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Synergistic deciphering of bioenergy production and electron transport characteristics to screen traditional Chinese medicine (TCM) for COVID-19 drug development

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ABSTRACT

Background: Traditional Chinese medicine (TCM) has been used as an "immune booster" for disease prevention and clinical treatment since ancient China. However, many studies were focused on the organic herbal extract rather than aqueous herbal extract (AHE; decoction). Due to the COVID-19 pandemics, this study tended to decipher phytochemical contents in the decoction of herbs and derived bioactivities (e.g., anti-oxidant and anti-inflammatory properties). As prior works revealed, the efficacy of Parkinson's medicines and antiviral flavonoid herbs was strongly governed by their bioenergy-stimulating proficiency.
Methods: Herbal extracts were prepared by using a traditional Chinese decoction pot. After filtration and evaporation, crude extracts were used to prepare sample solutions for various bioassays. The phytochemical content and bioactivities of AHEs were determined via ELISA microplate reader. Microbial fuel cells (MFCs) were used as a novel platform to evaluate bioenergy contents with electron-transfer characteristics for antiviral drug development.
Significant findings: Regarding 18 TCM herbal extracts for the prevention of SARS and H1N1 influenza, comparison on total polyphenol, flavonoid, condensed tannins and polysaccharides were conducted. Moreover, considerable total flavonoid contents were detected for 11 herb extracts. These AEHs were not only rich in phytonutrient contents but also plentiful in anti-oxidant and anti-inflammatory activities. Herbs with high polyphenol content had higher antioxidant activity. *Forsythia suspensa* extract expressed the highest inhibition against nitric oxide production for anti-inflammation. MFC bioenergy-stimulating studies also revealed that top ranking COVID-19 efficacious herbs were both bioenergy driven and electron mediated. That is, electron transfer-controlled bioenergy extraction was significant to antiviral characteristics for anti-COVID-19 drug development.

1. Introduction

Although herbal medication has been considered "lack of logical mechanism of action" behind clinical treatment, this first-attempt study tended to decipher that the bioenergy-extracting and electron transporting performance of medicinal herbs directly influence such disease curing performance (e.g., anti-COVID-19 efficacy as stated afterwards). Traditional Chinese medicine (TCM) practitioners (also known as *Zhongyi*) believe that every natural product own its nature (e.g., cold-, cool-, warm-, hot-natured) and flavor (e.g., sour, sweet, bitter, pungent, salty). As principles of TCM indicated, different properties influenced the balance between *Yin* and *Yang* and serve as a supply for different body parts [1]. Thus, Cantonese are known to incorporate herbal/medicinal soup (*yaoshan*) in daily diet to maintain balance of human body. Although Chinese medicine has been considered as "pseudoscience" without logical mechanism of action, TCM practitioners emphasized that every organ in the system (human body) will only work well when *Yin* and *Yang* are in balance. In

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addition, healthy *qi* (immune system) will be enriched as practices of TCM stated. If not, the system will develop syndromes and be prone to sickness caused by external invaders (evil qi). Interactive synergy and antagonism of diverse herbs in TCM directly influenced transient dynamics of *Yin* and *Yang* for health preservation. In particular, if organisms to cause infectious disease passed from various "carriers" (e.g., persons, animals, insects, contaminated food or water), such exogenous invasion would break through the balance of *Yin* and *Yang* and an epidemic outbreak in the community [2] may be inevitable. Here, for efficacious COVID-19 drug development, this novel study tended to uncover that such *yin*-and-*yang* treatment would be strongly associated with bioenergy extraction and electron transport phenomena, tending to decipher the possible logical mechanism of TCM action.

Since the first case of coronavirus disease 2019 (COVID-19) identified in Wuhan, China at late 2019, a newly discovered β-coronavirus of such contagious disease for the ongoing pandemic (i.e., severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)) was identified by the International Committee on Taxonomy of Viruses (ICTV). Due to the spread rate and range of affected regions and infected population, World Health Organization (WHO) stated SARS-CoV-2 as a pandemic on March 2, 2020 [3]. As reported, 96% of the SARS-CoV-2 genome is related to bat coronavirus, which beliefs to be the origin of this virus. SARS-CoV-2 interacts with the host's cell's angiotensin-converting enzyme II (ACE-2) for viral entry [4]. From the perspective of TCM practitioners, SARS-CoV-2 is caused by the accumulation of dampness (*shi qi*) from the external into the lungs (interior). Dampness will affect the movement of healthy *qi* in the body that cause the development of damp-heat toxin and congestion in the lungs; it is usually observed for patients in severe and critical stages. Heat development will deplete *qi* and *Yin* (especially on the lungs and spleen), generally observed during recovery. By understanding the syndrome developed as the disease progressed, the TCM practitioners select herbs with properties like notify qi to eliminate possible dampness, wind, and heat accumulated in the body [1,2]. Following the concept of "Jun-Chen-Zuo-Shi", these herbs would work together as a preventative TCM prescription against viral infection [1,5].

For SARS-CoV-2, TCM practitioners from different regions proposed different combinations of herbal formulae as preventative TCM prescriptions. Among 54 available herbs of preventive TCM formulae, nineteen herbs were commonly used (at least three times). Based on the previous studies [1,5], organic extracts of the herbs were mentioned to have diverse bioactivities (e.g., anti-oxidant, anti-inflammatory, or immunomodulatory characteristics). However, rare studies were conducted on aqueous herbal extracts (AHEs) (i.e., decoction), which is the traditional method for TCM preparation. As literature of SARS and H1N1 influenza prevention indicated [6], this study selected 18 TCM to uncover interactions between bioenergy potentials and the total phenolic, total flavonoids, total condensed tannins, and total polysaccharides in AHEs of a total 18 herbs. For the anti-oxidant activity of the extracts, free radical scavenging assay (DPPH) and ferric ion reducing anti-oxidant power (FRAP) assay were conducted. Then, nitric oxide (NO) inhibition was carried out to screen for extracts with anti-inflammatory activity. Lastly, the toxicity of the extract was determined by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium (MTT) reduction assay. These will be correlated to the aspects of biomedical science and bioenergy technology as fundamental chemical engineering principles of novel practical applications.

As a matter of fact, recent findings [6–11] indicated that several refreshment-oriented herbal medicines (e.g., *Lonicera japonica, Syzygium aromaticum, Camellia* green tea) and brain disorder-treated neurotransmitter medicines were found to be electrochemically active due to their promising electron-shuttling and bioenergy-stimulating capabilities. They possessed antioxidant polyphenolics structures; in particular, most of them contained abundant compositions bearing chemical structures with *ortho*-dihydroxyl substituents on benzene ring for outstanding electron-shuttling potentials. These seemed to point out that the electron transfer (ET)-steered and bioenergy-driven phenomena would be the augmenting mechanism behind their magnificent efficacy of disease treatment. Moreover, the electron shuttle (ES)-based postulate for antiviral activities stated herein was that with electron transport chain as the driving force, a series of protein complexes and ET-associated molecules in intracellular membrane space would mediate electron transfer between electron donors and acceptors via redox reactions of disease treatment. Apparently, ES-oriented medicinal species not only provides higher power of disease remedy, but also persist long duration of drug medication. For example, *ortho*-dihydroxyl substituents bearing flavonoids (e.g., fisetin, quercetin, rutin, luteolin) were well-known to own antiviral characteristics for pharmacological values. Regarding our novel platform of evaluating bioenergy contents, microbial fuel cells (MFCs) [6] as bioelectrochemical devices can exploit electrochemically energetic microbes to oxidize organic matter, transforming chemical energy to be bioelectric power. To minimize mass-transfer resistance for maximal bioelectricity-generating and/or stimulating capabilities, exogenous supplement of nutrient sources or electron shuttles (ESs; electrochemically active catalysts) [7] could be employed to considerably augment electron transfer (ET) phenomena, leading to noteworthy rises in bioelectric power output. In fact, prior studies indicated that bioenergy-extracting features were of great importance to clinical treatment of neurotransmitter-associated diseases (e.g., Parkinson's disease (PD)) [8,9]. For instance, prior first-attempt studies clearly revealed that *ortho*-dihydroxyl substituents (*o*-diOH)-bearing dopamine (DA), epinephrine (EP), levodopa) directly associated to control functions of the central nervous system in human brain and body were strongly correlated to bioenergy-stimulating and electron mediating *o*-diOH chemicals. Moreover, levodopa is even the most effective PD medicine. It can pass into human brain and is converted to dopamine. Furthermore, combined medication of carbidopa-levodopa (i.e., both *o*-diOH chemicals) is used to treat PD symptoms (e.g., shakiness, stiffness, difficulty in moving) to substantial side-effects. From bioenergy point of view, both *o*-diOH carrying ESs (i.e., levodopa and carbidopa) could synergistically augment and effectively persist the efficacy of PD medication very likely due to effective ET stimulation as prior studies pointed out [10–12]. These additional representations also indicated that bioenergy-stimulating and electron-transporting capabilities would be pre-requisite to provide efficacious power of disease treatment. Primarily, ES-associated drug treatment may imply that such disease remedy would be electron transfer chain directed, suggesting that electron-shuttling catalysis is of great significance to disease treatment.

This study went more forward to explore that the efficacious herbal composition(s) in COVID-19 TCM formulae were not only antioxidant abundant, but also ES rich to deal with severe inflammation caused by coronavirus infection. As aforementioned, since the mechanisms in prior serial studies indicated, *ortho* or *para*-dihydroxyl groups-bearing phenolic chemicals could act as ESs forming stable "radical-resonant" intermediates to foster the electron-transfer circumstances for compelling redox reaction(s) to be taken place [13]. These novel findings also uncovered that neurotransmitter-related diseases are in fact bioenergy extraction and electron transfer steered likely due to such encouraging electron-shuttling catalysis. Moreover, as literature [14–18] stated, chemical species carrying both *ortho*-dihydroxyl substituents and flavonoids structures (e.g., fisetin, rutin, quercetin, lueolin) could also exhibit diverse antiviral activities (e.g., coronavirus (CoV), dengue virus (DengV), Japanese encephalitis virus (JEV)). That was why such phenolic compositions rich-herbal medicines (e.g., *Camellia* tea, *Rheum rhabarbarum*) were often mentioned for antiviral treatment as well. Recently, due to global pandemics of COVID-19 since the end of 2019, top-priority task of COVID-19 drug

development should be first screened via antioxidant and anti-inflammatory assessment. Combined with above-mentioned drugs of antiviral capabilities and PD and neurotransmitters-based medicines, this first-attempt study thus explored whether therapeutic prescription TCM for COVID-19 treatment were also possibly bioenergy-stimulating and electron transfer-driven using MFCs as bioenergy evaluation platform. The ranking of ET-tailored capabilities of compound herbal medicines may directly exhibit which keystone herbal compositions for such compound COVID-19 drugs play crucial roles for treatment. Apparently, top-ranking medicinal herbs in 18 TCM of COVID-19 all showed nearly identical ranking of DPPH-activity, anti-inflammatory characteristics, and MFC bioenergy-generating characteristics in parallel. To best of our knowledge, this work was the first study to decipher possible COVID-19 drugs from the aspect of biomass energy expression and *Forsythia suspensa* was found to be the most bioenergy-abundant herb among all 18 TCM herbs.

2. Materials and methods

2.1. Sample preparation

According to Luo et al. [19], eighteen herbs (*Adenophora stricta, Astragalus membranaceus, Atractylodes chinensis, Atractylodes macrocephala, Citrus reticulate, Coicis semen, Cyrtomium fortune, Forsythia suspensa, Glycyrrhiza uralensis, Lonicera japonica, Lysimachia nummularia, Morus alba, Perilla frutescens, Phragmites australis, Platycodon grandifloras, Pogostemon cablin, Ophiopogon japonicus, Saposhnikovia divaricata*) were usually included in the preventative TCM prescription used in China. These herbs were collected from a local TCM drugstore available at Tainan City, Taiwan, for this study. The particle size of the sample was reduced by using a blender. The extraction was performed in a 1:20 (Herb: water) ratio was made in the traditional Chinese decoction pot until the volume of the water was reduced to ca. 200 mL for each sample. After vacuum filtration, the excess solvent was evaporated using a rotary evaporator. These crude extracts had been undergone a freeze-drying process to ensure that the sample was free of water.

2.2. Total phenolic content

A standard stock solution A was prepared by dissolving 10 mg of gallic acid in 1 mL of ethanol. Then, 50 μL of the standard solution. A was diluted to 1000 μL with ethanol to have standard stock solution B. Serial dilutions by a factor of two was used to prepare the standard solution with the different concentrations (e.g., 500, 250, 125, 62.5, 31.3, 15.6, and 7.81 μg/mL). On the other side, the sample solution (1.0 mg/mL) was obtained by diluting 10 mg/mL of the crude extract with ethanol. All test solutions were prepared by adding 500 μL of Folin-Ciocalteu reagent and 400 μL of Na_2CO_3 solution into the 100 μL of blank, standard solution, or sample solution. All standard and sample test solutions were prepared in triplicates for statistical significance. After 30 min, 200 μL of each solution were transferred into the 96 well microarray plate. Then, an ELISA microplate reader was used to measure the absorbance of these solutions at 600 nm.

2.3. Total flavonoids content

Five milligrams of rutin were dissolved in 1 mL of ethanol to prepare a 5 mg/mL standard stock solution A. Then, standard solution B was prepared from 80 μL of solution. A diluted to 1000 μL with ethanol. For the standard calibration curve, seven standard solutions (400, 200, 100, 50, 25, 12.5, and 6.25 μg/mL) were prepared by serial dilution from standard stock solution B. Meanwhile, the 10 mg/mL of crude extract was diluted to obtain the 1.0 mg/mL of sample solution. A 500 μL of the test solution (including the blank) was treated with 2% of $AlCl_3$ in methanol. After an hour of reaction duration, the absorbance of the reacted mixture at 430 nm was measured by an ELISA microplate reader. Each standard and sample solution were prepared in triplicate.

2.4. Total condensed tannins content

The standard stock solution A was prepared by dissolving 5 mg of catechin in 1 mL of ethanol. For standard stock solution B, 32 μL of the stock solution A was diluted to 1000 μL with ethanol. Then, seven standard solutions (160, 80, 40, 20, 10, 5.0, 2.5 μg/mL) were prepared through serial dilution from standard stock solution B. The sample solution was prepared from 50 μL of crude extract (10 mg/mL) with 250 μL of ethanol and 600 μL of vanillin agent. All test solutions were prepared in triplicate, and the absorbance (at 530 nm) was measured through an ELISA microplate reader.

2.5. Total polysaccharides content

Eppendorf tube containing 125 μL of crude extract (10 mg/mL) was reacted with 1 mL of ethanol for 24 h. Then, the solution was centrifuged at 3000 rpm for 10 min. The precipitate was collected and washed with ethanol. A sample stock solution was prepared by dissolving the precipitate in 1 mL of distilled deionized (D.D.) water. Four hundred microliter of the solution was further diluted to 1 mL with D.D. water and reacted with 500 μL of 5% phenol solution and 5 mL concentrated sulfuric acid. A 100 mL standard stock solution was prepared by dissolving 10 mg of glucose in D.D. water. Standard solutions with the following concentration were prepared to construct a standard calibration curve: 10.0, 7.0, 5.0, 3.0, 2.0, 1.0 and 0.5 μg/mL. These standard solutions were treated the same way as the sample solution. These reaction mixtures were left undisturbed for 15 min at 95 °C. After cooling, 200 μL of the test solutions were transferred to a 96-well microplate, and the absorbance (at 485 nm) was measured by an ELISA microplate reader.

2.6. DPPH assay

All test solutions and reagents used in the experiment were prepared in the darkroom to prevent light-sensitive reagents from decomposition. The standard stock solution was prepared from 5 mg of ascorbic acid in 1 mL D.D. water. A 100 μL of the standard stock solution was diluted to 1000 μL using ethanol. Then, a series of standard solutions (500, 250, 62.5, 31.25, 15.63, and 7.81 μg/mL) were prepared through serial dilution. In the microtiter plate, 100 μL of standard, extract, and control (ethanol) solution was treated with 150 μL of DPPH solution. After 30 min, the absorbance (at 517 nm) of the test solutions and blank (250 μL ethanol) were measured. The radical scavenging activity (%RSA) of the extracts was calculated using the equation below. While the 50% inhibition concentration (IC_{50}) was estimated using the regression determined at 50% RSA.

$$\%RSA = \frac{(A_{ctl} - A_{blk}) - (A_{spl} - A_{blk})}{(A_{ctl} - A_{blk})} \; x\; 100$$

$A_{ctl:}$ Absorbance of the control
A_{blk}*: Absorbance of the blank*
A_{spl}*: Absorbance of the sample*

2.7. FRAP assay

Standard stock solution (1000 μg/mL) was prepared by diluting 500 μg/mL Trolox stock solution with 500 μL of ethanol: D.D. water (2:3) mixture. A series of standard solutions were prepared through serial dilution from the stock solution. The following concentration was considered to construct the standard calibration curve: 1000, 500, 250, 125, 62.50, 31.25, and 15.63 μg/mL. The sample stock

solution (10 mg/mL) was obtained from dissolving 10 mg of crude extract in 1 mL of ethanol: D.D. water (2:3) mixture. Transfer 50 μL of the sample stock solution in a microplate then treated with 1450 μL of FRAP reagent. The standard solutions were also treated in the same way as the sample. All test solutions were prepared in triplicated. The limit of quantitation was determined by measuring twenty-four blanks. The absorbance of all solutions at 593 nm was measured on an ELISA microplate reader.

2.8. Cell culture

Murine macrophages cell line RAW 267.4 (bought from American Type Culture Cultivation) was treated Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS), 100 IU/mL penicillin, and 100 μg/mL of streptomycin (Gibco BRL). Cells were cultivated in a humidified incubator under 5% CO_2 at 37 °C.

2.9. MTT and NO inhibition assay

RAW 264.7 were seeded in a 96-well microplate, and each mL contained (4×10^5 cells) and cultured overnight. Then, extracts in different concentrations were added to the well. The cells were then treated with LPS solution (500 ng/mL) for 24 h. Afterward, the MTT (5 ng/mL) solution was added and incubated for another 4 h. The medium was removed, and isopropanol was added to dissolve the formazan. For the MTT assay, the absorbance of the sample at 570 nm was measured. While the Griess Reagent was added to the medium and the absorbance at 530 nm was measured for the NO inhibition assay.

2.10. MFC operation and microbial cultures

Regarding double chamber (DC)-MFC operation [8,9], the soaking areas of graphite anode and cathode (Grade: IGS743; Central Carbon Co., Ltd) with culture broth or electrolyte solutions ca. 0.001649 m^2 (i.e., the cathode and anode chamber (operating volume 200 mL) were selectively isolated by proton exchange membrane (DuPont™ Nafion® NR-212) in an immersed area ca. 0.000452 m^2 (ID = 1.2 cm). LB broth medium (Difco™ LB Broth, Miller; Luria Bertani) used for microbial growth in batch cultures and MFC chamber is composed of 10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl. LB medium consisted of crucial nutrients of cell growth and energy consumption (e.g., electron-transport chain). The electroactive microorganism-*Aeromonas hydrophilia* NIU01 (originally separated and identified from soil near Yi-Lan River, I-Lan County, Taiwan with favorable potentials of color removal of reactive red 141) was chosen. Moreover, to compare bioelectrochemical characteristics of pure microbes and mixed consortia, the mixed consortia-NP (originally isolated from pond water of National Ilan University, Taiwan) that was acclimatized to have promising color removal capabilities of azo dyes-sunset yellow FCF (SYF), allura red AC (AR), and tartrazine (T) were used for bioenergy formation in MFC modules. For double chamber-MFC, cathodic chamber consisted of 6.38 g $K_3Fe(CN)_6$ (potassium ferricyanide; BAKER ANALYZED, A.C.S. Reagent) and 17.42 g K_2HPO_4 (dipotassium hydrogen phosphate; SHOWA Co. Ltd.) that were completely dissolved in 200 mL distilled-deionized water. MFCs were performed at room temperature (25 °C). Inoculated bacterium NIU01 obtained from an isolated colony on a LB-streak agar plate was chosen for 12 h overnight (O/N) preculture in LB broth medium using a water bath shaker (Shinkwang, SKW-12; 30 °C, 125 rpm). Next, 1% (v/v) subcultured broth was inoculated into fresh autoclaved LB broth for 12 h culture (pH was not adjusted for these O/N flask subcultures). Test chemical(s) at 0.3 mM were then added in 200 mL cell broth (OD_{600}~2.0) of anodic chamber in double chamber MFC for bioelectricity generating inspection.

2.11. Power-generating determination

Time courses of electric current (I_{MFC}) and voltage (V_{MFC}) were automatically determined with a D/A system (DAS 5020; Jiehan Tech. Corp., Taiwan) [8,9]. To have identical basis for comparison, 1 KΩ external resistance was applied to MFCs. Power and current densities were determined by the formulae:

$$P_{density} = \frac{V_{MFC} \times I_{MFC}}{A_{Anode}}, \quad (1)$$

$$I_{density} = \frac{I_{MFC}}{A_{Anode}}, \quad (2)$$

where V_{MFC} and I_{MFC} could be evaluated with linear sweep voltammetry through a workstation for electrochemical analysis (Jiehan 5600, Jiehan Technology Corp., Taiwan). The parameter A_{anode} was the apparent working area of the graphite anode.

3. Result and discussion

3.1. Phytochemical content of AHEs

To construct basic contents of test herbs for comparative analysis upon bioenergy content and disease-treating potentials, the phytochemical ingredients present in the aqueous extract of 18 herbs were determined as described in Table 1. The phytonutrients could be individually evaluated via total polyphenol content (gallic acid-based), total polysaccharide content (glucose-based), total flavonoid content (rutin-based), and total condensed tannins (catechin-based).

As known, polyphenol compounds were classified into categories: flavonoid, phenolic acid and tannins [20]. These compounds could very possibly exhibit diverse anti-bacterial, anti-inflammatory, and anti-oxidant activities [21,22]. In fact, as aforementioned in Introduction, several polyphenol compounds were even reported to possess antiviral activity, especially flavonoids. By comparison upon test herbal extracts, total polyphenol contents obtained from *Forsythia suspensa* (Lian qiao, 60.97 ± 1.39 mg gem/g), *Lonicera japonica* (Jin yin hua, 55.63 ± 0.53 mg gem/g), and *Perilla frutescens* (Zi su ye, 43.14 ± 0.35 mg gem/g) were perceived to have high total polyphenol content (Table 1). As a matter of fact, these medicinal herbs were primarily employed to ease the inflammatory reaction, removing heat and toxins from human body. Moreover, several TCM prescriptions usually include these herbs for treating cold, fever, and even viral infections with high efficacy [23–25]. As Paraiso et al. [24] reported, possible antiviral mechanism of action (MOA) for polyphenols is to prevent the viral entry and replication of SARS-CoV-2. Some polyphenols were found to bind with the receptor-binding domain (RBD) of SARS-CoV-2, which may even interfere with combined interaction of the virus and its receptor. Furthermore, other polyphenols were revealed to regulate the ACE-2 receptor expression and function, further limiting the viral entry. Apart from preventing viral entry, polyphenols also disrupt the viral replication by inhibiting the viral proteases ($3CL^{pro}$ and PL^{pro}) and RNA polymerase (RdRp). As an immunoregulator, polyphenols were found to inhibit the pro-inflammatory cytokines and lower the IL-6 level in the plasma [24].

Flavonoids have always been crucial compounds for industrial applications. However, these compounds are known to have limited solubility in water, leading to relatively low flavonoids content detected in the extract as described herein. Moreover, the method used may also limit the detection of flavonoids in some extracts [25]. In this study, extracts of *Lysimachia nummularia* (Jin qian cao, 7.06± 0.28 mg/g), *Perilla frutescens* (Zi su ye, 5.76±0.16 mg/g), and *Morus alba* (Sang ye, 5.49±0.02 mg/g) were observed to have the highest flavonoid content among all (Table 1). As a group of polyphenols,

Table 1
Comparative list of phytochemical content of 18 aqueous herbal extracts of COVID-19 TCM.

Herbal sample	Total polyphenol content (mg *gem*/g)	Total flavonoid content (mg/g)	Total condensed tannin content (mg/g)	Total polysaccharide content (mg/g)
Adenophora stricta(Sha shen)	2.27 ± 0.08	Not Detected	3.21 ± 0.04	1.91 ± 0.04
Astragalus membranaceus (Huang qi)	4.95 ± 0.23	Not Detected	3.76 ± 0.05	1.13 ± 0.04
Atractylodes chinensis (Cang zhu)	9.80 ± 0.35	Not Detected	6.21 ± 0.15	1.51 ± 0.08
Atractylodes macrocephala(Bai zhu)	3.75 ± 0.03	4.24 ± 0.02	5.36 ± 0.03	9.20 ± 0.29
Citrus reticulata(Chen pi)	28.88 ± 0.00	1.67 ± 0.10	7.65 ± 0.03	0.76 ± 0.02
Coicis semen(Yi ren)	3.57 ± 0.07	Not Detected	15.11 ± 0.10	0.24 ± 0.00
Cyrtomium fortunei(Guan zhong)	7.49 ± 0.26	0.32 ± 0.02	4.20 ± 0.12	2.56 ± 0.05
Forsythia suspensa(Lian qiao)	60.97 ± 1.39	1.41 ± 0.06	14.49 ± 0.19	0.07 ± 0.00
Glycyrrhiza uralensis (Gan cao)	14.10 ± 0.14	2.74 ± 0.07	7.23 ± 0.09	0.62 ± 0.01
Lonicera japonica (Jin yin hua)	55.63 ± 0.53	0.65 ± 0.02	6.09 ± 0.12	0.39 ± 0.01
Lysimachia nummularia(Jin qian cao)	23.84 ± 0.26	7.06 ± 0.28	9.85 ± 0.02	0.59 ± 0.02
Morus alba(Sang ye)	38.09 ± 0.05	5.49 ± 0.02	3.72 ± 0.02	0.65 ± 0.01
Perilla frutescens(Zi su ye)	43.14 ± 0.35	5.76 ± 0.16	7.74 ± 0.07	4.05 ± 0.00
Phragmites australis(Lu gen)	13.74 ± 0.64	0.35 ± 0.01	6.32 ± 021	1.11 ± 0.03
Platycodon grandiflorus (Jie geng)	3.02 ± 0.07	Not Detected	4.61 ± 0.07	1.42 ± 0.04
Pogostemon cablin(Guang huo xiang)	25.54 ± 0.10	3.66 ± 0.05	16.23 ± 0.09	0.59 ± 0.05
Ophiopogon japonicus(Mai dong)	3.17 ± 0.15	Not Detected	6.36 ± 0.08	0.40 ± 0.02
Saposhnikovia divaricata(Fang feng)	6.35 ± 0.06	Not Detected	4.19 ± 0.02	0.12 ± 0.00
Standard curve	y = 5.2057x - 0.0109 r^2 = 0.9969	y = 12.684x + 0.0795 r^2 = 0.9971	y = 7.1651x - 0.0699 r^2 = 0.9937	y = 7.0708x + 0.0683 r^2 = 0.9911

flavonoids were also known to exhibit the MOA as aforementioned for their antiviral activity. Moreover, flavonoids could regulate signaling pathways as anti-inflammatory agents and immunomodulatory agents [26].

Regarding compositions other than flavonoids in plants, tannins or tannic acid (derivative of phenolic acids) are water-soluble polyphenols in plants. As secondary metabolites of plants, tannins are known to act as a defense mechanism against foreign microbes. Other than antimicrobial activity, tannins were reported to have other physiological activities (e.g., anti-oxidant, antimicrobial, and modulating immune responses) [27].

Recently, due to COVID-19 pandemics, the antiviral activity of tannins against SARS-CoV-2 could be triggered by inhibiting main viral protease ($3CL^{pro}$) and transmembrane protease serine 2 (TMPRSS2) [28]. As observed, *P. cablin* (16.23±0.09 mg/g), *C. Semen* (15.11 ± 0.10 mg/g), and *F. suspensa* (14.49 ± 0.19 mg/g) were the tannin-rich herbs (Table 1) that may serve as dual inhibitors for their activity against SARS-CoV-2.

Other than polyphenols, polysaccharides are also secondary metabolites known to have wide-ranged pharmacological potentials. For example, bioactive polysaccharides obtained from herbal extracts were reported to prevent hyperpigmentation by inhibiting melatonin production and tyrosinase. Moreover, these polysaccharides are natural macromolecular polymers that allow them to be considered for biomedical applications [29,30]. Here, *A. macrocephala* (9.20±0.29 mg/g), *P. frutescens* (4.05±0.00 mg/g) and *C. fortunei* (2.56 ± 0.05 mg/g) were found to be herbs with notable amounts of polysaccharides present in their extracts (Table 1). As literature stated, crude polysaccharides from leaves of *P. frutescencs* were observed to have an immunomodulatory effect and inhibit the production of nitric oxide (NO), tumor necrosis factor (TNF), and other pro-inflammatory cytokines [31].

In comparison, polysaccharides obtained from *A. macrocephaa* were found to have immune enhancer capability by upregulating the signaling pathways related to T-cell activation [32]. Considering others acting as immunomodulatory agents, bioactive polysaccharides obtained from herbal extracts were also known to exhibit antiviral activity against SARS-CoV-2 [33]. With comparison of these antiviral activity-associated contents at different levels, follow-up inspections tended to reveal the rankings of these antiviral activities also showed to be bioenergy expression-steered and electron mediation-associated as keystone neurotransmitters unveiled in prior works [8–11].

3.2. Antioxidant activity of AHEs

Aware that antioxidant and electron-shuttling activity could be indicators to probe whether test samples could conduct electrochemically mediated irreversible and reversible reactions, respectively. Simultaneous higher antioxidant content and bioenergy-stimulating (ES) capabilities simply suggest that such sample could persist its electrochemical catalysis for prolonged bioactivities (e.g., antiviral, antibacterial characteristics). In contrast, herbal samples with higher antioxidant, but lower bioenergy-generating capability directly imply that such sample owned designated bioactivities in a short period of time. That could explain why exploration of bioenergy-stimulating capabilities would be strongly correlated to the efficacy of herbal extract(s) for disease treatment (e.g., antiviral potentials). Aware that plants produce natural radical scavengers (antioxidants) as their defense mechanism against reactive oxygen species (ROS) generated due to the environmental stress. These antioxidants scavenge ROS by hydrogen atom transfer (HAT) and single electron transfer (SET) [27,34]. For this instance, two different bioassays defined on different probed reactions would be used for screening the anti-oxidant properties of herbal extract. DPPH would detect the number of antioxidants that could scavenge the free radical (diphenylpicrylhydrazyl) via HAT [35]. While FRAP would determine the antioxidants that reduced the Fe (III)/tripyridyltriazine complexes via SET [34]. Different indicator reactions to be responded would exhibit diverse reaction extents of distinct free-radical scavenging activities.

As Table 2 exhibited, comparison of the antioxidant activity of various herbal extracts was shown. Among all samples, the top ranking extracts of promising antioxidant activity were *F. suspensa* > L. *japonica* > *P. cablin* > *M. alba* > *P. frutescens* > L. *nummularia*. In common, these herbs were reported to have high polyphenol content (including tannins and flavonoids) as polyphenols compounds of remarkable antioxidant activity. In addition, the antioxidant activity of polyphenols highly depended on their structure, allowing them to scavenge a wide variety of ROS [22]. Hence, the amount of polyphenol content present in the extract could directly influence the antioxidant capacity of the herbs. While for herbs like *Astragalus membranaceus*, this result indicated that the amount of antioxidants present might be beyond the detection limit [36]. This may also suggest that such herb in this formula is an enhancer/stimulator to trigger interactive synergy of “main-effect” herbal compositions for effective antiviral expression. Furthermore, the result obtained from *in vitro* is not always equivalent to *in vivo*; therefore, the efficacy of

Table 2
Tabulated list of antioxidant activity of 18 aqueous herbal extracts of COVID-19 TCM.

Herbal Sample	DPPH (IC_{50} mg/g)	FRAP (mg/g)
Adenophora stricta (Sha shen)	Not Detected	Not Detected
Astragalus membranaceus (Huang qi)	Not Detected	Not Detected
Atractylodes chinensis (Cang zhu)	14.01 ± 0.80	0.59 ± 0.14
Atractylodes macrocephala (Bai zhu)	Not Detected	Not Detected
Citrus reticulata (Chen pi)	9.08 ± 1.21	2.44 ± 0.05
Coicis semen (Yi ren)	Not Detected	Not Detected
Cyrtomium fortunei (Guan zhong)	7.97 ± 0.96	9.95 ± 0.07
Forsythia suspensa (Lian qiao)	0.25 ± 0.02	102.40 ± 0.92
Glycyrrhiza uralensis (Gan cao)	11.83 ± 0.99	4.24 ± 0.05
Lonicera japonica (Jin yin hua)	1.45 ± 0.04	46.58 ± 0.21
Lysimachia nummularia (Jin qian cao)	4.64 ± 0.48	19.74 ± 0.16
Morus alba (Sang ye)	1.76 ± 0.19	44.50 ± 0.14
Perilla frutescens (Zi su ye)	2.00 ± 0.14	38.50 ± 0.17
Phragmites australis (Lu gen)	18.19 ± 0.98	6.88 ± 0.00
Platycodon grandiflorus (Jie geng)	Not Detected	Not Detected
Pogostemon cablin (Guang huo xiang)	1.66 ± 0.17	45.05 ± 0.19
Ophiopogon japonicus (Mai dong)	15.55 ± 1.87	Not Detected
Saposhnikovia divaricata (Fang feng)	Not Detected	Not Detected
Ascorbic acid	0.454 ± 0.011	Not Applicable
Standard curve	y = 92.596x + 7.8568 r^2 = 0.9969	y = 5.638x + 0.6778 r^2 = 0.9911

Table 3
Anti-inflammatory activity of the extracts.

Sample	NO inhibition IC_{50} (μg/mL)	Cell viability
Coicis semen (Yi ren)	484.69	Toxicity not detected
Forsythia suspensa (Lian qiao)	215.7	Toxicity not detected
Glycyrrhiza uralensis (Gan cao)	344.28	Toxicity not detected

these herbs in preventing viral infection could not simply be justified or denied by just performing solely an *in vitro* assay [37]. That is, further inspection may be inevitably required for conclusive remarks.

3.3. Anti-inflammatory activity of AHEs

According to WHO, COVID-19 may develop serious inflammatory symptoms of difficulty in breathing or shortness of breath. In particular, inflammation is part of the defense mechanisms once the immune system senses danger from a particular substance or external pathogens. However, an uncontrolled inflammatory response would lead to cytokines storm, overproduction of nitric oxide (NO), and other substances that would cause damages to tissues and organs [38]. Here, the anti-inflammatory capability of the extracts was screened based on their ability to hinder NO production, which was triggered by the lipopolysaccharide (LPS) of Gram-negative bacteria. Only three (i.e., *F. suspensa, G. uralensis,* and *C. semen*) aqueous herbal extracts (Table 3, Figs. 1–3) were observed to inhibit the NO production in LPS-stimulated RAW 264.7 cells [39]. Among others, aqueous extract of *C. semen* (Fig. 1) did have the highest anti-inflammatory activity likely due to its high polysaccharide content. Based on prior studies, these polysaccharides may act as a direct inhibitor for NO synthase (NOS) and other downstream mediators in the NO synthetic pathway. Furthermore, these polysaccharides may indirectly influence NO production by controlling the secretion of T helper (Th) 1 to restore the balance between Th1 and Th2 [40,41]. Other than polysaccharides, polyphenols in the extracts of *F. suspensa* and *G. uralensis* may also act as a direct and indirect inhibitor on the NO synthetic pathway. For example, the immune response may be modulated to prevent further activation of pro-inflammatory cytokines by controlling the ROS level in the plasma [36]. Thereby, these herbs seemed to be promising candidates for an anti-inflammatory agent with non-detectable toxicity levels (Table 3). On the other side, the other aqueous extracts may also exhibit anti-inflammatory activity by modulating different signaling pathways like nuclear factor kappa-light chain enhancer of activated B cells (NF-κB). For this instance, additional bioassays can be considered for screening the anti-inflammatory activity of these extracts.

3.4. MFC power density analysis

As aforementioned in Introduction, MOAs of antiviral activities for herbal extracts were strongly associated to effective expression of electron transport chain in the intracellular compartment. Exogenous supplement of ES would considerably catalyze such persistent electrochemical activation. Examples of refreshing herbal medicines and neurotransmitters as indicated earlier clearly demonstrated such promising feasibility. That is, bioenergy-extracting and electron-shuttling potentials of chemical species could be of great significance to possible potentials for clinical medication. That was why clinical treatment of keystone neurotransmitters and/or PD-related drugs (e.g., levodopa, dopamine, carbidopa) could directly affect the efficacy of treatment of PD and much further protect nervous system. Regarding their MOAs, levodopa is capable to cross the protective blood-brain barrier and used to increase dopamine concentrations in the PD

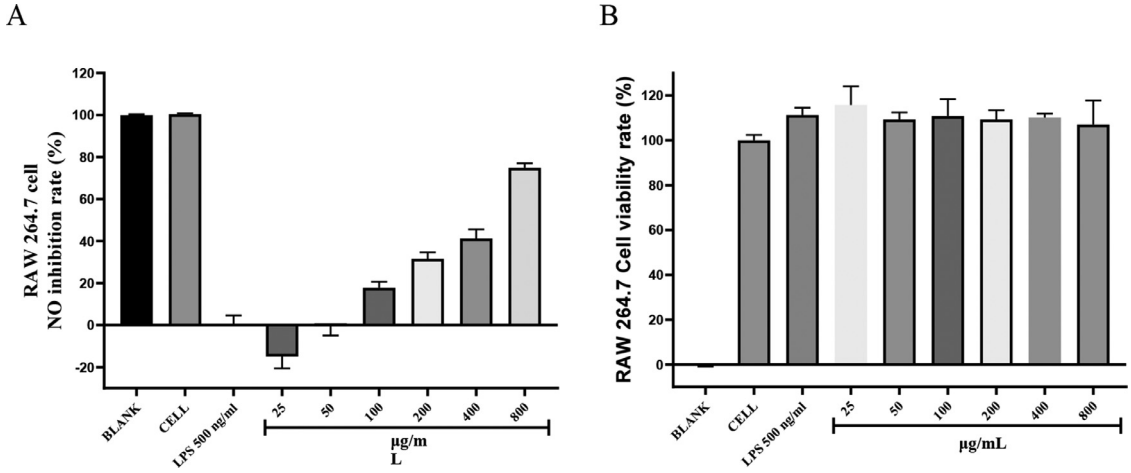


Fig. 1. (A) Inhibitory effect of *Coicis semen* extract on NO production by LPS-stimulated RAW 264.7 cells; (B) MTT survival rate on *Coicis semen* extract.

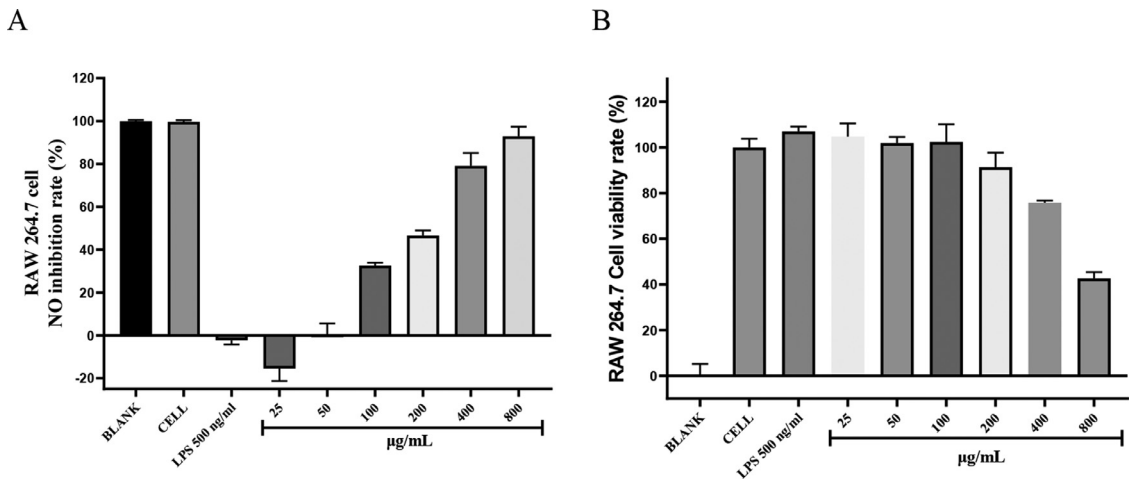


Fig. 2. (A) Inhibitory effect of *Forsythia suspensa* extract on NO production by LPS-stimulated RAW 264.7 cells; (B) MTT survival rate on *Forsythia suspensa* extract.

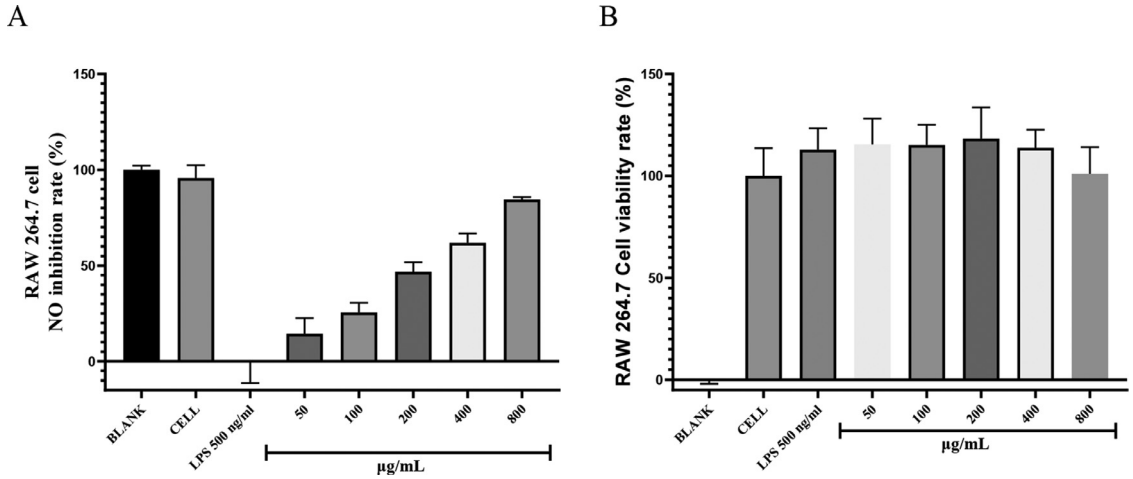


Fig. 3. . (A) Inhibitory effect of *Glycyrrhiza uralensis* extract on NO production by LPS-stimulated RAW 264.7 cells; (B) MTT survival rate on *Glycyrrhiza uralensis* extract.

treatment. Moreover, L-DOPA (i.e., levodopa) could mediate neurotrophic factor discharged by the brain and central nervous system. Levodopa is even the keystone precursor to neurotransmitters dopamine (DP), norepinephrine (NP) and epinephrine (EP). All these electron shuttles (ESs; DP, NP, EP) among all neurotransmitters are of great importance to be distinguished as catecholamines. Note that electrochemically-promising biocatalysts (i.e., ESs) cannot be consumed in the metabolic reaction and remain unchanged before and after biological treatment. That is, as "electroactive catalysts", ES-behaved drugs (e.g., *o*-diOH levodopa for PD treatment) electrochemically own the capabilities to persist drug efficacy in nature for a longer duration of disease treatment. Moreover, antiviral chemical species (e.g., *o*-diOH and flavonoid-bearing fisetin, rutin, quercetin, luteolin) often found in herbal extracts for clinical treatment) were also naturally exhibited strongly electrochemical capabilities for biomass energy extraction. Thus, this study went forward to suggest that antiviral compound formulae of herbal medicines for COVID-19 should contain crucial compositions of bioenergy extraction and electron transport to "reversibly catalyze" drug efficacy. Here, top 6 ranking herbal extracts were selected to evaluate bioenergy-expressing capabilities using two MFC systems (amplification factors in Figs. 4 and 5, Tables 4 and 5) with dopamine as a common basis for comparison as follows: Dopamine (3.49~3.64) > *P. cablin* (2.03 ± 0.05) ~ *F. suspensa* (1.94 ± 0.11) > *P. frutescens* (1.78 ± 0.06) > *L. japonica* (1.61 ± 0.07) > *L. nummularia*

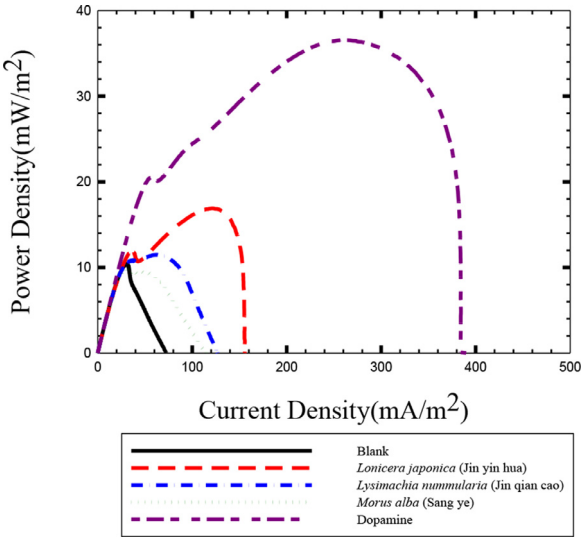


Fig. 4. Comparative profiles of power generation of *Lonicera japonica, Lysimachia nummularia, Morus alba* extracts using microbial fuel cells as a platform of bioenergy evaluation.

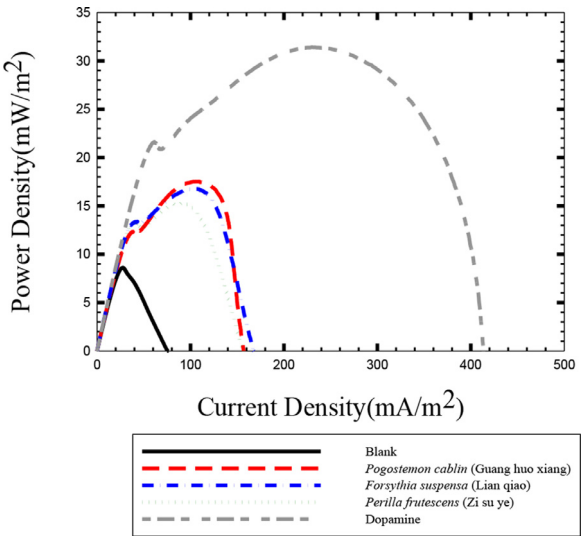


Fig. 5. Comparative profiles of power generation of *Pogostemon cablin, Forsythia suspensa, Perilla frutescens* extracts using microbial fuel cells as a platform of bioenergy evaluation.

(1.10 ± 0.08) > Blank > *M. alba* (0.94 ± 0.00). Note that herbal extracts are mixtures of several chemical species; thus, power density expression less than pure chemical dopamine would be anticipated due to the presence of other low bioenergy content species.

Evidently, aforementioned results clearly exhibited several significant points as follows: (a) the bioelectricity-generating capabilities for these herbal extracts nearly followed antioxidant activity (unit: IC_{50} mg/g) (e.g., DPPH: *F. suspensa* (0.25 ± 0.02) > *L. japonica* (1.45 ± 0.04) > *P. cablin* (1.66 ± 0.17) > *M. alba* (1.76 ± 0.19) > *P. frutescens* (2.00 ± 0.14) > *L. nummularia* (4.64 ± 0.48); (b) most of water extract dominant in polysaccharides seemed to be positively correlated to antiviral activities; (c) condensed tannin content well-known related to antibacterial activity, but not correlated to antiviral activity as stated herein; (d) top 6 ranking of flavonoid content (mg/g) showed *L. nummularia* (7.06 ± 0.28) > *P. frutescens* (5.76 ± 0.16) > *M. alba* (5.49 ± 0.02) > *A. macrocephala* (4.24 ± 0.02) > *P. cablin* (3.66 ± 0.05) > *G. uralensis* (2.74 ± 0.07), revealing that flavonoid content was nearly consistent with antioxidant (e.g., DPPH, FRAP) activities; (e) summary with anti-inflammatory analysis, *F. suspensa* seemed to be the most promising herb dealing with antiviral characteristics (e.g., COVID-19 treatment); (f) this PD ranking also clearly unveiled that the amplification factor with respect to the blank greater than ca. 2.0 seemed to be an appropriate level of strong electron transfer potential, suggesting convincing antiviral characteristics for COVID-19 herbal TCM. This first-attempt study also provided a twofold index of bioelectricity-generating amplification for pre-screening upon various herbal extracts for anti-viral or anti-COVID-19 drug development via electrochemical exploration.

As Table 6 showed, the active constituents of *P. cablin, F. suspensa* and *P. frutescens* with electron-shuttling properties for anti-COVID-19 were revealed. Literatures mentioned that apigenin, caffeic acid and chlorogenic acid three major active compounds of *P. cablin* have anti-COVID-19 activity for clinical treatment. In particular, caffeic acid and its derivatives (CAFDs) can be the inhibitors of SARS-CoV-2 and chlorogenic acid as a potential inhibitor for SARS-CoV-2 using molecular docking [42–46]. Forsythoside A and forsythoside B are the active constituents from *F. suspensa*, both of them also showed that activities against the influenza A virus which could be revealed beneficial effects to treat COVID-19 symptom [47]. On the other hand, forsythoside A is a potential anti-COVID-19 active compound of TCM Lianhuaqingwen capsule from China [48]. Apienin, luteolin and rosmarinic acid of *P. frutescens* have been showed their possibility potential for anti-COVID-19 as the inhibitors [43,49–51]. The active constituents- apigenin, caffeic acid and chlorogenic acid in *P. cablin* showed that anti-inflammtory activity of apigenin in the dose at 20 mg/kg for tested animals on escalating the inflammatory and oxidative response [52]. Caffeic acid and chlorogenic acid also inhibited pro-inflammatory TNF-α and H_2O_2-induced IL-8 production [53]. Forsythoside A and B of *F. suspensa* have been reported that forsythoside A and B inhibited virus-induced inflammatory pathways and viral replication through designated signaling pathways leads to downregulation of several proteins involved in viral infection [54]. For *P. frutescens* major compound luteolin could block the interaction of ligands (PAMPs) with the receptors (PRRs) and inhibits downstream activation signals [54,55]. Rosmarinic acid of *P. frutescens* could reduce LPS-induced vascular inflammation by inhibiting MAPK/NF-B signaling in smooth muscle cells. These active compounds express anti-inflammatroy and electron transport properties [56]. On the other hand, the TCM formula Jing Guan Fang (JGF) has been reported for Preventing SARS-CoV-2 Infection. This JGF contained 5 herbal medicine such as *Forsythia suspens*a, *Scutellaria baicalensis, Bupleurum chinese, Magnolia officinalis* and *Agastache rugose*. JGF provides evidence for JGF effectively blocks syncytia formation and inhibits SARS-CoV-2 plaque formation [57]. As aforementioned, among these active constituents at least 5/8 contained *ortho*-dihydroxyl substituents for electron-mediation, greatly augmenting their redox-catalyzing capabilities and interactive synergy with other compositions for antiviral characteristics to be effectively expressed.

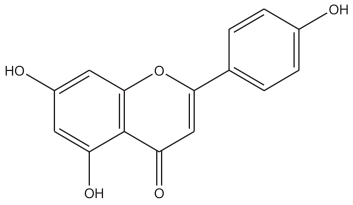
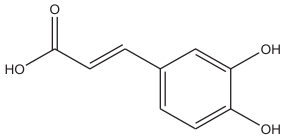
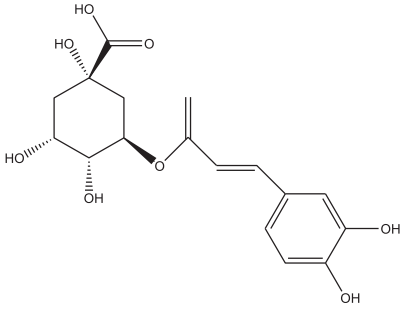
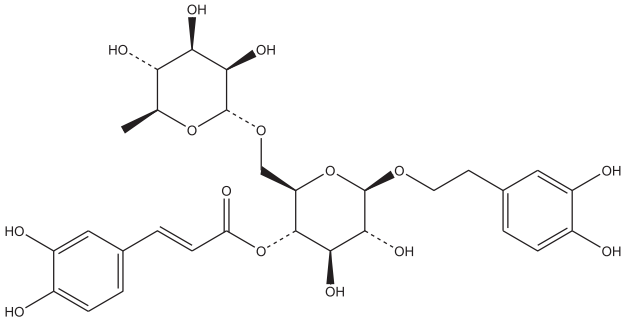
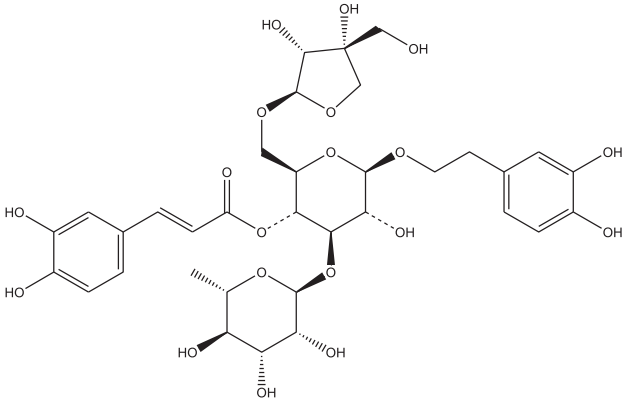
Table 4
Comparison upon maximum power density profiles of *Lonicera japonica, Lysimachia nummularia, Morus alba* extracts by microbial fuel cells.

	Blank	*Lonicera japonica* (Jin yin hua)	*Lysimachia nummularia* (Jin qian cao)	*Morus alba* (Sang ye)	Dopamine
Power Density	10.46 ± 0.27	16.91 ± 1.19	11.50 ± 1.18	9.86 ± 0.16	36.57 ± 2.34
Amplification factor		1.61 ± 0.07	1.10 ± 0.08	0.94 ± 0.00	3.49 ± 0.13

Table 5
Comparative list on maximum power density profiles of *Pogostemon cablin, Forsythia suspensa, Perilla frutescens* extracts by microbial fuel cells.

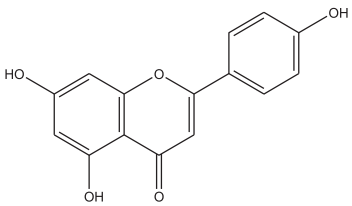
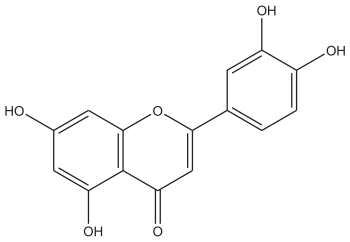
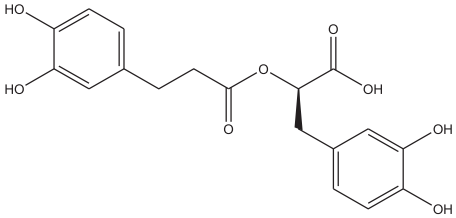
	Blank	*Pogostemon cablin* (Guang huo xiang)	*Forsythia suspensa* (Lian qiao)	*Perilla frutescens* (Zi su ye)	Dopamine
Power Density	8.63 ± 0.26	17.54 ± 0.96	16.78 ± 1.47	15.31 ± 0.09	31.42 ± 1.43
Amplification factor		2.03 ± 0.05	1.94 ± 0.11	1.78 ± 0.06	3.64 ± 0.06

Table 6
Active constituents of *Pogostemon cablin*, *Forsythia suspensa* and *Perilla frutescens* with electron shuttles property for anti-COVID-19.

Plant	Compound	Chemical structure	Refs.
Pogostemon cablin	Apigenin		[42,43]
	Caffeic acid		[44,45]
	Chlorogenic acid		[44,46]
Forsythia suspensa	Forsythoside A		[47,48]
	Forsythoside B		[47]

(*continued*)

Table 6 (*Continued*)

Plant	Compound	Chemical structure	Refs.
Perilla frutescens	Apigenin		[43,49]
	Luteolin		[49,50]
	Rosmarinic acid		[49,51]

4. Conclusion

Phytochemical content analysis, antioxidant, anti-inflammatory and bioenergy evaluation using microbial fuel cells have been established for selected 18 TCM herbs for treatment of SARS and H1N1. This study also pointed out that *F. suspensa* was possibly "main-effect" herbal species dealing with rate-determining step(s) of anti-COVID-19 activities. The bioenergy-oriented bioassay systems (i.e., MFCs) could help to electrochemically select the potential drug(s) for COVID-19 treatment and twofold index of power amplification could be applicable for screening of new drug development. It is expected not only to provide new bioassay methods but also to efficaciously discover candidate herbal for anti-COVID-19 in current situation of the pandemic.

Declaration of Competing Interest

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

Declaration of Competing Interest

None.

Acknowledgments

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