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Interactive deciphering electron-shuttling characteristics of *Coffea arabica* leaves and potential bioenergy-steered anti-SARS-CoV-2 RdRp inhibitor via microbial fuel cells

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**ABSTRACT**

Due to the pandemics of COVID-19, herbal medicine has recently been explored for possible antiviral treatment and prevention via novel platform of microbial fuel cells. It was revealed that *Coffea arabica* leaves was very appropriate for anti-COVID-19 drug development. Antioxidant and anti-inflammatory tests exhibited the most promising activities for *C. arabica* ethanol extracts and drying approaches were implemented on the leaf samples prior to ethanol extraction. Ethanol extracts of *C. arabica* leaves were applied to bioenergy evaluation via DC-MFCs, clearly revealing that air-dried leaves (CA-A-EtOH) exhibited the highest bioenergy-stimulating capabilities (ca. 2.72 fold of power amplification to the blank). Furthermore, molecular docking analysis was implemented to decipher the potential of *C. arabica* leaves metabolites. Chlorogenic acid (~6.5 kcal/mol) owned the highest binding affinity with RdRp of SARS-CoV-2, showing a much lower average RMSF value than an apo-protein. This study suggested *C. arabica* leaves as an encouraging medicinal herb against SARS-CoV-2.

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1. Introduction

*Coffeea* are plants categorized as shrubs or small trees that belong to a flowering genus in the family Rubiaceae (Nayeem et al., 2011). They produce red or purple fruits, often called “coffee cherries” where the caffeine-containing seeds are popularly used for beverages, flavoring and even in cosmetic industries. It was even widely known as coffee and these plants are native to tropical countries in South America, Africa and Asia which even rank top one of the world’s most treasured and widely traded commodity (Perrois et al., 2015; Caracostea et al., 2021). Two most principal varieties account for the majority of the world’s coffee production of *Coffea arabica* (also known as “Arabica”) and *Coffea canephora* (also known as “Robusta”).* C. arabica* is preferred for its sweeter taste, while *C. canephora* is characterized due to its high caffeine content (Cagliani et al., 2013; Patay et al., 2016).

As literature indicated, significant attentions on secondary metabolites of coffee seed extracts have been paid towards not only sensory profile improving for food and beverage, but also the medicinal benefits.

**Abbreviations:** *C. arabica*, *Coffea arabica*; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; RdRp, RNA-dependent RNA polymerase; MFCs, Microbial fuel cell; DC-MFCs, Dual Chamber-Microbial Fuel Cells; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, Ferric ion reducing antioxidant power; CA-H2O, Water extract of *C. arabica* leaves by 40°C oven-dried; CA-40-EtOH, EtOH extract of *C. arabica* leaves by 40°C oven-dried; CA-EA, Ethyl acetate extract of *C. arabica* leaves by 40°C oven-dried; CA-AC, Acetonate extract of *C. arabica* leaves by 40°C oven-dried; CA-HX, Hexane extract of *C. arabica* leaves by 40°C oven-dried; CA-EA-EtOH, EtOH extract of *C. arabica* leaves by air-dried; CA-F-EtOH, EtOH extract of *C. arabica* leaves by freeze-dried; CA-80-EtOH, EtOH extract of *C. arabica* leaves by 80°C oven-dried; RMSF, Root-mean-square fluctuation; BBB, Blood-brain barrier; ADMET, Absorption-distribution-metabolism-excretion-toxicity; QSAR, Quantitative-structure-activity relationship.

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augmented for its bioactive metabolites. In fact, coffee pulp as the first by-product acquired from coffee cherry processing has been reported to contain major chemical components (e.g., chlorogenic acid, caffeine, epicatechin, isochlorogenic acid and rutin) of health value (Ontawong et al., 2021). Some components have also been identified, including alkaloids, organic acids, fatty acids, glycosides, amino acids and some organic volatile compounds (Kryksak, 2017; Miao et al., 2022; Zhang et al., 2019). Bioactive compounds in coffee beans have been identified to present antioxidant, antimicrobial and even anti-inflammatory activities (Khochapong et al., 2021; Ciaramelli et al., 2019; Pergolizzi et al., 2020). As a matter of fact, coffee studies are chiefly focused on secondary metabolites in cherries or seeds which greatly vary depending on factors (e.g., roasting temperature, modes of fermentation, coffee variety, degree of coffee ripeness, soil quality). Recently, advanced metabolomics have triggered rapidly developing of instrumentation in areas (e.g., nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS)) to provide detailed identification of these vital and minute compositions in seed extracts. In fact, natural products present in coffee beans were associated with numerous health benefits and even medicinal potential since they are popularly present in human diets. Functional daily drink substances of C. arabica leaves with antiviral activity using a molecular docking method to target RdRp of SARS-CoV-2 were implemented for conclusive remarks.

2. Methods

2.1. Materials

The leaves of C. arabica were obtained from Dawu Township, Taichung city, Taiwan by December 2020. Collected plant sample (No. CJCUC-001) was then authenticated by Dr. Chia-Jung Lee from College of Pharmacy of Taipei Medical University, Taiwan.

2.2. Sample preparation and extraction

The leaf samples of C. arabica were treated via three different drying methods (freeze-dried, air-dried and oven-dried). For oven-dried sample, the temperature was maintained on 40 °C for 3 days and 80 °C for 1 day. Then, the plant samples were grinded prior to extraction. At the ratio of 3:2 (sample: solvent), the samples were extracted using different solvents: water, ethanol, ethyl acetate and hexane. For the organic solvents, reflux extraction (2 h) was applied. While the water extraction (decoction) was implemented by using a traditional Chinese decoction pot until 200 mL of the extract was obtained. Vacuum filtration was carried out to collect the crude extracts and the excess solvents were then removed by rotary evaporator.

2.3. Determination of total polyphenol content

A one mg/mL of sample solution in ethanol was prepared from ten milligrams per millilitre (10 mg/mL) of C. arabica leaves extract solution. Then, 10 µL of the sample solution was treated with 500 µL Folin’s reagent and 400 µL Na₂CO₃. A 10 mg/mL of stock solution A was prepared by dissolving 10 mg of gallic acid in 1 mL of ethanol. Followed by 50 µL of the stock solution A was serially diluted to 1000 µL using ethanol. Through serial dilution, 500-, 250-, 125-, 62.5-, and 31.25 µg/mL of standard solutions were prepared. Same as sample solution preparation, 100 µL of standard solutions and blanks were also treated with 500 µL Folin’s reagent and 400 µL Na₂CO₃. All testing solutions were analyzed in triplicates using ELISA microplate reader at a wavelength of 600 nm. A calibration curve was constructed and used for calculation (Tsai et al., 2022).

2.4. Determination of total flavonoid content

The sample solution (1 mg/mL) was prepared by diluting 10 mg/mL of the sample with ethanol. A 0.4 mg/mL of rutin stock solution was prepared and diluted to prepare the standard solutions with the following concentrations: 400, 200-, 100-, 50.0-, 25.0-, 12.5- and 6.25 µg/mL. Followed by five hundred microliters (500 µL) of the testing solutions (i.e., sample, standard and blank) was incubated for 1 h, after treating with 2.0% AlCl₃. Standard solutions and the samples were prepared in triplicates and analyzed on ELISA reader at 430 nm wavelength (Tsai et al., 2022).

2.5. Determination of total condensed tannin content

Fifty microliter of leaf extract solution (10 mg/mL) was diluted with 250 µL of ethanol and treated with 600 µL of Vanillin reagent. Through serial dilution, standard solutions (i.e., 160-, 80-, 40-, 20-, 10-, 5.0-, 2.5 µg/mL) was prepared from 160 µg/mL of catechin solution in ethanol. Then, 600 µL of Vanillin reagent was supplemented to 300 µL of standard solutions and blank (ethanol). The absorbance of the solutions (at 530 nm) was measured by using ELISA microplate reader. All testing solutions were prepared in triplicates to ensure data reproducibility.
2.6. Determination of DPPH free radical scavenging activity

The ascorbic acid solution (0.5 mg/mL in ethanol) was serially diluted to obtain concentrations of 500.0, 250.0-, 125.0-, 62.50-, 31.25-, 15.63-, and 7.813 μg/mL of standard solution. In a microplate reader, one hundred microliters (100 μL) of the standard solutions, control (ethanol) and extracts were taken and 150 μL of DPPH (200 μM) solution was added, and then incubated for 30 min. All testing solutions must be simultaneously treated with DPPH solution. The testing solutions (treated with DPPH) and blank (250 μL) in a microplate plate was analyzed in ELISA microplate reader at 517 nm (Tsai et al., 2022). To calculate the percentage radical scavenging activity, the equation below was used, and the activity was plotted against its corresponding concentrations. Through linear regression, the 50% inhibitory concentration (IC50) was estimated which was determined at 50% radical scavenging activity.

\[
\% \text{RSA} = \left( \frac{A_{\text{ctl}} - A_{\text{blk}}}{A_{\text{sp}} - A_{\text{blk}}} \right) \times 100
\]

Different extracts were added to each well and treated with LPS (500 ng/mL) for a period of 24 h. MTT (5 mg/mL) were added to each well and incubated for another 4 h. The spent cell media were eventually removed and isopropanol was used to dissolve formazan overnight. The cell culture plates were analyzed at 570 nm using an ELISA plate reader (Tsai et al., 2022).

2.11. Nitric oxide (NO) inhibition assay

The 96-well plates were seeded with cell suspension containing 4 × 10^4 cells per well. Different extracts were added to each well and treated with LPS (500 ng/mL) for a period of 24 h. Nitric oxide concentration was determined from the collected spent cell media after the addition of the Griess reagent with the use of ELISA plate reader at 560 nm (Tsai et al., 2022). The NO percentage inhibition was calculated using the equation: % NO = \{1 - (T/C)\}, where T and C represent the mean optical density of LPS-stimulated RAW 264.7 cells with and without sample extracts respectively.

2.12. Double chamber-microbial fuel cells (DC-MFCs)

With immersed area of ca. 0.000452 m² (ID = 1.2 cm), the graphite anode and cathode (grade, IGS743; Central Carbon Co., Ltd.) were soaked in culture broth or electrolyte solutions (ca. 0.001649 m², the cathode and anode chamber of operating volume 200 mL). Both chambers were selectively isolated by proton exchange membrane (DuPont Nafion® NR-212). For microbial growth in batch cultures, LB broth medium (Difco LB Broth, Miller; Luria-Bertani) containing 10 g/L tryptone, 5 g/L yeast extract, and 10 g/L NaCl was used in MFC chamber. Electroactive bacterium- *Shewanella halodis* WLP72 originally enriched and isolated to have favorable capabilities of azo dye removal and bioelectricity generation was used as seeding bacterial cultures in MFCs. Under room temperature, the cathodic chamber containing 6.38 g of K2Fe(CN)6 (potassium ferricyanide; BAKER ANALYZED, A.C.S. Reagent) and 17.42 g of K2HPO4 (dipotassium hydrogen phosphate; SHOWA Co. Ltd.) in 200 mL of distilled-deionized water was used. Using a water bath shaker (Shinkwang, SKW-12; 30 °C, 125 rpm), inoculated bacterium WLP72 obtained from an isolated colony on an LB-streak agar plate was pre-cultured in LB broth medium for 12 h. Approx. 1% (v/v) sub-cultured broth was inoculated in a freshly pre-autoclaved LB broth for a 12 h culture (pH was not adjusted for these O/N flask subcultures). For quantitative evaluation of bioelectricity-generating performance, the test sample(s) (e.g., herbal extract) were then placed on a 200 mL of cell broth (OD600 ~ 2.0-2.2) of the anodic chamber in double chamber MFC (Chen et al., 2021).

For power-generating determination, a D/A system (DAS 5020; Jiehan Tech. Corp., Taiwan) was used to determine the time courses of electric current (I_{MFC}) and voltage (V_{MFC}). Then, a 1 kΩ external resistance was applied to MFCs to have identical basis for comparison. While the power and current densities were determined by the equation below:

\[
\text{Density} = \frac{V_{\text{MFC}} \times I_{\text{MFC}}}{A_{\text{anode}}} \tag{1}
\]

\[
\text{Identity} = \frac{I_{\text{MFC}}}{A_{\text{anode}}} \tag{2}
\]

A_{anode} = apparent working area of the graphite anode

For V_{MFC} and I_{MFC}, this two parameters could be evaluated with linear sweep voltammetry through a workstation for electrochemical analysis (Jiehan 5600, Jiehan Technology Corp., Taiwan).
2.13. Determining pharmacokinetic properties and pharmacophore of the ligands

All compounds were obtained from PubChem (chem.ncbi.nlm.nih.gov) in sdf format and were converted to pdbqt, and mol2 format by OpenBabel (O’Boyle et al., 2011). The pdbqt files were used for docking analysis. Then, the canonical SMILES (from PubChem) of the compounds with good binding affinity were submitted to ADMET 2.0 server (Xiong et al., 2021) for drug-likeness and ADMET screening. While, mol2 files were submitted to PharmaGist (Schniedman-Duhovny et al., 2008) for pharmacophore modeling.

2.14. Protein Preparation and Validation

The Viral RdRp of SARS-CoV-2 (PDB: 7BTF) was obtained from Protein Data Bank (PDB, https://www.rcsb.org) and processed in Biovia Discovery Studio 2021 (Discovery Studio Visualizer, 2020) and AutoDock tools 4.2 (Rizvi et al., 2003). The binding site (Coordinates: x = 127.0598, y = 124.6167, and z = 128.7631; Size: x = 14, y = 14, and z = 14) of the protein were defined in Biovia Discovery Studio 2021. Followed by, removing the heteroatoms and water molecules from the protein. Then, the polar hydrogens, Kollman charge, missing atom, and AutoDock4 atom types were added into the pre-processed protein by AutoDock Tools 4.2. After processing, the proteins were submitted to UCLA-DOE-LAB SAVES v6.0 (saves.mbi.ucla.edu) and Ramachandran plot server (Anderson et al., 2005).

2.15. Molecular docking and molecular dynamics analysis

AutoDock Vina (Ansari et al., 2021) was used for docking analysis and the computer system used was Windows 11 with AMD Ryzen 7 3700U, Radeon Vega Mobile Gfx 2.30 GHz, and 8.00 GB RAM. Afterward, compounds with good binding affinity (compared to the positive control) were subjected to molecular dynamics simulation. According to the coarse-grained simulation, the root-mean-square fluctuation plot (RMSF) and interaction model (within 10 ns) of the apo-protein and protein-ligand complexes were obtained from CABS-Flex 2.0 (Ding et al., 2020; Hariyadi et al., 2020).

3. Result and discussion

3.1. Determination of total polyphenol, total flavonoid and total condensed tannin

To reveal antioxidant activities as possible antiviral activities, the quantitative measures of phytochemical constituents for total polyphenols, total flavonoids and total condensed tannins in the C. arabica leaves extracts using various solvents were carried out. The calibration curves clearly exhibited promising linear relationship of signal against the concentration of reference standards, with determination coefficient ($R^2$) greater than 0.99. That is, the amount of polyphenols, flavonoids and condensed tannins in all the samples well fell within the quantitation limits.

As Table A (Supplementary Information) indicated, phytochemical profiles of C. arabica leaves extracted using five different solvents of varying polarities were summarized. The total polyphenol contents were in decreasing order of mg Gallic Acid Equivalent (GAE)/g of extracts were shown as follows: 244.342 ± 2.107 (CA-40-EtOH) > 187.892 ± 19.371 (CA-AC) > 183.618 ± 2.063 (CA-H2O) > 151.505 ± 5.778 (CA-EA) > 115.89 ± 1.964 (CA-HX). Ethanol extraction seemed to be the most applicable solvent to gain the maximal amounts of the polyphenols. The amounts of total flavonoids extracted in decreasing order (unit: mg of rutin/g of extract) were indicated as follows: 30.885 ± 2.627 (CA-EA) > 25.903 ± 2.44 (CA-40-EtOH) > 22.655 ± 1.944 (CA-AC) > 14.65 ± 0.704 (CA-H2O) > 8.242 ± 0.968 (CA-HX). The solvent ethyl acetate provided the highest yield of the total flavonoid content from the C. arabica leaves. The amount of total condensed tannin (unit in mg of catechin/g of extract) were listed as follows: 40.237 ± 0.0864 (CA-AC) > 39.809 ± 1.308 (CA-40-EtOH) > 26.928 ± 0.102 (CA-EA) > 26.126 ± 0.61 (CA-HX) > 12.943 ± 0.11 (CA-H2O). There were very minute differences between the yield from acetone and ethanol, although acetone yield seemed to be the highest. In addition, no significant difference was observed from the yield using ethyl acetate and hexane solvents. For comparison with identical extracts, both antioxidant assays (e.g., DPPH and FRAP assays) were performed. FRAP assay of the leaf extracts (expressed in mg of Trolox/g) in the decreasing order of antioxidant activity were as follows: 2.249 ± 0.034 (CA-40-EtOH) > 2.013 ± 0.009 (CA-H2O) > 0.615 ± 0.026 (CA-AC) > 0.494 ± 0.018 (CA-EA) > 0.176 ± 0.017 (CA-HX). As indicated herein, ethanol extract exhibited the highest antioxidant activity via FRAP assay. On the other hand, DPPH assay measures the percentage inhibition using ascorbic acid as standard and an inverse relationship was found to be present. The lowest inhibitory value (IC50) implies the highest antioxidant activity. DPPH assay in an increasing order of antioxidant activities was ranked as follows: 0.516 ± 0.005 (CA-40-EtOH) < 1.11 ± 0.024 (CA-H2O) < 5.125 ± 0.027 (CA-EA) < 5.807 ± 0.086 (CA-AC). Nearly negligible antioxidant activity was detected for hexane leaf extract. Both FRAP and DPPH assays were in consistent with the highest antioxidant activity using ethanol followed by water as extracting solvents. Moreover, as Table A (Supplementary Information) showed, high amounts of total polyphenols in the ethanol extract were well correlated with the obtained antioxidant activities as aforementioned. This finding indicated that ethanol extraction was the most favorable and acceptable to maximal yield effective constituents in test samples. To understand the impact of sample processing in bioactivity of the extract, further analysis on the ethanolic leaf extract with various drying processes was implemented (Table 1).

Regarding the phytochemical analysis, the results reveal that ethanol extracts show the highest total phenol content. Similar results from the study of Campa et al. (2012) highlights the presence polyphenols (e.g., mangiferin, chlorogenic acid, caffeic acid, and ferulic acid) in C. arabica. These extracts may well be correlated to the results from the antioxidant assays. The differences for the extracting solvents for the total flavonoids and condensed tannins can be attributed to the difference in the polarity in the solvents. The solubility of the compounds in the solvent used for extraction is thus an important factor (Vijayalaxmi et al., 2015). The highest antioxidant activity (mg Trolox/g) in the FRAP assay was obtained from the CA-40-EtOH extraction and negligible quantities were obtained from the rest of the conditions. Moreover, DPPH assay demonstrated the highest antioxidant activity using extracts from CA-F-EtOH (Table 2). As reported by Ngamsuk et al. (2019), drying C. arabica leaves (DY50) at 50 °C does yield a higher total polyphenolic content and a higher DPPH radical scavenging activity than DY40 (dried by 40 °C). Hence, the amount of total polyphenol content present from the plant is correlated with the DPPH radical scavenging activity of the extract (Klattisim et al., 2018). Although, the total polyphenolic content

Table 1

| Phytochemical content analysis of C. arabica leaves at numerous drying conditions. | Extract | Total polyphenol content analysis (mg/g) | Total flavonoid content analysis (mg/g) | Total condensed tannin content analysis (mg/g) |
|---|---|---|---|---|
| C. arabica Leaves | CA-F- EtOH | 185.773 ± 0.02 | 226.069 ± 0.007 | 152.019 ± 0.003 |
| | CA-A- EtOH | 95.124 ± 0.016 | 167.949 ± 0.018 | 100.435 ± 0.002 |
| | CA-40- EtOH | 244.342 ± 2.107 | 25.903 ± 2.44 | 39.809 ± 1.308 |
| | CA-80- EtOH | 169.594 ± 0.008 | 238.348 ± 0.008 | 127.322 ± 0.006 |
extracted from the leaves may increase as the temperature increases. On the other side, this might not be the case once the drying temperature exceeded its optimal temperature. Based on the patented procedures, the other side, this might not be the case once the drying temperature exceeded its optimal temperature. Based on the patented procedures, the optimal temperature for drying the plant sample was 30–60 °C. Consider drying the plant sample under the temperature of 80 °C, the total polyphenolic content of the sample decreased along with its antioxidant capacity. This may be due to thermal degradation of polyphenolic compounds present in the sample which is affected by factors like solvent type and treatment time. (Ricci et al., 2019).

In fact, due to considerable market demand of coffee beans, there were plentiful literature mentioned about improvement of the flavor profile of their coffee-associated product(s). Although many claims indicated that caffeine in coffee may have many adverse effects, there were still several studies showing health benefits of coffee utilization. For example, considerable studies focused on metabolites in coffee beans (e.g., metabolites depended upon coffee quality) (Ansari et al., 2021; Ding et al., 2020). As previously mentioned, there were limited literature depicted about uses of coffee leaves although some countries have popularly utilized coffee leaves as daily tea drink (Hariyadi et al., 2020; Novita et al., 2018). Dietary antioxidants have been claimed to exist in coffee leaves and some are even utilized as a source of novel food (Monteiro et al., 2020). Here, this first-attempt study explored the possibility of adopting phytochemicals as feasible source of antioxidants in C. arabica leaves using different extraction and drying methods. In fact, leaves are abundant in coffee plantations and they were usually considered to be no economic viability to exploit such raw materials. Here, considering sustainable recycling for value-added applications, this work disclosed chemical constituents of coffee leaves and inspected detailed insights on different bioprocesses for product bioconversions of recovery.

Aware that phytochemicals are bioactive nutrient plant chemicals possibly provide health benefits to reduce risk of some diseases. They also participate in a multitude of biological processes and chemical reactions (Pang et al., 2021). Organisms rely on plant food attain their health and physiological benefits via different routes in the complex biochemical pathways in cells. These pathways deal with energy metabolism, cellular regeneration and repair, modulation of the immune response, elimination of unwanted metabolites or cancer prevention (Linnewiel-Hermoni et al., 2015). Some of these vital plant compounds (e.g., flavonoids, anthocyanins, carotenoids) contain aromatic moieties, conjugated systems or both in their chemical structure (Shimizu et al., 2010; Gupta and Prakash, 2014). Such structures are of great significance to effectively participate in electron transferring or redox mediating processes in biotic and abiotic reactions. These compounds provided from plant-based food may even facilitate extracellular electron transfer reactions of gut microbiome through digestion for efficient bioenergy utilization (Duda-Chodak et al., 2015).

To disclose possible potential of bioenergy medicine, significant characteristics of phytoneutrients (e.g., total polyphenols, total flavonoids, and total condensed tannins) were exhibited from the extracts of C. arabica leaves using various solvents and different drying techniques. This scheme was applied to maximize the yield of the phytochemicals in the crude extract since distinctive solvents can solubilize different concentrations of specific functional group-bearing compounds from coffee leaves (Marchea et al., 2019).

As polyphenolic contents were reported to have capabilities of disease treatment, of course total polyphenolics from the extracts of coffee leaves were first determined. In fact, plant polyphenolics as vital secondary metabolites are elucidated to manipulate regulatory metabolisms and immune responses of significance to diverse organisms (Shin et al., 2020). In particular, they could considerably promote antioxidant and anti-inflammatory properties in plant foods. Recent studies even revealed potentials to prevent viral infections (e.g., anti-COVID-19) (Cory et al., 2018; Montenegro-Landivar et al., 2021). Next group of molecules to be investigated are the condensed tannins where they were bearing several ortho-dihydroxyol substituent-bearing polyphenols with electron-shuttling potential for bioenergy stimulation. They are synthesized from the precursor compound flavan-3-ol which are related to catechin and epicatechin from Camellia green tea (Ju et al., 2021; Abu et al., 2016). In some cases, flavanols are recognized as catechin which were present in many plant foods (e.g., grapes and cocoa beans) often in the form of (+)-catechin and (-) epicatechin. Moreover, the monomeric form of flavanols are the catechin and in the polymeric form which are also known as proanthocyanidin (Markovic, 2015). The third major group includes flavonoids which are polyphenols with diverse forms of either an aglycones or glycosides. Their structure is comprised of 15 carbons with a common skeleton of phenyl-benzo-γ-pyran, composed of two phenyl rings and a heterocyclic pyran ring. In fact, flavonoids in plants usually carry different functional groups (e.g., flavonols, flavones, flavonoids, flavanones, anthocyanidins, and isoflavones). Total flavonoids are expressed in terms of rutin concentration for analysis. Studies on the bioactivities of flavonoids have demonstrated its medicinal properties (e.g., antioxidant, anti-carcinogenic and offer many physiologically protective roles in organisms) (Ganeshpurkar and Saluja, 2017; Gullón et al., 2017; Kopustinskiene et al., 2020).

### 3.2. Antioxidant activity of C. arabica leaves extracts

Regarding solvents used for extraction, apparently ethanol provided the most encouraging yield in terms of total polyphenols and rank second for condensed tannins and flavonoids (Table A in Supplementary Information). Apparently, antioxidant activities exhibited for both DPPH and FRAP assays were primarily due to the total polyphenols in the crude extract. Phenolic hydroxyl groups can engage in a cease reaction with reactive oxygen and reactive nitrogen species that inhibit the cycle of producing new radicals (Bernatoniene and Kopustinskiene, 2018). Among the solvents chosen for extraction, ethanol could extract the maximal contents of high antioxidant activities. Thus, different drying approaches of coffee leaves prior to extraction were implemented. Evidently, drying approaches of leaf samples followed by ethanol extraction offered higher yields compared to the other solvents (i.e., ethyl acetate, water, hexane, and acetone). In terms of yield and antioxidant activity, the freeze-dried leaves exhibited the optimal content among the other drying methods prior to ethanol extraction (Table 2).

As the flavonoids, polyphenols and condensed tannins have been regularly mentioned in literature to exhibit antioxidant characteristics, this study employed these two methods for measuring antioxidant activities of plant extracts. The DPPH assay that is based on the hydrogen atom donating ability of plant extract assayed from the decolorization of methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The molecules of DPPH generate a purple hue in methanol solution which turns to a yellow-colored solution in the presence of antioxidants. Here, the antioxidant activities were reported as IC50 (Half Maximal Inhibitory Concentration) values which can be interpolated from the concentration of the extract that can scavenge 50% of DPPH free radicals (Lee et al.,

### Table 2

| Extract | DPPH assay IC50 (mg/mL) | FRAP assay (mg trolox/g extract) |
|---------|-------------------------|----------------------------------|
| CA-H2O  | 1.11 ± 0.024            | 2.013 ± 0.009                    |
| CA-40%  | 0.516 ± 0.005           | 2.249 ± 0.034                    |
| EtOH    | 5.125 ± 0.027           | 0.494 ± 0.018                    |
| CA-EA   | 5.807 ± 0.086           | 0.615 ± 0.026                    |
| CA-HX   | ND                      | 0.176 ± 0.017                    |
| CA-F-EtOH| 0.278 ± 0.005           | 0.024 ± 0.001                    |
| CA-A-EtOH| 0.451 ± 0.014           | 0.017 ± 0.001                    |
| CA-80%  | 0.327 ± 0.006           | 0.023 ± 0.001                    |
| EtOH    | 0.235 ± 0.016           | acidic                            |
Moreover, the other assay known as FRAP (ferric reducing antioxidant power) is based on the principle of an electron transfer-based reaction where measurements are based on the conversion of ferric ion (Fe³⁺)-ligand complex to intensely blue-colored ferrous (Fe²⁺) complex by antioxidants in acid solution (pH 3.6). Antioxidant activity is quantitatively evaluated as the increase in absorbance at 593 nm, and results are expressed as μmol Fe²⁺ equivalents or relative to an antioxidant standard (Benzie et al., 1996). Trolox is a water-soluble Vitamin E analog and the preferred commercially available reference standard for this analysis.

3.3. High pressure liquid chromatography analysis of C. arabica leaves extracts marker substances

To specifically identify whether different treated extracts could own dissimilar characteristics of medicinal properties, HPLC profile for the ethanol extract of the coffee leaves with the different drying conditions were employed (Table 3 and Fig. 1). This study intentionally selected four bioactive compounds (i.e., caffeine, mangiferin, chlorogenic acid and catechin) as fingerprint chemical species for comparative analysis. As indicated, catechin is evidently the most abundant in the CA-40-EtOH extract (ca. 3.43 ± 0.11 mg/g). CA-80-EtOH was in the highest amount of caffeine (48.71 ± 0.30 mg/g) followed by CA-A-EtOH (30.12 ± 0.66 mg/g), then CA-F-EtOH and CA-40-EtOH were both approximately in similar quantities (~19 mg/g). Chlorogenic acid was the top-level in CA-80-EtOH (19.61 ± 0.16 mg/g) and lowest at CA-40-EtOH (4.14 ± 0.05 mg/g). Mangiferin exhibited the top-ranking amounts in CA-40-EtOH (16.73 ± 0.27 mg/g) extract and lowest with CA-A-EtOH. Apparently, Table 3 and Fig. 1 provided the inconsistent profile for the variation of these four bioactive substances.

The crude extracts from ethanol extraction were also subjected to HPLC analysis (Table 3). Undeniably, there was no such the best drying approach for recovery of caffeine, mangiferin, chlorogenic acid and catechin. HPLC analyses were also consistent with the CA-40-EtOH extract since the greatest amount of total polyphenols as aforementioned was consistent with the high amount of catechin from HPLC analysis. Furthermore, CA-80-EtOH ranked second in the amount of catechin as HPLC analysis indicated, but this extract exhibited the highest amount of total flavonoids. These findings clearly unveiled the quantitative consistency of spectrophotometric assays and HPLC analyses. Certain forms of flavonoids especially under the sub-group of flavanols are closely related to catechin (Bernatoniene and Kopusinskieni, 2018) and our data showed that the catechin (from HPLC) was most likely measured under total flavonoids or total polyphenols using spectrophotometry.

3.4. Cell viability assay

To reveal whether extracts were biocompatible and non-toxic to be considered for further biomedical uses, cytotoxicity assessment on the various coffee leaves extracts. The water extract showed a slightly higher amplification of power density compared to the blank (CA-H₂O (10.13 ± 0.52 (1.36±0.13)) > blank (7.45 ± 0.33 (1.00)). This indicated that chemical components from the water leaf extract were capable of electron mediating for bioelectricity generation. Comparative analysis upon power-generating capabilities of water and ethanol leaf extracts with respect to the blank was implemented. In general, ethanol extracts of coffee leaves exhibited higher power density compared to the water leaf extract. The ranking of power density of various coffee leaf extracts (unit: mW/m²) was shown as follows (subscript was amplification factor with respect to the blank) (Fig. 3):

Table 3

| Extracts | Marker substance | (mg/g) |
|----------|------------------|--------|
|          | Catechin         |        |
| CA-F-EtOH| 0.76 ± 0.04     | 13.48 ± 0.18 |
| CA-A-EtOH| 1.08 ± 0.02     | 6.15 ± 0.11  |
| CA-40-EtOH| 3.43 ± 0.11    | 4.14 ± 0.05  |
| CA-80-EtOH| 2.04 ± 0.05    | 19.61 ± 0.15 |
|          | Chlorogenic acid |        |
| CA-F-EtOH|              |        |
| CA-A-EtOH|              |        |
| CA-40-EtOH|             |        |
| CA-80-EtOH|             |        |
|          | Caffeine         |        |
| CA-F-EtOH|              |        |
| CA-A-EtOH|              |        |
| CA-40-EtOH|             |        |
| CA-80-EtOH|             |        |
|          | Mangiferin       |        |
| CA-F-EtOH| 0.76 ± 0.04     | 13.48 ± 0.18 |
| CA-A-EtOH| 1.08 ± 0.02     | 6.15 ± 0.11  |
| CA-40-EtOH| 3.43 ± 0.11    | 4.14 ± 0.05  |
| CA-80-EtOH| 2.04 ± 0.05    | 19.61 ± 0.15 |

3.5. Nitric oxide (NO) inhibition assay

Table 4 and Fig. 2 showed the capabilities of various leaf extracts to inhibit the production of NO in vitro using stimulated RAW264.7 cell lines. NO is a known signaling molecule that plays a crucial role in cellular inflammatory response. Their production triggers a cascade of events, leading to an inflammatory response in organisms. Among the solvents used for extraction, only ethanol extracts exhibited capability to slightly inhibit nitric oxide (IC₅₀ = 274.89 µg/mL). As Fig. 2 showed, the ranking of different ethanol extracts in a decreasing order for NO inhibition was shown as follows: 274.89 µg/mL (CA-40-EtOH) < 544.25 µg/mL (CA-80-EtOH) < 629.22 µg/mL (CA-A-EtOH) < 698.33 µg/mL (CA-F-EtOH). The CA-40-EtOH extract exhibited the best NO inhibition due to the lowest values of IC₅₀ to inhibit nitric oxide synthesis in vitro. Apparently, both MTT and NO inhibition assays supported that coffee leaves were feasible material for re-purposing like food or medicine-oriented applications.

The results of the NO inhibition assay reveal a direct correlation to the polyphenol content. Known mechanisms of anti-inflammatory activity of caffeine as a competitive inhibitor for adenosine receptors and another mechanism is through the inhibition of the cyclooxygenase enzyme and prostaglandin synthesis reduction (Galami et al., 2013). Aside from the polyphenols, condensed tannis and flavonoids detected in the C. arabica leaves, specific fingerprint compounds (e.g., catechin, caffeine, chlorogenic acid and mangiferin) were also determined. Mangiferin as earlier identified in mangoes (M. indica Linn) belongs to a group of compounds called xanthones. They are crucial to provide a wider range of pharmacological values (e.g., antioxidant, anticancer, antimicrobial, anti-atherosclerotic, anti-allergic, anti-inflammatory, analgesic, and immunomodulatory characteristics) (Mei et al., 2021; Walla et al., 2021). As a matter of fact, xanthones were powerful anti-oxidants in nature, as Table 1 showed good antioxidant activities for both DPPH and FRAP assays. This could be at least partly contributed by mangiferin, since HPLC analysis exhibited high quantities in both CA-F-EtOH and CA-40-EtOH extracts. Conversely, chlorogenic acid is a polyphenol and an ester of caffeic acid and quinic acid. They owned medicinal properties (e.g., antioxidant, anti-inflammatory, anti-diabetic and anti-obesity activities) (Lu et al., 2020; Göckeen and Sanlier, 2019; Loader et al., 2017).

3.6. Power Density Determination of the Leaf Extracts Using Dual Chamber Microbial Fuel Cells (DC-MFCs)

As performance of biomass energy expression would be crucial to influence whether efficacious electrochemical catalysis was taken place, various extracts were thus provided to implement bioenergy evaluation via DC-MFCs. Table 5 and Fig. 3 showed the power density profiles of various coffee leaves extracts. The water extract showed a slightly higher amplification of power density compared to the blank (CA-H₂O (10.13 ± 0.52 (1.36±0.13)) > blank (7.45 ± 0.33 (1.00)). This indicated that chemical components from the water leaf extract were capable of electron mediating for bioelectricity generation. Comparative analysis upon power-generating capabilities of water and ethanol leaf extracts with respect to the blank was implemented. In general, ethanol extracts of coffee leaves exhibited higher power density compared to the water leaf extract. The ranking of power density of various coffee leaf extracts (unit: mW/m²) was shown as follows (subscript was amplification factor with respect to the blank) (Fig. 3):

(1) MFC-A: CA-40-EtOH (16.84 ± 3.59(1.46±0.54)) > blank (11.57 ± 1.83(1.00))
(2) MFC-B: CA-H₂O (10.13 ± 0.52(1.36±0.13)) > blank (7.45 ± 0.33(1.00))
(3) MFC-C: CA-A-EtOH (26.43 ± 0.00 (2.72 ± 0.45)) > CA-F-EtOH (24.58 ± 4.04 (2.53 ± 0.84)) > CA-80-EtOH (21.31 ± 4.67 (2.20 ± 0.85)) > blank (9.70 ± 1.61 (1.00)).

Of course, the highest amplification factor (AF) of power density was obtained from CA-A-EtOH (ca. 2.72 ± 0.45 fold). The decreasing AF trend for the power generation output is as follows: CA-F-EtOH (2.53 ± 0.84) > CA-80-EtOH (2.20 ± 0.85) > CA-40-EtOH (1.46 ± 0.54) > CA-H₂O (1.36 ± 0.13). Of course, the phytochemicals obtained from ethanol extracts would be the most electrochemically active to bioenergy generation in DC-MFCs. As prior study pointed out, the amplification factor with respect to the blank greater than ca. 2.0 would be an appropriate level of strong electron transfer potential to have convincing antiviral characteristics (i.e., “twofold index”). That is, ethanol extracts would be the most promising candidates of antiviral activities as prior work revealed (Tsai et al., 2022).

As aforementioned analysis indicated, coffee leaves possessed chemical groups that contain aromatic rings and a system of double bonds (Fig. 3(B)). The antioxidant properties were attributed to the capabilities of chemical moieties to act as reducing agents in redox-
mediating processes. However, the presence of electron donating and electron withdrawing substituents present in the chemical structures all greatly affect these antioxidant capabilities (Sys et al., 2017; Chen et al., 2016). Leaf extracts are composed of different proportions of phytochemicals and some share similarities with the parent chemical structures. These variations may be due to different electrochemical properties of extracts via chemical reactions. Both DPPH and FRAP assays also pointed out electron transfer potentials of test samples. This study showed differences in their antioxidant activities presumably due to various types and nature of molecules present in the phytochemicals of leaf extract. Moreover, power density profiles in DC-MFCs also suggested promising electron-mediating potentials of the leaf extracts, considerably stimulating electron transfer capabilities of electroactive microbes in MFCs. That is, these extracts may own synergistic nature to trigger redox mediation-oriented potentials for medicinal purposes (e.g., neurotransmitter-related diseases and Parkinson’s disease as indicated

Table 4
Cell Viability and NO Inhibition Assay of RAW264.7 cells of crude extracts from C. arabica leaves.

| Crude extract | IC<sub>50</sub> (µg/mL) | NO inhibition (µg/mL) | Cell viability |
|---------------|--------------------------|-----------------------|----------------|
| C. arabica Leaves |
| CA-H<sub>2</sub>O | – | – | – |
| CA-40-EtOH | 274.89 | Non toxicity | Non toxicity |
| CA-EA | – | Non toxicity | Non toxicity |
| CA-AC | – | Non toxicity | Non toxicity |
| CA-HX | – | Non toxicity | Non toxicity |
| CA-F-EtOH | 698.33 | Non toxicity | Non toxicity |
| CA-A-EtOH | 629.22 | Non toxicity | Non toxicity |
| CA-80-EtOH | 544.25 | Non toxicity | Non toxicity |

Fig. 1. (continued).
Fig. 2. Effect of crude extracts from the seeds of (a) CA-40-EtOH; (b) CA-F-EtOH; (c) CA-A-EtOH (d) CA-80-EtOH on cell viability of RAW264.7 cells.
in prior works) (Guo et al., 2019; Tsai et al., 2022). Although this study showed antioxidant properties of extracts, their pharmacological interactions in either synergy or antagonism were still remained to be further disclosed. Chemical profiling of the crude extract may be an important step in follow-up studies to decipher detailed insights on such possible interaction of secondary metabolites as exhibited afterwards.

3.7. Protein validation

To have more detailed assessment, protein model after processing was validated first before any docking analysis. Different algorithms were used to ensure the structural validity of the protein, and the results were indicated in Fig. 4. Amino acids in the protein with preferable and unpreferable conformations were shown in the Ramachandran Plot (Fig. 4A) and the quality of the non-bonding interaction was examined by ERRAT (Fig. 4C). In addition, the Verify 3D supported the compatibility of the amino acid sequence to its three-dimensional structure (Dym et al., 2011; Eisenberg et al., 1997).

3.8. In silico screening of the anti-SARS-CoV-2 properties of the phytochemicals

After protein validation, the drug-likeness of the compounds were determined, and only two (catechin and caffeine) of them passed Lipinski’s rule of five (Benet et al., 2016). However, this is not sufficient to exclude the viability of other two compounds for the screening process. For instance, although positive control of this study (remdesivir) did not pass Lipinski’s rule, it was still considered for further development and released into the market as an anti-viral drug. Furthermore, Doak et al. proposed that the criteria for drug-likeness could be extended further than just the rule of five (Doak et al., 2014). Therefore, the docking analysis was performed, and three of the phytochemicals have a higher binding affinity (Table 6) over the positive control. The binding interaction between the protein and ligands was shown in Figure A, B and 5, and the amino acid residues responsible for the interaction were tabulated in Table 7. (Fig. 5)

Among thousands of amino acids, 15 amino acids (two sheets, two turns, four helices, and five coils) were observed to interact with the three compounds. Out of 15, Arg 555 (Coil), Asp 623 (Helix), and Asp 691 (Turn) were commonly found to interact with the compounds. The remaining amino acids (Arg 553, Ala 554, Thr 556, Val 557, Arg 624, Thr 680, Thr 681, Ser 682, Thr 687, Ala 688, Ser 759) would interact with either one or two of the compounds during binding. Commonly, these residues usually interacted with hydrogen bonding, van der Waals, and carbon-hydrogen bond.

Among all, chlorogenic acid had the highest binding energy affinity to the viral RdRp, and ten amino acids from the protein were responsible for the interaction. Most of them interacted with the ligand by van der Waals force which was known to be a determinant of complex formation. Furthermore, van der Waals forces were considered as an essential

### Table 5

| Type of Extract | Amplification factor of power generation |
|----------------|-----------------------------------------|
| CA-H2O         | 1.36 ± 0.13                             |
| CA-40-EtOH     | 1.46 ± 0.54                             |
| CA-A-EtOH      | 2.72 ± 0.45                             |
| CA-F-EtOH      | 2.53 ± 0.84                             |
| CA-80-EtOH     | 2.20 ± 0.85                             |

Fig. 3. (A) Comparison of power-density profiles of different coffee extracts supplemented DC-MFCs (replicated blanks were applied to guarantee data reproducibility of DC-MFCs), (B) The postulated electron-shuttling scheme for antiviral activities where resonance forms (2) and (3) as intermediates for the redox mediator of catechin (ED: electron donor) to attack target virus.
Table 6
Drug-likeness and binding affinity of the phytochemicals found in coffee leaves and the positive control.

| Compound Name | Binding affinity (kcal/mol) | Molecular Formula | Molecular Weight (Da) | Log P | HBD | HBA | TPSA (Å²) |
|---------------|----------------------------|-------------------|-----------------------|-------|-----|-----|-----------|
| Catechin      | -6.2                       | C_{13}H_{14}O_{6} | 290.27                | 1.343 | 5   | 6   | 110.38    |
| Chlorogenic acid | -6.5                 | C_{16}H_{18}O_{9} | 354.31                | 0.331 | 6   | 9   | 164.75    |
| Caffeine      | -4.7                       | C_{10}H_{12}N_{2}O_{2} | 194.19             | 0.048 | 0   | 6   | 61.82     |
| Mangiferin    | -6.2                       | C_{19}H_{18}O_{11} | 422.30                | 0.136 | 8   | 11  | 201.28    |
| Remdesivir (Control) | -6.1                  | C_{27}H_{35}N_{6}O_{8}P | 602.23               | 1.761 | 5   | 14  | 204.28    |

Log P: Lipophilicity; HBD: Hydrogen-bond Donor; HBA: Hydrogen-bond Acceptor; TPSA: Total polar surface area

Table 7
List of amino acid residues in viral RdRp observed to interact with the phytochemicals and the average RMSF value of each protein-ligand complex.

| Hydrogen Bonding | Catechin | Chlorogenic acid | Mangiferin | Caffeine |
|------------------|----------|-----------------|------------|----------|
| Thr 556 (2.21 Å), Asp 623 (3.01 Å) | Arg 553 (2.16 Å), Ser 682 (2.78 Å), Ala 688 (2.77 Å), Thr 556 (2.87 Å) | Arg 553 (2.58 Å), Arg 555 (2.42 Å) | – |
| Thr 687 (2.26 Å) | Asp 623 (3.38 Å) | Arg 623 (3.38 Å, 3.56 Å) | Asp 623 (3.38 Å, 3.56 Å) | – |

Van der Waals

| Ala 554, Arg 624, Thr 680, Ala 688, Asn 691, Ser 759 | Arg 555, Val 557, Thr 681, Asn 691, Ser 759 | Arg 555, Arg 624, Ser 682, Asp 684, Ala 685, Asn 691 | Arg 555, Thr 556, Thr 680, Ser 681, Ser 682, Asn 691 |
| – | Asp 623 (3.52 Å) | Arg 623 (3.38 Å, 3.56 Å) | Asp 623 (3.54 Å, 4.10 Å) |

Carbon Hydrogen Bond

| e-Anion | Arg 555 (3.30 Å) | – | – | Asp 623 (3.54 Å, 4.10 Å) |
| e-Alkyl | Ala 688 (5.36 Å) | Ala 688 (4.72 Å) | – | – |
| Average RMSF value | 0.8359 ± 0.023 Å | 0.8197 ± 0.026 Å | 0.8653 ± 0.025 Å | Not applicable |
Fig. 5. Binding interaction of SARS-CoV-2 Viral RdRp and chlorogenic acid, (A) Ligand binding sphere and protein, (B) Two-dimensional display of the interaction, (C) Ramachandran plot of Apoprotein, (D) Hydrophobic property of binding pocket, (E) H-bond property of binding pocket, (F) Ramachandran plot of the ligand-interacting amino acid residues.

Fig. 6. Pharmacophore descriptor of phytochemicals with promising binding affinity.
factor for molecular recognition of the binding pocket and affect the ligand’s binding affinity (Bitencourt-Ferreira et al., 2019). Other than van der Waals forces, hydrogen bonding and carbon hydrogen bond contributed to the binding of chlorogenic acid. Of course, combined interactions between the ligand and protein would be related to the pharmacophore descriptor of compounds (Fig. 6). Mainly, the hydroxyl group in the compounds were the one acting as the hydrogen-bond acceptor and donor. Thereby, majority of the hydrogen bonding interaction present in the protein-ligand complex could be due to the hydroxyl groups in the compounds (Raschka et al., 2018). While, the aromatic function groups were observed to interact with the protein either by π-alkyl or x-anion.

3.9. Molecular dynamics simulation of the protein-ligand complexes

A molecular dynamics simulation was performed with the complexes to understand the stability and impact of ligand binding to the protein. As the average RMSF values of the complexes (Table 7) and the model and RMSF plot yield from the simulation (Figure C, D, 7) presented, the most promising candidate, chlorogenic acid had a stiffer protein-ligand complex than the other two complexes. The average motion of the amino acid residues in the protein is reduced after ligand binding (Grünberg et al., 2006). This phenomenon was also observed on Remdesivir, which was known to have a strong ligand binding to viral RdRp and terminate the viral transcription (Bardal et al., 2011). Hence, chlorogenic acid could be a promising anti-viral candidate with the same mechanism of action as remdesivir revealed.

3.10. Pharmacokinetics properties of the compounds

To predict the possible pharmacokinetics properties of the compounds, an ADMET screening was also employed on the compounds (Fig. 8). Compared to the control, the bioavailability of the phytochemicals was higher due to their affinity to plasma protein. As literature reported, high plasma protein binding increased the chance of the chemicals was higher due to their affinity to plasma protein. As literature reported, high plasma protein binding increased the chance of the chemicals was higher due to their affinity to plasma protein. As literature reported, high plasma protein binding increased the chance of the bioavailability of the phytochemicals (e.g., polyphenols, flavonoids and condensed tannins) were explored. Oven dried leaf samples at 40 °C of the leaf samples prior to ethanol extraction (CA-40-EtOH) provided the highest contents of total polyphenols in the extract, suggesting the most promising antioxidant activity as FRAP assay revealed. Cytotoxicity studies also showed that all ethanol extracts elucidated excellent cell viability while CA-40-EtOH offered the highest anti-inflammatory activity using RAW264.7 cell lines. HPLC analyses of C. arabica leaves extracts confirmed the presence of catechin, caffeine, mangiferin and chlorogenic acid. Molecular docking analyses of these metabolites against RdRp of SARS-CoV-2 demonstrated that chlorogenic acid had the highest binding affinity over the RdRp of SARS-CoV-2 and a much lower average RMSF value than an apoprotein. Thus, a stiffer viral RdRp protein formed could highly affect the transcription process, leading to pre-termination of the whole viral replication process (Khan et al., 2021). Followed by, catechin and mangiferin expressed an equal binding affinity to the protein. The top three rank compounds do belong to different sub-groups of polyphenolic compounds which were known to have diverse bioactivity including anti-viral nature. Among all, caffeine indeed revealed a low binding affinity (~ 4.7 kcal/mol) over the target and less non-bonding interaction to the protein. As observed, caffeine interacted with six amino acid residues (from viral RdRp) either by van der Waals or x-anion. Unlike the other three, hydrogen bonding interaction was observed that would strengthen the protein-ligand complex formed (Raschka et al., 2018). In summary, this study provided some potential hits for anti-viral drug that can act as RdRp inhibitor. Hence, follow-up studies may do QSAR studies to further improve the pharmacokinetic and efficacy of these compounds for next phase toward clinical trial.

4. Conclusion

Medicinal potential groups of phytochemicals (e.g., polyphenols, flavonoids and condensed tannins) were explored. Oven dried leaf samples at 40 °C of the leaf samples prior to ethanol extraction (CA-40-EtOH) provided the highest contents of total polyphenols in the extract, suggesting the most promising antioxidant activity as FRAP assay revealed. Cytotoxicity studies also showed that all ethanol extracts elucidated excellent cell viability while CA-40-EtOH offered the highest anti-inflammatory activity using RAW264.7 cell lines. HPLC analyses of C. arabica leaves extracts confirmed the presence of catechin, caffeine, mangiferin and chlorogenic acid. Molecular docking analyses of these metabolites against RdRp of SARS-CoV-2 demonstrated that chlorogenic acid had the highest binding affinity over the RdRp of SARS-CoV-2 and a much lower average RMSF value than an apoprotein. Thus, a stiffer viral RdRp protein formed could highly affect the transcription process, leading to pre-termination of the whole viral replication process (Khan et al., 2021). Followed by, catechin and mangiferin expressed an equal binding affinity to the protein. The top three rank compounds do belong to different sub-groups of polyphenolic compounds which were known to have diverse bioactivity including anti-viral nature. Among all, caffeine indeed revealed a low binding affinity (~ 4.7 kcal/mol) over the target and less non-bonding interaction to the protein. As observed, caffeine interacted with six amino acid residues (from viral RdRp) either by van der Waals or x-anion. Unlike the other three, hydrogen bonding interaction was observed that would strengthen the protein-ligand complex formed (Raschka et al., 2018). In summary, this study provided some potential hits for anti-viral drug that can act as RdRp inhibitor. Hence, follow-up studies may do QSAR studies to further improve the pharmacokinetic and efficacy of these compounds for next phase toward clinical trial.

Fig. 7. Molecular dynamics simulation of the viral RdRp bound with chlorogenic acid. (A) RMSF values of amino acid residues of Apoprotein vs protein-ligand complex and control. Three-dimensional models of (B) Changes in Apoprotein in 0 ns (Yellow) and 10 ns (Violet), (C) Changes in Protein-ligand complex in 0 ns (Yellow) and 10 ns (Teal), and (D) overlap model of Apo-protein (Violet) vs protein-ligand (Teal) complex (at 10 ns).
acid owned the highest binding affinity and a much lower average RMSF value than the apoprotein. In general, *C. arabica* leaves metabolites exhibited various degrees of inhibition against RdRp which may imply follow-up exploration in future studies as either sole compounds or using a combinatorial approach. Extracts from the plant were also used as electroactive mediators in MFCs. Apparently, air-dried leaves (CA-A-EtOH) exhibited the maximal amplification factor of power density (2.72 fold) to the blank. Detailed analyses suggested that *C. arabica* leaves contained crucial compositions of antioxidants and the electron-mediating chemicals to stimulate power generation in DC-MFCs. This work provided new model to further exploration upon electrochemically-active substances and their therapeutic potential for biomedical applications. *C. arabica* leaves could even more feasible to be considered as raw materials for bioprocesses and as a source of nutraceuticals.

Credit authorship contribution statement

Po-Wei Tsai and Bor-Yann Chen conceived and designed the experiments; Cheng-Yang Hsieh, Jasmine U. Ting, Chih-Ling Chen, Hsiao-Chuan Yang and Hsing-Yu Tsai performed the experiments; Po-Wei Tsai, Lemmeul L. Tayo, Chia-Jung Lee, Chung-Chuan Hsueh and Bor-Yann Chen analyzed the data; Po-Wei Tsai, Lemmeul L. Tayo, Jasmine U. Ting and Bor-Yann Chen wrote the paper. All authors have approved this final version of the manuscript for submission.

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.

Data Availability

No data was used for the research described in the article.
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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2022.115944.

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