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Research article

Quality and production enhancement of fish mint, *Houttuynia cordata* Thunb., cultivated in a hydroponic planting system with designed plant growth-promoting additives

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ABSTRACT

Fish mint, *Houttuynia cordata* Thunb. (HCT) is an edible vegetable that has also been used in traditional folk medicines. As both a medicinal herb and a dietary source, HCT has been clinically proven to be a pivotal ingredient in formulas administered to alleviate COVID-19 symptoms. With the increasing market demand for imported materials, ensuring the quality consistency of HCT becomes a significant concern. In this study, the growing time for hydroponically-cultivated HCT with seaweed extract and amino acids added (HCTW) reduced by half compared to conventional soil-cultivated HCT (HCTS). Key quantified components in HCTW, flavonoid glycosides and caffeoylquinic acid derivatives, exhibited a 143% increase over HCTS. These crucial constituents were responsible for possessing antioxidant activity (IC_{50} < 25 μg/mL) and anti-nitrite oxide production (IC_{50} < 20 μg/mL). An economically-designed hydroponic system with appropriate

Abbreviations: COVID-19, coronavirus disease 2019; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; DPPH, 1,1-diphenyl-2-picrylhydrazyl; EC, electricity conductivity; ELISA, enzyme-linked immunosorbent assay; HCT, *Houttuynia cordata* Thunb; HPLC, high-performance liquid chromatography; IFN, Interferons; IL, Interleukin; iNOS, inducible nitric oxide synthase; LED, light-emitting diode; LPS, lipo-polysaccharides; NF-κB, nuclear factor-kappa B; NO, nitric oxide; PBS, phosphate buffered saline; PGE2, prostaglandin E2; SARS, severe acute respiratory syndrome; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TCM, Traditional Chinese Medicine; TNF-α, tumor necrosis factor-alpha; TLR, toll-like receptor; UV, ultraviolet.

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additives is proposed to replace HCTS with improvements of growth time, overall production yields, and bioactive qualities.

1. Introduction

Houttuynia cordata Thunb. (HCT), the sole species of the genus *Houttuynia* belonging to the Saururaceae family, is an edible vegetable, functional food and has long been used in traditional folk medicine. It is a leafy vegetable that is cultivated with regularity and utilized as a fresh herbal garnish. The unique taste attributed to its foliage stems from the presence of decanoyl acetaldehyde (Houttuynine), a volatile compound renowned for its distinct "fishy" flavor profile. Consequently, this remarkable taste has bestowed upon it the appellation of "fish mint" and "Yuxingcao" in Chinese [1,2].

This flowering and perennial plant is widely distributed in China, India, Japan, Korea, Southeast Asia, and Taiwan, and it especially grows in moist and shady hillside with an altitude of 300–2600 m. It is widely utilized in diverse culinary traditions, e.g., in India, it is commonly added to salads, sauces, and cooked dishes featuring fish and vegetables. In Japan and Korea, the HCT is valued for its potential medicinal qualities and are commonly steeped to prepare herbal tea. In Vietnamese cuisine, the plant is incorporated into grilled meat and noodle salad recipes [2].

As an affinal drug and diet source, HCT is traditionally used to relieve fever, resolve toxin, reduce swelling, promote urination, and treat lung-related symptoms, such as lung abscess, phlegm, cough, dyspnea, and pneumonia [2,3]. For the folk zootherapy use, wild-grown HCT and its extract can be mixed with feedings as an oral supplementation to prevent or treat swine diseases, e.g., to overcome the viruses causing reproductive failures, breeding, respiratory distress, poor growth performance, and mortality [4]. Dietary HCT extracted powder may also exhibit the potential for improvements in growth performance, nutrient digestibility, fecal microbial shedding and meat quality in weaning and finishing pigs [5].

During the outbreak of severe acute respiratory syndrome (SARS) virus pandemic in 2003, HCT was introduced to the public as one of the most important components in Traditional Chinese Medicine (TCM) formula to ameliorate SARS patients in Taiwan and Hong Kong [3]. In 2020, the National Research Institute of Chinese Medicine, under the Ministry of Health and Welfare in Taiwan (NRICM), developed two additional Traditional Chinese Medicine (TCM) formulas, namely, Chingguan Yihau (NRICM101) and Chingguan Erhau (NRICM102), in which HCT played a major and pivotal role as the key ingredient. These formulas were specifically created to alleviate the symptoms associated with coronavirus disease 2019 (COVID-19). According to the outcome of a clinical trial, NRICM101 and NRICM102 were significantly associated with a lower risk of intubation/ICU admission or death among patients with mild-to-severe COVID-19. In addition, modern pharmacology researches has revealed the potential of HCT in anti-viral, anti-inflammation, anti-tumor, antioxidant, and anti-bacterial activities, which lead to the protection of lungs, kidneys, heart, and digestive system [6,7]. The anti-viral and anti-inflammatory activity of HCT are known to be related to its flavonoid and flavonoid glycoside content, including hyperoside, quercetin, quercetin-3-*O*-rhamoside, and quercetin-7-*O*-rhamoside [2,8,9].

The increasing utilities of HCT in ethnopharmacology and natural product chemistry is accompanied by the globalization of TCM. With an annual growth rate of between 5 and 15%, the market for TCM, including raw medicinal materials and products, has expanded since the late 1990s to worldwide today according to the records of The World Bank. The annual TCM production from China reached US$48 billion while the export value amounted to US$3.72 billion in 2015, with 70% of them being raw or its processed materials [10]. Followed this, the global development of the medicinal plant industry is highly dependent on the imported raw materials, including HCT. Despite established rules and national standards, concerns regarding unstable quality control due to new and unregulated supply chains, as well as contamination from heavy metals and pesticides residues, continue to persist [11]. To achieve the sufficiency in supplying high-quality HCT, the government and industry in Taiwan have dedicated to develop agricultural policies and productivity with the purpose of scaling up locally-sourced HCT to meet the rising demand in the market. However, most of these HCT are cultivated on soil by following conventional agriculture practices, where the chemical composition of HCT might be affected by the weather, harvest seasons, drying processes and other environmental factors. Hence, a hydroponic planting system, which is a soil-free cultivation technique with reproducibility that grows plant in nutrient solution, is considered as an alternative to replace traditional farming methods [12]. Besides that, a well-controlled indoor environment with light-emitting diode (LED) equipment for lighting control, as well as regulated temperature and humidity, can optimize the growth condition of plants and minimize the effects of seasonality [13]. In addition, a hydroponic solution can be biofortified to supply additional nutrients, such as minerals and growth-promoting bacteria, to the plants in order to meet their specific needs and control their quality [12,14,15]. However, the startup cost may be relatively higher compared with traditional farming due to more expensive land in urban area, cost of machinery and the installation of an extensive range of LED devices [13].

In this study, our research team developed a hydroponic planting model in a partially-opened cultivation carrier with ceiling/side-windows and an air ventilation system installed. The newly designed system for exposing plants to sunlight is a cost-effective alternative to use LED devices as the primary light source for cultivating HCT. The hydroponic solution was developed by our research team specifically for HCT cultivation, comprising plant fertilizers (nitrogen, phosphate and potassium) and HCT growth-promoting additives, either one from vitamin B groups, seaweed extract, amino acids and microorganisms. This study aimed to investigate the differences in overall production yields, along with the quality, as indicated by comparing their fingerprints of high-performance liquid chromatography (HPLC) among HCT cultivated from traditional farming system and different hydroponic planting systems. The HPLC fingerprints were established based on caffeoylquinic acid derivatives and flavonoid glycoside contents, including 5-*O*-caffeoylquinic acid (**1**), 3-*O*-caffeoylquinic acid (**2**), 4-*O*-caffeoylquinic acid (**3**), rutin (**4**), hyperoside (**5**), isoquercetin (**6**), and quercitrin (**7**). The

role of crucial constituents included in HCT as well as HCT-containing formulas has also been discussed and clarified based on their antioxidant properties and capacity to inhibit nitric oxide (NO) production activities.

The outcomes of this study aimed to fulfill the hypotheses of utilizing an economically-designed hydroponic agricultural system with the addition of specific additives to enhance the quality of HCT, including factors such as growth time, overall production yields, and biofunctional components.

2. Materials and methods

2.1. Chemicals and reagents

All solvents, including acetonitrile, methanol, and ethanol, used for extraction and HPLC were purchased from HY Biocare Chemical Enterprise (New York, USA) and Merck (Merck KGaA, Darmstadt, Germany). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Dulbecco's modified Eagle's medium (DMEM), fetal calf serum, penicillin, and streptomycin, respectively, were purchased from Corning and Sigma-Aldrich company (St. Louis, USA). 5-*O*-caffeoylquinic acid, 3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, rutin, hyperoside, isoquercitrin, and quercitrin were isolated from the water extract of HCTW and these chemical structures were further identified with NMR spectroscopic data. Standard compounds used for quantitative analysis were purchased from ChemFaces (Hubei, China) and Sigma-Aldrich (St. Louis, USA).

2.2. Plant materials and growth conditions

The plant materials were cultivated in the plantation farm of the We-Win Bio-Medical Co., Ltd., Taiwan, and identified by Dr. Chia-Ching Liaw, Curator of Herbarium, NRICM, Ministry of Health and Welfare, Taiwan. The voucher specimen (No. NRICM-20210712-HB) was deposited in the Herbarium of NRICM.

HCT seedlings were divided into two groups. 80 seedlings were soil-cultivated in an opened environment, while 320 seedlings were cultivated in our developed hydroponic planting system with a basic hydroponic solution (without HCT growth-promoting additives) in a partially-opened cultivation carrier in the beginning (located in the plantation farm of We-Win Bio-Medical Co., Ltd, Taiwan). The cultivation carrier was installed with an air ventilation system which operated for 24 h and was exposed to sunlight through ceiling and side-windows. The temperature inside the cultivation carrier was maintained at ca. 25 °C.

After 20 days of growth in the basic hydroponic solution, HCT young shoots that reached a height of 3–3.5 cm were selected. 300 shoots were divided into 5 groups, with each group containing 60 HCT young shoots. As presented in Table 1, the groups of young shoots were then supplied with five different hydroponic solutions: four groups, i.e., HCTW-A, HCTW-B, HCTW-C and HCTW-D, were further cultivated with specially designed HCT growth-promoting additives. Meanwhile, HCTW was cultivated with basic plant fertilizers and nutrition as a reference control. In summary, a mixture of plant fertilizers (18%) (Guoo Bao Agriculture Co., Ltd.), Naturelogic® plant extracts (80%) (WE-WIN BIO-MEDICAL Co., Ltd.) and HCT growth-promoting additives (2%) was diluted with water by 500-fold to prepare a 20 L solution. Each hydroponic solution was added with 50 mL of the freshly-prepared mixture mentioned above once a month. The pH values and electricity conductivity (EC) values of the hydroponic solution were maintained in the range of 5.5–7.0 and 0.4–2.0 s/cm, respectively.

All HCT plants were harvested when the average length of leaves reached 6.5–7.5 cm. HCT which grew in the traditional farming system (soil-cultivated HCT, HCTS), took around 60–90 days. Remarkably, HCT grown in the hydroponic planting system (hydroponically-cultivated HCT, HCTW) spent only one half the growing time compared with HCTS, which was just 30–45 days.

2.3. Extraction and calculation of extraction yields

Firstly, fresh HCT plants were dried in shady areas at room temperature. The aerial part of HCT was powdered and well-mixed; then, 10 g of powdered HCT leaves and stems were weighted and extracted with 500 mL of 95% ethanol solution. After the mixture was sonicated for 1 h at room temperature, it was filtered with Whatman Filter No.1 and evaporated to dryness using a rotary evaporator. The extraction procedures were repeated two times to yield HCT crude extracts. The extraction yields (%) of HCT were calculated by the formula below:

Table 1
Hydroponic solution used for cultivation of HCT in hydroponic planting system.

Hydroponic solution	Composition (%)	HCTW	HCTW-A	HCTW-B	HCTW-C	HCTW-D
Plant fertilizers	18	Plant fertilizers (Guoo Bao Agriculture Co., Ltd.) (6.0% of total nitrogen, 6.0% of phosphoric anhydride, and 6.0% of potassium oxide)				
Basic nutrients	80	Not added	Naturelogic® plant extracts (WE-WIN BIO-MEDICAL Co., Ltd.) (Specific composition of HCT, *Taiwanofungus camphoratus*, *Chamaecyparis obtusa*, and *Calocedrus formosana* leaves extracts)			
HCT growth-promoting additive	2	Not added	Vitamin B groups	Seaweed extract (derived from *Phaeophyceae*)	Amino acids	Microorganisms (Nitrogen fixing bacteria, lactic acid bacteria, phosphate solubilizing bacteria, *saccharomyces*, potassium solubilizing bacteria, *Bacillus subtilis*)

[dry weight after extraction (mg) / dry weight before extraction (mg)] × 100

2.4. HPLC analysis

Briefly, 5 mg of each HCT crude extract was dissolved in 1 mL of methanol, followed by filtration with 0.45 μm nylon membrane filters, to prepare samples with concentration of 5 mg/mL. HPLC analysis was carried out by using Shimadzu LC-2050C 3D (Shimadzu Inc., Kyoto, Japan) equipped with pumps and a PDA-UV detector. The C_{18} column (Cosmosil $5C_{18}$-AR-II, 4.6 × 250 mm) was used for analysis. Deionized water with 0.2% of acetic acid (solvent A) and acetonitrile with 0.2% of acetic acid (solvent B) were used as mobile phases for programmed gradient with the following solvent B concentration: 10% in 0 min, 20% in 15 min, 20% in 20 min, 90% in 30 min, 100% in 35 min and 100% in 45 min, followed by a 10 min of re-equilibration. The flow rate was maintained at 1.0 mL/min. The injection volume for all samples was 10 μL and the wavelength for UV detection was set at 254 nm.

In this experiment, 5-*O*-caffeoylquinic acid (**1**), 3-*O*-caffeoylquinic acid (**2**), 4-*O*-caffeoylquinic acid (**3**), rutin (**4**), hyperoside (**5**), isoquercetin (**6**), and quercitrin (**7**) were used as standards for analysis. Briefly, 1 mg of pure compound was dissolved in 1 mL of methanol, followed by further dilution with methanol to prepare samples with concentration of 250 μg/mL. The injection volume for these samples were 0.1 μL, 0.3 μL, 1 μL, 2 μL, 10 μL, 25 μL, and 50 μL while the HPLC analysis method was as mentioned above. The UV peak areas were plotted against the final concentrations to generate a calibration curve for quantitative analysis (Fig. S1).

2.5. Scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay

The radical scavenging activity of standard compounds on DPPH free radical was measured using the method of Shimada et al. with minor modifications [16]. The aliquot of each sample (120 μL, 10–200 μg/mL), or α-tocopherol (10–100 μg/mL) was mixed with 30 μL of 0.75 mM DPPH methanol solution in 96-wells microplate. The mixture was shaken vigorously with orbital shaker in the dark at room temperature for 30 min. The absorbance was then measured at 517 nm with enzyme-linked immunosorbent assay (ELISA) reader. Methanol was used as negative control to replace the sample in the react solution. The DPPH radical scavenging activity of each compound was compared to the negative control and α-tocopherol as positive control. The final results were presented as IC_{50} value, which is the concentration of sample required to cause 50% inhibition against DPPH radicals in react solution.

2.6. Cell culture and cell viability assay

The macrophage cells RAW 264.7 were obtained from ATCC (Rockville, MD, U.S.A.) and cultured in Dulbecco's Modified Eagle Medium (DMEM) medium containing 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin/streptomycin, 2 mM L-glutamine, 0.1 mM non-essential amino acid (NEAA), 1.0 mM sodium pyruvate, 4.5 g/L glucose and 3.7 g/L of $NaHCO_3$. Cells were cultured in an incubator at 37 °C in a humidified atmosphere of 5 % CO_2. The viability was determined using a 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay based on the previous study with modification [17]. Cells were seeded on a 96-well plate at a density of 2×10^4 in each well for 24 h and then treated with different compounds (20 μg/mL) for another 24 h. After treatment, all samples were incubated with MTT (final concentration: 0.1 mg/mL) for 2 h in an incubator at 37 °C. Finally, the supernatant was replaced with DMSO (200 μL) and mixed well. The optical density (OD) was measured at 570 nm using a multimode microplate reader.

2.7. NO measurement

The estimation of anti-nitric oxide (NO) production capacities of the key compounds was adapted from previous study [18]. Cells were plated in 96-wells culture plate at a density of 2×10^4 cells/well and stimulated with 1 μg/mL of LPS in the presence or absence of tested compounds (20 μg/mL) for 24 h simultaneously. All compounds were dissolved in DMSO, further diluted with sterile PBS, and sterilized via a 0.2 μm filter. Nitrite accumulation in the medium was used as an indicator of NO production which was measured by adding Griess reagent (1% sulfanilamide and 0.1% naphthylenediamine in 5% phosphoric acid). Sodium nitrite ($NaNO_2$) was used to generate a standard curve, and nitrite production was determined by measuring optical density at 540 nm. All experiments were performed in triplicate. NO production by LPS stimulation was designated as 100% for each experiment. Quercetin, as the positive control, was a well-known inducible nitric oxide synthase (iNOS) inhibitor.

2.8. Statistical analysis

All values were expressed as means ± standard deviation (SD) of at least triplicate experiments. SPSS 20.0 (authorized by Office of Library and Information Services, Kaohsiung Medical University) were used to evaluate the statistically significant differences through one-way analysis of variance (ANOVA) followed by Tukey's test.

3. Results and discussion

3.1. Effects of hydroponic planting system and HCT growth-promoting additives on growing time and extraction yield of HCT

HCT plants were harvested from both soil cultivation system and hydroponic planting systems when they were mature, at which point the average length of their leaves reached 6.5–7.5 cm. Interestingly, despite the different hydroponic solution used in these groups, the average growing time for HCT grown in the hydroponic planting system, which took around 30–45 days, was twice as faster as that for HCT grown in the soil cultivation system, which took around 60–90 days (data not shown).

The ethanolic extracts of the leaves and stems (aerial part) of HCT from all experimental groups were obtained, including HCT grown in a soil cultivation system (HCTS) and HCT grown in hydroponic planting systems with different hydroponic solutions: basic plant fertilizer without HCT growth-promoting additives (HCTW), and basic plant fertilizer with one of the HCT growth-promoting additives from vitamin B groups (HCTW-A), seaweed extract (HCTW-B), amino acids (HCTW-C), and microorganisms (HCTW-D), respectively. Based on Table 2, the extraction yields of HCTS remained the highest (10.91% on average), compared with all the HCT grown in hydroponic planting systems. Among the hydroponic groups, the extraction yields of four groups, each with one of the HCT growth-promoting additives, were higher than HCTW in the following orders: HCTW-B (7.84%) > HCTW-A (6.89%) > HCTW-D (6.36%) > HCTW-C (5.92%) > HCTW (5.12%). These results suggested that the addition of the above HCT growth-promoting additives could provide extra nutrients to produce more biomass extractable by ethanol. Especially, HCTW-B achieved 71.86% of the HCTS yield. On the other hand, HCTW without nutritional supplement only achieved 46.93%, not even half, of the extraction yield of HCTS.

Referring to some previous studies, hydroponic planting systems could possibly reduce the growing time, and meanwhile, increase the bioactive components of the target plant in a suitable hydroponic solution formula. One example would be *Panax ginseng* Meyer, in which a short-term cultivation method was successfully established to reduce the cultivation time from over five years to around 120 days, with significantly improved total ginsenoside contents [19]. Another study also reported that the application of spirulina extract to hydroponically grown lettuce could reduce their growing time by six days and increase their yield by 12.5% [20]. Taken together, although the extraction yield of HCTW might be lower than that of HCTS, the shorter growing time of HCTW could increase the overall production yields of HCT, while the extraction yields can be further enhanced with HCT growth-promoting additives in hydroponic solutions. During the same time period, HCTW-B formula can potentially produce up to 143% of the yield of HCTS.

3.2. Effects of hydroponic planting system and HCT growth-promoting additives on chemical composition of HCT

The chemical compounds present in HCT plants are responsible for the diverse range of bioactivities they exhibit. The main bioactive components identified were found to be flavonoid glycosides, with quercitrin as the main biomarker for quality control of HCT according to the "Taiwan Herbal Pharmacopeia, the 4th edition", which is a basis for quality assurance in TCM implemented by the government of Taiwan. In terms of parts of HCT plants, most of the flavonoid glycosides were found in their flowers, followed by leaves, fruits, and stems. In a noteworthy finding, the constituents present in the aboveground stems and leaves were found to be totally distinct from those found in the underground parts [21]. This might be the reason why HCT and the products of HCT containing formulas in the market are mainly utilizing their aerial parts. For this reason, the leaves and stems of HCT were mainly used for extraction in this study, and the contents of their flavonoid glycosides were suitable to be used as standards for the quality control of HCT grown in the soil cultivation system and the hydroponic planting systems. Caffeoylquinic acid derivatives have been reported to be isolated from HCT [22], but their biofunctional roles in HCT and HCT containing formulas were very few to be mentioned. However, many bioactivities of caffeoylquinic acid derivatives isolated from other plants have been demonstrated, such as antioxidation, anti-bacterial, anti-cancer, and anti-diabetic, anti-inflammatory, anti-parasitic, anti-viral and neuroprotective [23]. In this study, caffeoylquinic acid derivatives were also identified clearly as key major components in HCT through our analysis method. Hence, we defined two groups of functional chemicals in HCT: caffeoylquinic acid derivatives and flavonoid glycosides. According to Fig. 1A, all of the HPLC profiles, regardless of their experiment groups, showed the presence of seven compounds, which were identified as 5-*O*-caffeoylquinic acid (**1**), 3-*O*-caffeoylquinic acid (**2**), 4-*O*-caffeoylquinic acid (**3**), rutin (**4**), hyperoside (**5**), isoquercetin (**6**), and quercitrin (**7**) (Fig. 1B).

Nevertheless, their chemical compositions were different based on the analysis results as shown in Table 3 and Fig. 2. In HCTS, the chemical contents of compounds **1**–**7** were 5.56 ± 0.16, 4.47 ± 0.10, 4.39 ± 0.09, 1.56 ± 0.02, 8.11 ± 0.10, 3.40 ± 0.05 and 11.14 ± 0.14 mg/g, respectively. Compound **7** was the most abundant one. However, in the same sample weight, all of these compounds were decreased significantly ($P < 0.01$) in their contents when HCT plants were grown in the hydroponic planting system. HCTW had the

Table 2
Extraction yield (%) of HCT samples.

Cultivation method	HCT samples	Extraction yield (%)
Traditional farming system	HCTS	10.91
Hydroponic planting system	HCTW	5.12
	HCTW-A	6.89
	HCTW-B	7.84
	HCTW-C	5.92
	HCTW-D	6.36

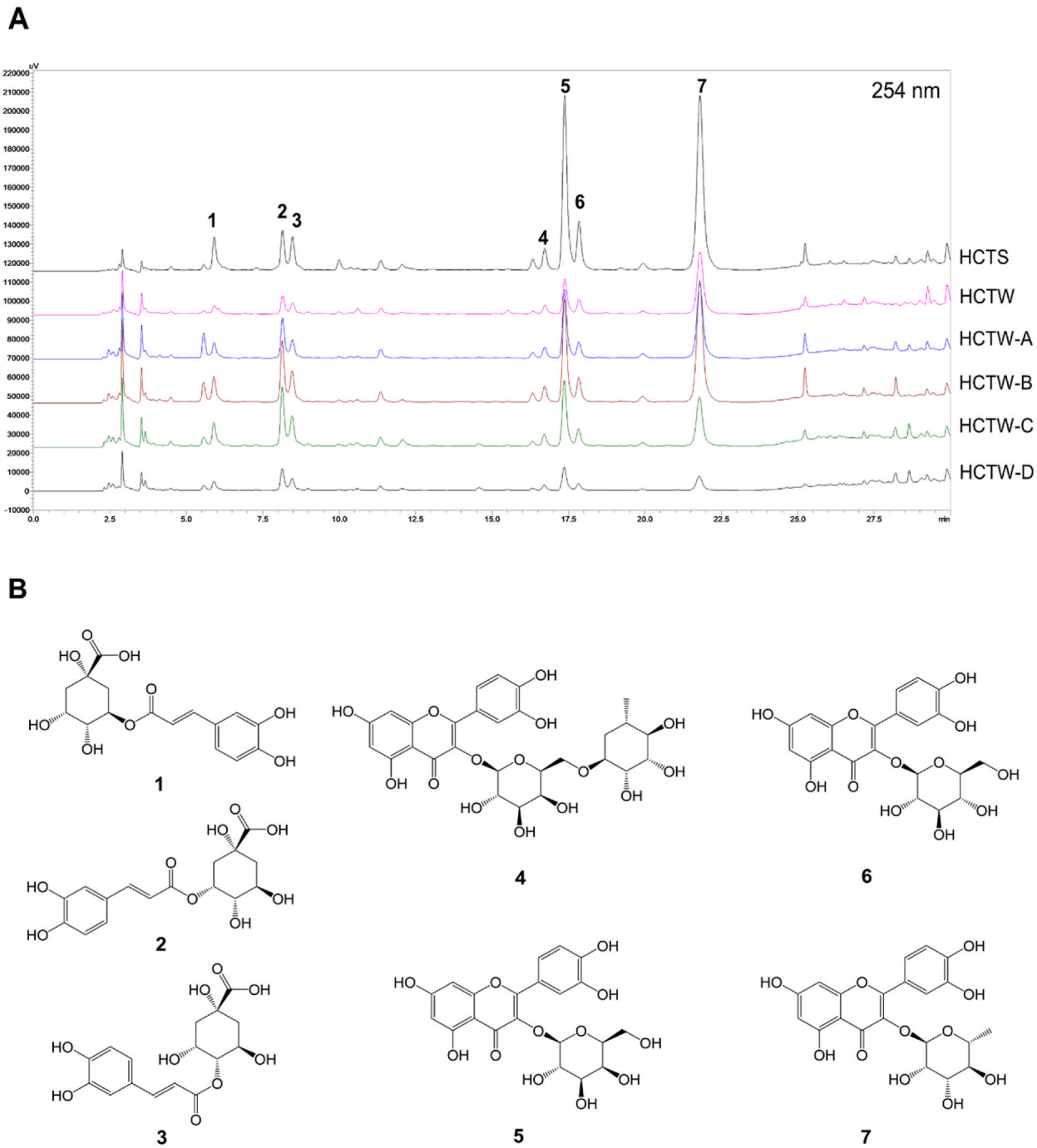

Fig. 1. (A) HPLC fingerprints of HCTS, HCTW, HCTW-A, HCTW-B, HCTW-C and HCTW-D with seven identified compounds: 5-*O*-caffeoylquinic acid (**1**), 3-*O*-caffeoylquinic acid (**2**), 4-*O*-caffeoylquinic acid (**3**), rutin (**4**), hyperoside (**5**), isoquercitrin (**6**), and quercitrin (**7**). (B) The chemical structures of the compounds **1**–**7**.

lowest contents of standard compounds with their relative % ratios ranged from 12.41% to 44.23% of those in HCTS (Table 4). However, the addition of HCT plant growth-promoting additives in the hydroponic systems could improve the overall chemical compositions, except for those in HCTW-D (the microorganism group), even though the extraction yield of HCTW-D was higher than HCTW. Overall, HCTW-B showed the most significant increase ($P < 0.01$ for all compounds), where the relative % ratios of the compounds ranged from 50.29% to 131.10% in comparison with those of HCTS in the same sample weight. The total flavonoid glycoside content of HCTW-B, comprising compounds **4** to **7**, was the highest among the hydroponic groups with the total relative %

Table 3
Quantitative analysis of HCT samples.

Compounds	Chemical content (mg/g)					
	HCTS	HCTW	HCTW-A	HCTW-B	HCTW-C	HCTW-D
5-*O*-Caffeoylquinic acid (**1**)	5.56 ± 0.16	0.69 ± 0.01$^{\#\#}$	2.54 ± 0.02**	3.13 ± 0.03**	3.35 ± 0.05**	1.23 ± 0.02**
3-*O*-Caffeoylquinic acid (**2**)	4.47 ± 0.10	1.81 ± 0.04$^{\#\#}$	4.00 ± 0.03**	5.86 ± 0.05**	5.70 ± 0.09**	2.10 ± 0.04*
4-*O*-Caffeoylquinic acid (**3**)	4.39 ± 0.09	1.50 ± 0.04$^{\#\#}$	2.69 ± 0.02**	3.91 ± 0.14**	3.86 ± 0.08**	1.63 ± 0.03
Rutin (**4**)	1.56 ± 0.02	0.69 ± 0.02$^{\#\#}$	0.83 ± 0.01**	1.14 ± 0.01**	0.87 ± 0.02**	0.41 ± 0.01**
Hyperoside (**5**)	8.11 ± 0.10	1.72 ± 0.04$^{\#\#}$	3.24 ± 0.03**	4.70 ± 0.03**	3.10 ± 0.06**	1.14 ± 0.02**
Isoquercetin (**6**)	3.40 ± 0.05	1.10 ± 0.02$^{\#\#}$	1.21 ± 0.01*	1.71 ± 0.02**	1.29 ± 0.02**	0.56 ± 0.01**
Quercitrin (**7**)	11.14 ± 0.14	3.88 ± 0.10$^{\#\#}$	4.70 ± 0.04**	7.47 ± 0.06**	3.14 ± 0.06**	0.91 ± 0.02**

($^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared to HCTS; $^{*}P < 0.05$, $^{**}P < 0.01$ compared to HCTW).

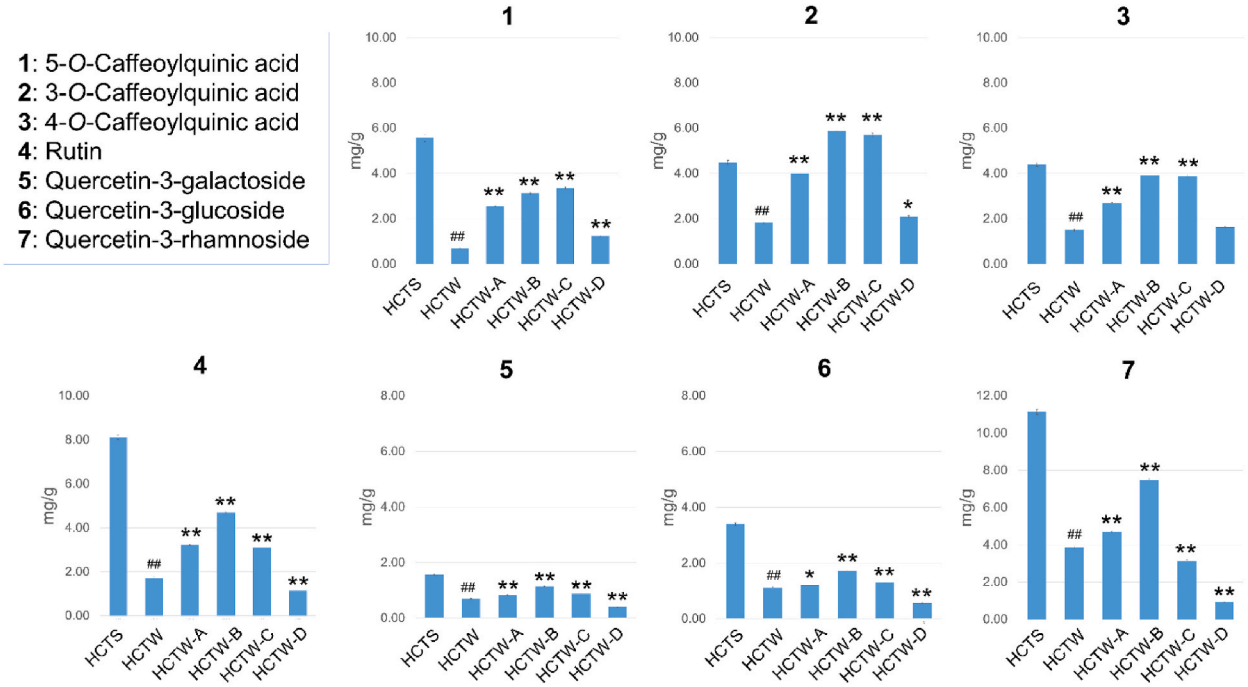

Fig. 2. The chemical content (mg/g) of compounds **1**–**7** in HCTS, HCTW, HCTW-A, HCTW-B, HCTW-C and HCTW-D were quantified using HPLC analysis ($\#P < 0.05$, $\#\#P < 0.01$ compared to HCTS; $^{*}P < 0.05$, $^{**}P < 0.01$ compared to HCTW).

Table 4
Relative ratio (%) of standard compounds in HCTW (including HCTW-A to D) compared with HCTS.

Compounds	Relative ratio of standard compounds compared with HCTS (%)					
	HCTS	HCTW	HCTW-A	HCTW-B	HCTW-C	HCTW-D
5-*O*-Caffeoylquinic acid (**1**)	100.00	12.41	45.68	56.29	60.25	22.12
3-*O*-Caffeoylquinic acid (**2**)	100.00	40.49	89.49	131.10	127.52	46.98
4-*O*-Caffeoylquinic acid (**3**)	100.00	34.17	61.28	89.07	87.93	37.13
Total average of 1–3	**100.00**	**27.74**	**64.01**	**89.46**	**89.53**	**34.40**
Rutin (**4**)	100.00	44.23	53.21	73.08	55.77	26.28
Hyperoside (**5**)	100.00	21.21	39.95	57.95	38.22	14.06
Isoquercetin (**6**)	100.00	32.35	35.59	50.29	37.94	16.47
Quercitrin (**7**)	100.00	34.83	42.19	67.06	28.19	8.17
Total average of 4–7	**100.00**	**30.52**	**41.22**	**62.04**	**34.70**	**12.47**

($^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared to HCTS; $^{*}P < 0.05$, $^{**}P < 0.01$ compared to HCTW).

ratio reaching up to 62.04% of those in HCTS in the same sample weight. Surprisingly, although the total flavonoid glycoside content of HCTW-C was not increased as much as those in HCTW-B, the content of compounds **1**–**3** was higher than the other hydroponic groups, amounting to 89.53% of total caffeoylquinic acid derivatives content in those of HCTS in the same sample weight. As previously

mentioned, in consideration of one-half growing time of HCT in hydroponic systems, the total flavonoid glycosides of HCTW-B and the total caffeoylquinic acid derivatives of HCTW-C could be 20% and 95%, respectively, more than those of HCTS in the same cultivation time period.

Due to the insignificant accumulation of abundant bioactive compounds, the conditions of HCTW-A and HCTW-D would not be further explored. Instead, the discussion will focus on HCTW-B (seaweed extract) and HCTW-C (amino acids) as HCT growth-promoting additives. A few studies have shown that biofortification of plant with seaweed-derived extract could enhance their secondary metabolites production, whether in traditional soil cultivation system or hydroponic planting system. It has been reported that the total phenolics and flavonoid glycoside contents in spinach were increased by the supplementation of brown seaweed *Ascophyllum nodosum* extract [24]. The application of seaweed-derived extract, known as Kelpak®, as well as their isolated compounds, eckol and phloroglucinol, has also been shown to increase the amount of bioactive compounds comprising of flavonoids and phenolic acids in *Eucomis autumnalis* (a type of traditional medicine in South Africa) [25]. Thus, the increase of flavonoid glycosides content, growth rate and extraction yields of HCTW-B might be attributed to the synergistic effect of numerous bio-stimulants found in seaweed extracts, such as plant hormones, phlorotannins and oligosaccharides, which help to improve the growth of plant and increase their tolerance to environmental stress [26].

Besides seaweed extract, past researches also showed that the application of amino acids could recover the negative effects of autotoxicity condition occurred in hydroponically-grown plants by improving their growth rate, production yield, and flowering, as shown in strawberry plants and *Eustoma grandiflorum* [27,28]. In *Urtica pilulifera* L, which is a type of medicinal plant, the increase in growth parameters and chemical composition were observed with the foliar application of amino acid, compared with untreated group [29].The increase in total caffeic acid derivatives amount has been reported in the aforementioned paper of the different plant material, which is similar to the findings about HCTW-C in our study. It was suggested that the better production of caffeoylquinic acid derivatives was due to the role of tyrosine and phenylalanine as the precursors in shikimate pathway, which is involved in the synthesis of caffeic acid and their derivatives are involved [29].

All of our results indicated that HCTW-B formula was the best formula to increase the overall chemical contents, especially flavonoid glycosides, of HCT grown in hydroponic planting system and had the potential to be improved for better effect, while HCTW-C formula might be useful for the production of caffeoylquinic acid derivatives in HCT plant. This study showed that different HCT plant growth-promoting additives can be used in cultivation of HCT plant depending on the different needs in the market. Interestingly, there are existing studies about the combination use of seaweed extract and amino acids in hydroponic planting system to improve the overall growth and physiochemical quality of grapes and bell peppers [30,31], which provides us with good insight into the possible adjustment of the combination of HCTW-B and HCTW-C formula in future studies.

3.3. Antioxidant and Anti-NO activities of key compounds of HCT

The antioxidant and anti-inflammatory properties of HCT play an important in its therapeutic potential [6]. We investigated whether the key compounds **1**–**7** possess antioxidant activity using DPPH assay. As shown in Table 4, all compounds exhibited excellent antioxidant activity with free radical removal effect of more than 90% at 200 μg/mL, and their IC_{50} values ranged from 15.95 to 24.69 μg/mL (α-tocopherol as positive control, $IC_{50} = 14.67 \pm 0.49$ μg/mL). Notably, the caffeoylquinic acid derivatives (**1**–**3**) showed better antioxidant activities than the flavonoid glycosides, and 5-*O*-caffeoylquinic acid (**1**) exhibited the strongest antioxidant effect ($IC_{50} = 15.95 \pm 0.19$ μg/mL).

The antioxidant properties of HCT extracts, including aqueous, ethyl acetate and methanolic extracts, have been studied *in vivo* with mechanisms involving the suppression of glutathione, superoxide dismutase, catalase, or malondialdehyde level [32,33]. Most studies of HCT on antioxidant effects were attributed to the isolated flavonoid glycosides, including quercetin and their glycosides. Quercetin, hyperoside, and quercitrin demonstrated antioxidant activity via DPPH assay with IC_{50} value of 0.83, 1.16, and 9.07 μM, respectively [34]. In addition, Cho et al. also reported the remarkable and dose-dependent inhibitory activities of these compounds on lipid peroxidation [35]. The results of the current study suggested that not only flavonoid glycosides, but caffeoylquinic acid derivatives are also the important antioxidants in HCT.

Next, we tested the anti-inflammatory effect of the key compounds (**1**–**7**) through the anti-NO production assay. All compounds

Table 5
Anti-oxidative and anti-NO production activities of compounds **1**–**7**.

Compounds	DPPH assay		Anti-NO production	
	removal effect (%)[a]	IC_{50} (μg/mL)	Anti-NO (%)[b]	IC_{50} (μg/mL)
5-*O*-Caffeoylquinic acid (**1**)	95.02 ± 0.95	15.95 ± 0.22	92.20 ± 1.47	13.32 ± 0.29
3-*O*-Caffeoylquinic acid (**2**)	95.70 ± 0.13	17.23 ± 0.19	98.71 ± 1.06	6.21 ± 0.19
4-*O*-Caffeoylquinic acid (**3**)	94.54 ± 0.60	19.80 ± 0.74	93.32 ± 1.84	9.51 ± 0.23
Rutin (**4**)	91.25 ± 0.76	24.69 ± 1.36	89.65 ± 2.80	12.36 ± 0.18
Hyperoside (**5**)	92.34 ± 0.33	19.83 ± 1.31	98.45 ± 2.34	7.41 ± 0.15
Isoquercetin (**6**)	91.73 ± 0.51	21.29 ± 0.89	85.33 ± 1.06	16.31 ± 0.14
Quercitrin (**7**)	92.48 ± 0.26	19.51 ± 0.46	95.19 ± 1.67	9.29 ± 0.24

[a] The removal effect (%) were at 200 μg/mL. Positive control for DPPH assay: Vit. E (α-Tocopherol) $IC_{50} = 14.67 \pm 0.49$ μg/mL.
[b] Cells were treated with LPS (1 μg/mL) or in combination with tasted agents (20 μg/mL) for 24 h. Data were normalized against LPS alone group. Positive control for anti-NO assay: Quercetin: $IC_{50} = 4.01 \pm 0.06$ μM.

remarkably decreased LPS-induced NO production by more than 85% at the dose of 20 μg/mL in RAW 264.7 (Table 5). Cell viability analysis showed that HCT showed little to no cytotoxic at a concentration of 20 μg/mL (data not shown). To be noted, 3-*O*-caffeoylquinic acid (**3**) and hyperoside (**6**) even showed their anti-NO effect of up to 98%, with their IC_{50} values of 6.21 ± 0.19 and 7.41 ± 0.15 μg/mL, respectively (Quercetin as positive control, IC_{50} = 4.01 ± 0.06 μM). These results indicated that the seven identified compounds (**1**–**7**) including caffeoylquinic acid derivatives and flavonoid glycosides could contribute to the anti-inflammatory effect of HCT by suppressing NO production. In fact, several studies reported that different extracts of HCT, including those from water, *n*-butanol, and ethanol exhibited anti-inflammatory effects [21]. Furthermore, certain compounds isolated from HCT have been reported to exhibit anti-inflammatory activity, including houttuynin, sodium houttuyfonate, houttuynamide B, and 2-undecanone and polysaccharides [36,37].

Regarding the anti-inflammation properties of flavonoid and flavonoid glycosides in HCT, treatment with flavonoid-rich extracts of HCT helped to constrain the TLR3, TLR4, and TLR7 agonist-stimulated cytokine secretion, NF-κB p65 phosphorylation, and nuclear translocation in RAW 264.7 cells. To be specific, both hyperoside and quercitrin showed significant inhibitory effects on TLR3-activated IL-6 and IFN-β secretion [8]. Besides that, the oral administration of quercitrin isolated from HCT to the rat could reduce the hid paw edema induced by carrageenan, dextran, histamine, serotonin, and bradykinin in a dose-dependent manner, thus validating its anti-inflammatory potential [9]. So far, there have been no studies on the anti-inflammatory potential of caffeic acid derivatives isolated from HCT. However, the composition of phenolic acids was identified in the aqueous and methanolic extracts of HCT fermentation products with anti-inflammation activity, including syringic, vanillic, *p*-hydroxybenzoic and ferulic acids [38]. In our study, we found that both caffeoylquinic acid derivatives and flavonoid glycoside found in HCT provided potent antioxidant and anti-inflammatory activities, which can be validated as biomarkers in quality control of HCT.

4. Conclusion

In conclusion, we established the hydroponic planting systems for HCT cultivation in this study, which could shorten the growing time of HCT by one-fold in comparison with traditional soil cultivation. Although the extraction yields and chemical compositions of hydroponic-cultivated HCT might not be as high as those of soil-cultivated HCT, the addition of HCT growth-promoting additives could greatly enhance them differently. HCTW-B formula with seaweed extract produced HCT with the highest extraction yield and total bioactive chemical contents, especially flavonoid glycosides. Moreover, HCTW-C formula with amino acids produced HCT with the highest yield of total caffeoylquinic acid derivatives. The key chemical markers in the HPLC profile were identified as bioactive compounds with antioxidant and anti-inflammatory activities, and thus can serve for quality control of HCT. Taken together, the overall production yield of HCT cultivated in hydroponic planting system can be increased due to shorter growing time with consistent quality attributed to the role of specific HCT growth-promoting additives. The possible combination of HCTW-B and HCTW-C might be suggested.

The hypotheses of utilizing a hydroponic agricultural system with the addition of appropriate additives to produce high-quality HCT were confirmed based on solid evidence. In future studies, the formula of hydroponic solutions can be refined to produce HCT with better quality for different needs in the market, and even has the potential to replace traditional farming methods.

Chemical compounds studied in this article

5-*O*-caffeoylquinic acid (PubChem CID: 162999449); 3-*O*-caffeoylquinic acid (PubChem CID: 1794427); 4-*O*-caffeoylquinic acid (PubChem CID: 9798666); rutin (PubChem CID: 5280805); hyperoside (PubChem CID: 5281643), isoquercitrin (PubChem CID: 5280804), and quercitrin (PubChem CID: 5280459).

Data availability statement

All the data related to this study are available in the research article and supporting information.

CRediT authorship contribution statement

Yen Chi Loo: Writing – original draft, Investigation, Conceptualization, Writing – review & editing. **Yi-Hong Tsai:** Writing – review & editing, Writing – original draft, Validation, Data curation, Conceptualization, Formal analysis. **Hsieh Chen:** Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Hui-Ping Hsieh:** Resources, Funding acquisition, Conceptualization. **Yen-Chang Chen:** Resources, Funding acquisition, Conceptualization. **Hsueh-Er Chen:** Investigation, Formal analysis, Data curation, Methodology. **Zhi-Hu Lin:** Data curation, Formal analysis, Investigation, Methodology, Resources, Validation. **Hung-Tse Huang:** Validation, Resources, Methodology, Investigation, Formal analysis, Data curation. **I-Min Liu:** Writing – review & editing, Funding acquisition, Formal analysis. **Chia-Ching Liaw:** Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Fang-Rong Chang:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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.

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Appendix A. Supplementary data

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References

[1] L. Han-Xing, On the evolution and distribution in saururaceae, Plant Divers 17 (3) (1995) 1.
[2] F. Jiangang, D. Ling, L. Zhang, L. Hongmei, *Houttuynia cordata* Thunb: a review of phytochemistry and pharmacology and quality control, Chin. Med. 4 (3) (2013).
[3] K.-M. Lau, K.-M. Lee, C.-M. Koon, C.S.-F. Cheung, C.-P. Lau, H.-M. Ho, M.Y.-H. Lee, S.W.-N. Au, C.H.-K. Cheng, C. Bik-San Lau, Immunomodulatory and anti-SARS activities of *Houttuynia cordata*, J. Ethnopharmacol. 118 (1) (2008) 79–85.
[4] W. Ruansit, W. Charerntantanakul, Oral supplementation of *Houttuynia cordata* extract reduces viremia in PRRSV-1 modified-live virus-vaccinated pigs in response to the HP-PRRSV-2 challenge, Front. Immunol. 13 (2022) 929338.
[5] J. Cho, S. Zhang, I.-H. Kim, Effects of anti-diarrhoeal herbs on growth performance, nutrient digestibility, and meat quality in pigs, Asian-Australas. J. Anim. Sci. 25 (11) (2012) 1595.
[6] Z. Wu, X. Deng, Q. Hu, X. Xiao, J. Jiang, X. Ma, M. Wu, *Houttuynia cordata* Thunb: an ethnopharmacological review, Front. Pharmacol. 12 (2021) 714694.
[7] Y.-H. Tseng, S.J.-S. Lin, S.-M. Hou, C.-H. Wang, S.-P. Cheng, K.-Y. Tseng, M.-Y. Lee, S.-M. Lee, Y.-C. Huang, C.-J. Lin, Curbing COVID-19 progression and mortality with traditional Chinese medicine among hospitalized patients with COVID-19: a propensity score-matched analysis, Pharmacol. Res. 184 (2022) 106412.
[8] L.-j. Ling, Y. Lu, Y.-y. Zhang, H.-y. Zhu, P. Tu, H. Li, D.-f. Chen, Flavonoids from *Houttuynia cordata* attenuate H1N1-induced acute lung injury in mice via inhibition of influenza virus and Toll-like receptor signalling, Phytomedicine 67 (2020) 153150.
[9] K. Taguchi, Y. Hagiwara, K. Kajiyama, Y. Suzuki, Pharmacological studies of Houttuyniae herba: the anti-inflammatory effect of quercitrin, Yakugaku Zasshi: J. Pharm. Soc. Jpn. 113 (4) (1993) 327–333.
[10] J. Xu, Z. Xia, Traditional Chinese Medicine (TCM)–Does its contemporary business booming and globalization really reconfirm its medical efficacy & safety? Medicine in Drug Discovery 1 (2019) 100003.
[11] J. Xiao, X. Xu, F. Wang, J. Ma, M. Liao, Y. Shi, Q. Fang, H. Cao, Analysis of exposure to pesticide residues from Traditional Chinese Medicine, J. Hazard Mater. 365 (2019) 857–867.
[12] P. Sambo, C. Nicoletto, A. Giro, Y. Pii, F. Valentinuzzi, T. Mimmo, P. Lugli, G. Orzes, F. Mazzetto, S. Astolfi, Hydroponic solutions for soilless production systems: issues and opportunities in a smart agriculture perspective, Front. Plant Sci. 10 (2019) 923.
[13] K. Benke, B. Tomkins, Future food-production systems: vertical farming and controlled-environment agriculture, Sustain: Sci. Pract. Policy 13 (1) (2017) 13–26.
[14] M. Schiavon, S. dall'Acqua, A. Mietto, E.A. Pilon-Smits, P. Sambo, A. Masi, M. Malagoli, Selenium fertilization alters the chemical composition and antioxidant constituents of tomato (*Solanum lycopersicon* L.), J. Agric. Food Chem. 61 (44) (2013) 10542–10554.
[15] B.R. Glick, Plant growth-promoting bacteria: mechanisms and applications, Scientifica 2012 (2012).
[16] K. Shimada, K. Fujikawa, K. Yahara, T. Nakamura, Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion, J. Agric. Food Chem. 40 (6) (1992) 945–948.
[17] J.M. Edmondson, L.S. Armstrong, A.O. Martinez, A rapid and simple MTT-based spectrophotometric assay for determining drug sensitivity in monolayer cultures, J. Tissue Cult. Methods 11 (1988) 15–17.
[18] L.-M. Yang Kuo, L.-J. Zhang, H.-T. Huang, Z.-H. Lin, C.-C. Liaw, H.-L. Cheng, K.-H. Lee, S.L. Morris-Natschke, Y.-H. Kuo, H.-O. Ho, Antioxidant lignans and chromone glycosides from *Eurya japonica*, J. Nat. Prod 76 (4) (2013) 580–587.
[19] J.Y. Lee, H. Yang, T.K. Lee, C.H. Lee, J.W. Seo, J.-E. Kim, S.Y. Kim, J.H.Y. Park, K.W. Lee, A short-term, hydroponic-culture of ginseng results in a significant increase in the anti-oxidative activity and bioactive components, Food Sci. Biotechnol. 29 (2020) 1007–1012.
[20] L. Lerer, C. Kamaleson, Growth, yield, and quality in hydroponic vertical farming–Effects of Phycocyanin-rich Spirulina Extract, Preprints 2020 (2020) 110354.
[21] S. Rafiq, H. Hao, M. Ijaz, A. Raza, Pharmacological effects of *Houttuynia cordata* Thunb (H. Cordata): a comprehensive review, Pharm. Times 15 (9) (2022) 1079.
[22] L. Yu-Syuan, L. Kuan-Hung, W. Chun-Wei, C. Yu-Sen, Effects of temperatures on growth, physiological, and antioxidant characteristics in *Houttuynia cordata*, Not. Bot. Horti. Agrobot 49 (4) (2021) 12536.
[23] W. Liu, J. Li, X. Zhang, Y. Zu, Y. Yang, W. Liu, Z. Xu, H. Gao, X. Sun, X. Jiang, Current advances in naturally occurring caffeoylquinic acids: structure, bioactivity, and synthesis, J. Agric. Food Chem. 68 (39) (2020) 10489–10516.
[24] D. Fan, D.M. Hodges, J. Zhang, C.W. Kirby, X. Ji, S.J. Locke, A.T. Critchley, B. Prithiviraj, Commercial extract of the brown seaweed *Ascophyllum nodosum* enhances phenolic antioxidant content of spinach (*Spinacia oleracea* L.) which protects *Caenorhabditis elegans* against oxidative and thermal stress, Food Chem. 124 (1) (2011) 195–202.
[25] A.O. Aremu, L. Plačková, J. Gruz, O. Bíba, O. Novák, W.A. Stirk, K. Doležal, J. Van Staden, Seaweed-derived biostimulant (Kelpak®) influences endogenous cytokinins and bioactive compounds in hydroponically grown *Eucomis autumnalis*, J. Plant Growth Regul. 35 (2016) 151–162.

[26] W.A. Stirk, K.R. Rengasamy, M.G. Kulkarni, J. van Staden, Plant biostimulants from seaweed: an overview, The chemical biology of plant biostimulants (2020) 31–55.
[27] M.R. Talukder, M. Asaduzzaman, H. Tanaka, T. Asao, Light-emitting diodes and exogenous amino acids application improve growth and yield of strawberry plants cultivated in recycled hydroponics, Sci. Horti 239 (2018) 93–103.
[28] M.F. Mondal, M. Asaduzzaman, H. Tanaka, T. Asao, Effects of amino acids on the growth and flowering of *Eustoma grandiflorum* under autotoxicity in closed hydroponic culture, Sci. Horti 192 (2015) 453–459.
[29] H. Wahba, H. Motawe, A. Ibrahim, Growth and chemical composition of *Urtica pilulifera* L. plant as influenced by foliar application of some amino acids, J. Mater. Environ. Sci. 6 (2) (2015) 499–509.
[30] A.S. Khan, B. Ahmad, M.J. Jaskani, R. Ahmad, A.U. Malik, Foliar application of mixture of amino acids and seaweed (*Ascophylum nodosum*) extract improve growth and physicochemical properties of grapes, Int. J. Agric. Biol. 14 (3) (2012) 383–388.
[31] R.I. Khan, I.A. Hafiz, M. Shafique, T. Ahmad, I. Ahmed, A.A. Qureshi, Effect of pre-harvest foliar application of amino acids and seaweed (*Ascophylum nodosum*) extract on growth, yield, and storage life of different bell pepper (*Capsicum annuum* L.) cultivars grown under hydroponic conditions, J. Plant Nutr. 41 (18) (2018) 2309–2319.
[32] C. Kang, H. Lee, D.-Y. Hah, J.H. Heo, C.H. Kim, E. Kim, J.S. Kim, Protective effects of *Houttuynia cordata* Thunb. on gentamicin-induced oxidative stress and nephrotoxicity in rats, Toxicol. Res. 29 (2013) 61–67.
[33] L. Tian, X. Shi, L. Yu, J. Zhu, R. Ma, X. Yang, Chemical composition and hepatoprotective effects of polyphenol-rich extract from *Houttuynia cordata* tea, J. Agric. Food Chem. 60 (18) (2012) 4641–4648.
[34] E. Cho, T. Yokozawa, D. Rhyu, S. Kim, N. Shibahara, J. Park, Study on the inhibitory effects of Korean medicinal plants and their main compounds on the 1, 1-diphenyl-2-picrylhydrazyl radical, Phytomedicine 10 (6–7) (2003) 544–551.
[35] E.J. Cho, T. Yokozawa, D.Y. Rhyu, H.Y. Kim, N. Shibahara, J.C. Park, The inhibitory effects of 12 medicinal plants and their component compounds on lipid peroxidation, The Am. J. Chinese Med 31 (6) (2003) 907–917.
[36] J. Ahn, H.-S. Chae, Y.-W. Chin, J. Kim, Alkaloids from aerial parts of *Houttuynia cordata* and their anti-inflammatory activity, Bioorganic. Med. Chem. Lett 27 (12) (2017) 2807–2811.
[37] D. Kim, D. Park, J. Kyung, Y.-H. Yang, E.-K. Choi, Y.-B. Lee, H.-K. Kim, B.Y. Hwang, Y.-B. Kim, Anti-inflammatory effects of *Houttuynia cordata* supercritical extract in carrageenan-air pouch inflammation model, Lab. Anim. Res 28 (2) (2012) 137–140.
[38] K. Woranam, G. Senawong, S. Utaiwat, S. Yunchalard, J. Sattayasai, T. Senawong, Anti-inflammatory activity of the dietary supplement *Houttuynia cordata* fermentation product in RAW264. 7 cells and Wistar rats, PLoS One 15 (3) (2020) e0230645.