC and ABTS were selected to examine the *in vitro* antioxidant effects of Lianqiao extracts.

The free radical scavenging capacities of Lianqiao water and ethanol extracts are shown in Fig. 6. The HOSC, RDSC and ABTS values of the water extracts were 448.48, 66.36 and 121.29 $\mu\text{mol TE/g}$, respectively, which were significantly greater than those of the ethanol extracts (154.04, 3.55 and 33.83 $\mu\text{mol TE/g}$, respectively). Overall, the results suggested that both Lianqiao water and ethanol extracts possessed considerable antioxidant capacities, whereas Lianqiao water extracts had a superior antioxidant capacity than ethanol extract. Specifically, for the HOSC assay, the extracts of Lianqiao showed significant efficacy in scavenging hydroxyl radicals, which is of clinical importance due to the critical role that hydroxyl radicals play in irreversible damage caused by oxidative stress in our body [35]. It is worth noting that the antiradical activity inhibited by using stable free radicals, such as DPPH and ABTS, may not be fully representative of the antioxidant activity of the tested samples, and they do not exist in biological systems [35]. Therefore, further research is needed to validate the antioxidant activity of Lianqiao extracts with an *in vivo* study design.

In conclusion, this present study managed to identify the chemical components of Lianqiao water and ethanol extracts, and Cistanoside F was found in Lianqiao extracts for the first time. This study contributed to a preliminarily understanding of the effects of Lianqiao water and ethanol extracts on preventing and/or treating SARS-CoV-2-related infections by evaluating the SARS-CoV-2 spike protein and ACE2 interaction, ACE2 enzyme activity and antioxidant capacities. The Lianqiao water extract showed stronger inhibition on the activity of ACE2 than the ethanol extract, but its inhibitory effects on the binding of SARS-CoV-2 spike protein and ACE2 were weaker than that of ethanol. In addition, the scavenging capacities of water extract on $\text{HO}^\bullet$, $\text{DPPH}^\bullet$ and $\text{ABTS}^{\bullet+}$ were stronger than those of ethanol extract. These suggest that the extraction solvents matter and are likely to result in different extracted components and bioactivities. Taken together, this study provides scientific support for the application of Lianqiao as a potential integrant for the prevention and treatment of SARS-CoV-2 infection and contributes to the insights into an alternative remedy for coronavirus infection by utilizing Lianqiao extracts.

Declarations

The authors declare no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical Approval: Not applicable.

Authors' contributions

Conceptualization, Liangli Yu; Formal analysis, Boyan Gao and Hanshu Zhu; Funding acquisition, Boyan Gao and Liangli Yu; Investigation, Boyan Gao, Hanshu Zhu, Zhihao Liu, Thomas Wang, Xiaohua He, Jianghao Sun, Yanfang Li, Xianli Wu, Pamela Pehrsson and Yaqiong Zhang; Methodology, Boyan Gao, Zhihao Liu and Yanfang Li; Writing – original draft, Boyan Gao; Writing, review editing, Boyan Gao, Yuanhang Yao and Liangli Yu. All authors reviewed the manuscript.

Availability of data and materials

The data that support the findings of this study are available on request from the corresponding author Y.Y.

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Tables

Table 1. Characterization of compounds identified in Lianqiao (*Forsythia suspensa* fruit)

ID	Positive mode (ESI ⁺)				Negative mode (ESI ⁻)				Formula	Name	Struc
	Retention time	Exptl. [M+H] ⁺	Fragment ions	Mass error (ppm)	Retention time	Exptl. [M-H] ⁻	Fragment ions	Mass error (ppm)			
1.	1.03	127.03889	nd	-0.635	nd	nd	nd	nd	C ₆ H ₆ O ₃	Benzene-1,2,4-triol	
1.	2.78	268.10385	136.0612	-0.673	nd	nd	nd	nd	C ₁₀ H ₁₃ O ₄ N ₅	Adenosine	
1.	3.14	169.04961	151.0385, 123.0436	0.442	3.24	167.03466	123.0447, 108.0213	4.638	C ₈ H ₈ O ₄	Vanillic acid	
1.	3.55	317.12292	299.1143, 263.0929, 221.0820, 137.0603	-0.549	3.72	315.10797	179.0567 , 161.0459	1.669	C ₁₄ H ₂₀ O ₈	Isomer of Hydroxytyrosol glucoside	
1.	3.82	165.05450	147.0434 , 119.0486	-0.731	nd	nd	nd	nd	C ₉ H ₈ O ₃	Isomer of p-Coumaric acid	
1.	nd	nd	nd	nd	4.34	191.05573	145.0500, 129.0552, 101.0602	3.745	C ₇ H ₁₂ O ₆	Quinic acid	
1.	4.83	155.07012	137.0592, 109.0644	-0.972	nd	nd	nd	nd	C ₈ H ₁₀ O ₃	Halleridone	
1.	nd	nd	nd	nd	5.74	153.01794	109.0284	-1.929	C ₇ H ₆ O ₄	Protocatechuic acid	
1.	6.49	317.12283	299.1146 , 281.1040	-0.833	nd	nd	nd	nd	C ₁₄ H ₂₀ O ₈	Isomer of Hydroxytyrosol glucoside	
1.	nd	nd	nd	nd	7.89	315.07126	153.0197 , 109.0295	0.640	C ₁₃ H ₁₆ O ₉	Protocatechuic acid-O-glucopyranoside	
1.	9.40	139.03885	111.0436, 93.0694	-0.867	10.15	137.02404	93.0342	5.251	C ₇ H ₆ O ₃	Isomer of 3,4-dihydroxybenzaldehyde	
1.	9.97	139.03783	111.0433	-8.204	9.32	137.02316	120.8092, 103.4394	-1.172	C ₇ H ₆ O ₃	Isomer of 3,4-dihydroxybenzaldehyde	
1.	10.45	317.12305	299.1147 , 281.1026, 263.0923	-0.139	10.28	315.10757	153.0561, 135.0455	0.400	C ₁₄ H ₂₀ O ₈	Isomer of Hydroxytyrosol glucoside	
1.	nd	nd	nd	nd	10.69	353.08662	191.0551, 179.0340	-0.251	C ₁₆ H ₁₈ O ₉	Isomer of Chlorogenic acid	
1.	nd	nd	nd	nd	11.20	341.08682	179.0339	0.327	C ₁₅ H ₁₈ O ₉	Isomer of Caffeic acid 3-glucoside	
1.	nd	nd	nd	nd	11.43	375.12872	213.0763 , 125.0603	0.391	C ₁₆ H ₂₄ O ₁₀	Loganic acid	
1.	nd	nd	nd	nd	11.62	389.10786	345.1174, 183.0654	0.057	C ₁₆ H ₂₂ O ₁₁	Secologanoside	
1.	nd	nd	nd	nd	14.68	487.14523	469.1335 , 427.1229, 397.1122,	1.258	C ₂₁ H ₂₈ O ₁₃	Cistanoside F	

							233.0445, 203.0340, 179.0341				
1.	14.40	181.04939	163.0387	-0.802	14.80	179.03457	135.0444	3.825	C ₉ H ₈ O ₄	Isomer of Caffeic acid	
1.	14.45	463.18079	317.1227, 301.1280	-0.459	14.85	461.16580	315.1071, 205.0709, 135.0445	0.970	C ₂₀ H ₃₀ O ₁₂	Forsythoside E	
1.	nd	nd	nd	nd	15.00	353.08679	191.0551	0.231	C ₁₆ H ₁₈ O ₉	Isomer of Chlorogenic acid	
1.	nd	nd	nd	nd	17.73	493.13428	475.1233	0.461	C ₂₃ H ₂₆ O ₁₂	Derhamnosyl suspensaside	
1.	nd	nd	nd	nd	17.75	163.03867	146.3310, 135.0436, 118.9913	-1.844	C ₉ H ₈ O ₃	Isomer of p-Coumaric acid	
1.	18.20	477.13864	459.1276, 325.0911, 163.0387	-1.044	nd	nd	nd	nd	C ₂₃ H ₂₄ O ₁₁	Plantasioside	
1.	18.43	165.05457	147.0435	-0.307	18.67	163.03963	119.0498	4.044	C ₉ H ₈ O ₃	Isomer of p-Coumaric acid	
1.	22.13	625.21222	607.2369, 589.2271	-0.762	22.27	623.19714	461.1649, 443.1544	-0.150	C ₂₉ H ₃₆ O ₁₅	Forsythoside H	
1.	nd	nd	nd	nd	23.14	477.13669	315.1053, 179.0331, 161.0226	-5.131	C ₂₃ H ₂₆ O ₁₁	Calceolarioside A	
1.	23.20	625.21246	479.1540, 471.1494, 325.0915	-0.379	nd	nd	nd	nd	C ₂₉ H ₃₆ O ₁₅	Forsythoside A	
1.	23.40	623.19672	477.1382, 325.0912	-0.524	23.22	621.18140	487.1441, 469.1337, 459.1494	0.005	C ₂₉ H ₃₄ O ₁₅	Suspensaside A	
1.	nd	nd	nd	nd	24.49	519.18640	357.1328	0.601	C ₂₆ H ₃₂ O ₁₁	Pinoresinol-4'-O-glucopyranoside	
1.	24.80	189.11209	171.1012, 125.0957	-0.241	24.67	187.09706	125.0967	3.071	C ₉ H ₁₆ O ₄	Azelaic acid	
1.	25.89	521.20148	359.1483	-0.495	26.20	519.18646	357.1331	0.716	C ₂₆ H ₃₂ O ₁₁	Epipinoresinol-4'-β-D-glucopyranoside	
1.	26.01	375.14349	357.1327	-0.905	26.12	373.12838	343.1174, 313.1071	0.538	C ₂₀ H ₂₂ O ₇	(+)-1-hydroxylpinoresinol	
1.	26.08	359.14876	341.1375, 223.0961, 137.0594	-0.431	nd	nd	nd	nd	C ₂₀ H ₂₂ O ₆	Isomer of Pinoresinol	
1.	29.23	389.15921	371.1485, 247.0963, 217.0858	-0.693	29.52	387.14408	372.1202	0.647	C ₂₁ H ₂₄ O ₇	Forsythialan B	
1.	31.11	335.22156	317.2100,	-0.376	nd	nd	nd	nd	C ₂₀ H ₃₀ O ₄	Isomer of Dehydropinifolic acid	

			289.2160								
1.	31.49	359.14883	341.1378, 223.0962, 137.0595	-0.236	31.46	357.13330	342.1093, 313.1433, 209.0810	0.098	C ₂₀ H ₂₂ O ₆	Matairesinol	
1.	32.70	359.14868	341.1376, 311.1272, 235.0961, 217.0858	-0.654	nd	nd	nd	nd	C ₂₀ H ₂₂ O ₆	Isomer of Pinoresinol	
1.	32.78	335.22141	317.2107, 299.2003	-0.823	nd	nd	nd	nd	C ₂₀ H ₃₀ O ₄	Isomer of Dehydropinifolic acid	
1.	32.81	359.14627	341.1359, 235.0946	-7.364	nd	nd	nd	nd	C ₂₀ H ₂₂ O ₆	Isomer of Pinoresinol	
1.	33.55	335.22177	317.2105, 299.2001, 289.2157	0.251	nd	nd	nd	nd	C ₂₀ H ₃₀ O ₄	Isomer of Dehydropinifolic acid	
1.	34.45	335.22156	317.2102, 299.1998, 287.1999	-0.376	nd	nd	nd	nd	C ₂₀ H ₃₀ O ₄	Isomer of Dehydropinifolic acid	

Exptl. [M + H]⁺ represents experimental *m/z* of molecular ion in positive mode; Exptl. [M – H][–] represents experimental *m/z* of molecular ion in negative mode; Ref represents references; WE: water extracts of Lianqiao; EE: ethanol extracts of Lianqiao; n/d represents not detectable.

Figures

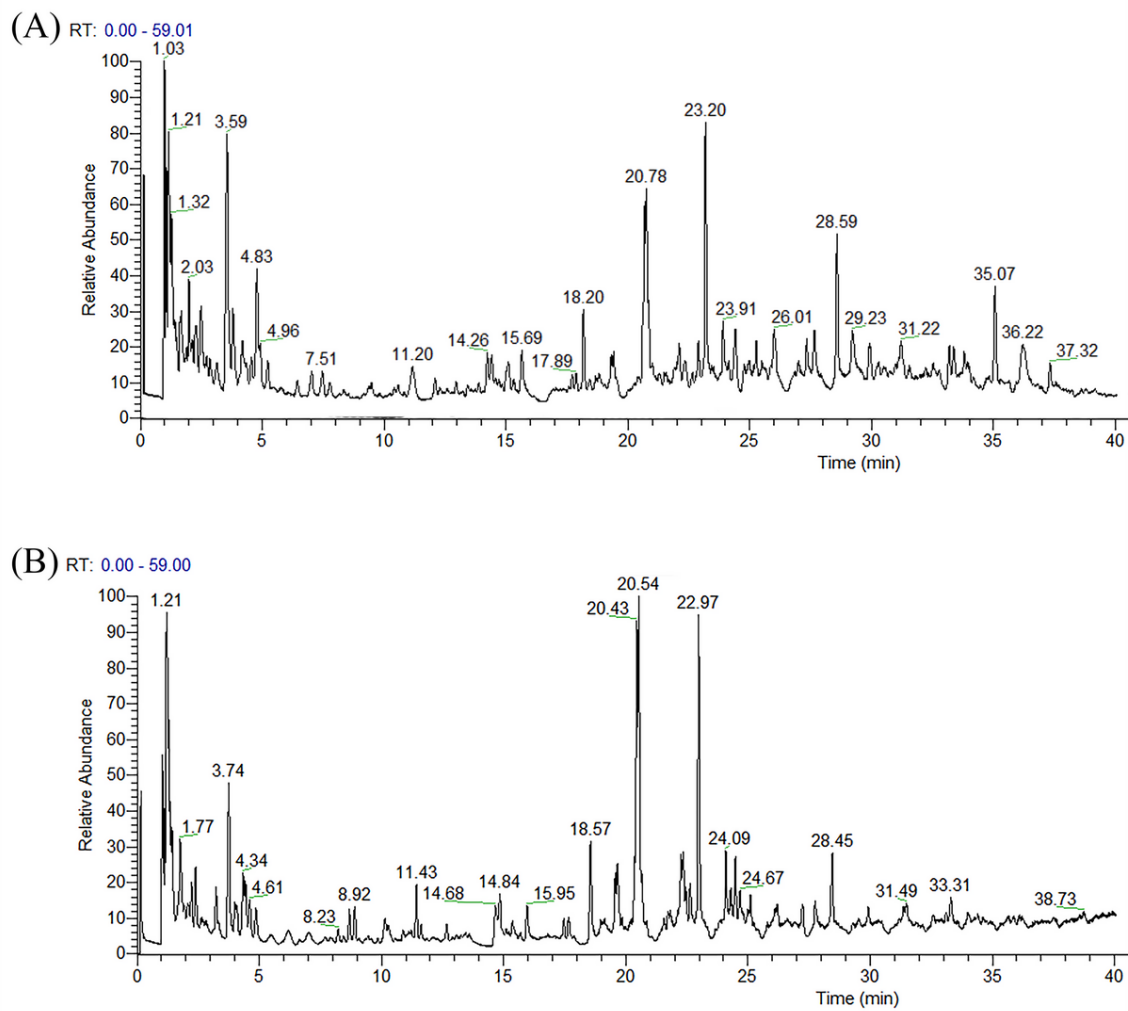


Figure 1

Chromatogram of Lianqiao water extract (WE) in positive (A) and negative (B) ionization mode.

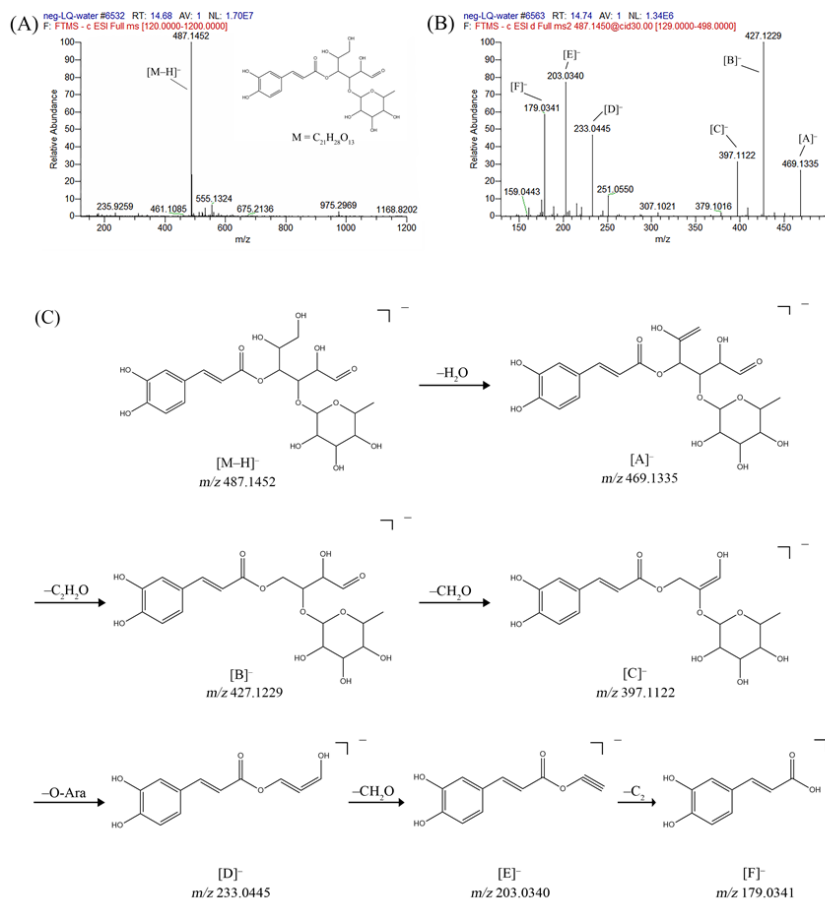


Figure 2

Identification of cistanoside F (compound 18). (A) The full scan mass spectrum in negative ionization mode, (B) The MS² fragments in negative ionization mode, (C) demonstration of inferred fragmentation of [M - H]⁻ at m/z 487.1452.

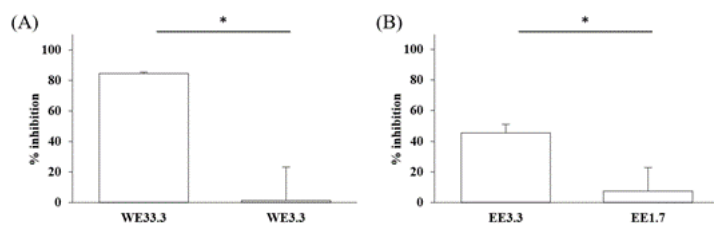


Figure 3

The ability of Lianqiao (A) water and (B) ethanol extract to inhibit the binding of SARS-CoV-2's spike protein to ACE2 at two concentrations: 33.3 and 3.3 mg dry Lianqiao /mL for water extracts and 3.3 and 1.7 mg dry Lianqiao /mL for ethanol extracts. Results are represented as mean $\pm$ standard deviation of triplicate experiments and * represent a statistical difference between the two groups ($p < 0.05$).

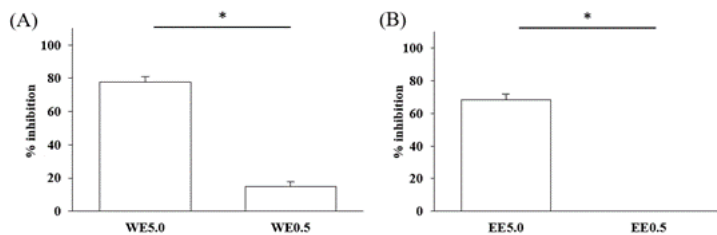


Figure 4

The ability of Lianqiao (A) water and (B) ethanol extracts to inhibit ACE2 activity at two concentrations: 5.0 and 0.5 mg dry Lianqiao /mL. Values are represented as mean ± standard deviation of triplicate experiments and * represent a statistical difference between the two groups ($p < 0.05$).

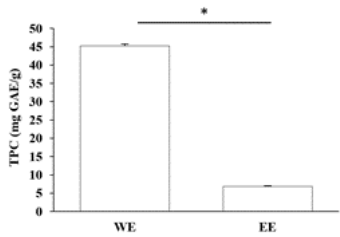


Figure 5

Total phenolic content (TPC) of Lianqiao water extracts (WE) and ethanol extracts (EE). Results are expressed as mean ± standard deviation of triplicate experiments and * represent a statistical difference between the two groups ($p < 0.05$).

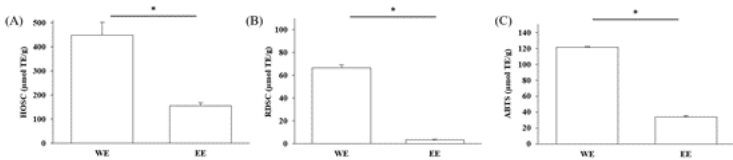


Figure 6

The relative free radical scavenging capabilities of Lianqiao water extracts (WE) and ethanol extracts (EE) against (A) HO•, (B) DPPH• and (C) ABTS•+. Results are expressed as mean ± standard deviation of triplicate experiments and * represent a statistical difference between the two groups ($p < 0.05$). All values are reported on a dry Lianqiao weight basis.