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ENHANCED ANTIOXIDANT AND ANTI-INFLAMMATORY OF HYDROPONIC PENNYWORT (*Centella asiatica*) TUBERS VIA ULTRASOUND-ASSISTED EXTRACTION

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ABSTRACT

Hydroponically cultivated pennywort tubers (*Centella asiatica*) represent a promising healthy food source that contributes to present and future food security. This is the first time pennywort tubers of *Centella asiatica* have been explored for their composition and biological activities. The study quantified total polyphenol and flavonoid contents using a UV-Vis spectrophotometer, identified bioactive compounds through LC-MS analysis, and evaluated pharmacological potential via biological assays. The radical scavenging was examined through the DPPH and ABTS assays, while the inhibition of nitric oxide production was assessed for anti-inflammatory medication. LC-MS signals confirmed the introduction of 10 groups (18 metabolites), comprising terpenes, flavonoids, and other phytochemicals in the extract. The obtained IC_{50} values were 253.45 µg/mL, 205 µg/mL, and 254.4 µg/mL for DPPH, ABTS, and NO inhibition, respectively, without inducing cytotoxicity in RAW 264.7 cells. This phytochemical and bioactivity profile provides a platform for optimised cultivation strategies, boosting the popularity of hydroponic crops, and supporting either nutraceutical or resilient food supply chains.

Keywords: *Centella asiatica* Tubers, Hydroponic, Antimicrobial, Antioxidant, Anti-Inflammation.

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INTRODUCTION

Centella asiatica (*C. asiatica*) has been used in traditional therapies for many years, such as Ayurveda.[1] It appeared in many phytopharmacological investigations due to the renowned neuroprotective, anti-inflammatory, antioxidant, antimicrobial, and wound-healing effects.[2] Besides, it contains many compounds, including triterpenoids, saponins, flavonoids, and essential oils. So far, *C. asiatica* continues to be recognised as a key source for discovering scientifically validated herbal therapies.[3] In conventional agriculture, plants obtain nutrients from organic matter in the soil, yet a substantial portion of water is absorbed into the ground and remains unavailable for plant uptake. This limitation is particularly pronounced in tropical and subtropical regions, where intense solar radiation and arid, cracked soils hinder cultivation and crop development. To overcome these barriers, hydroponics has emerged as a modern alternative, offering advantages in space utilisation, water efficiency, and accelerated plant growth.[4] In this system, instead of using soil, crops rely on nutrient-enriched water solutions containing essential elements such as phosphorus, nitrogen, potassium, and trace minerals. Hydroponics plays a pivotal role in large-scale commercial production, enhancing efficiency, reducing pesticide dependence, and contributing to sustainable strategies for meeting global food demand.[5] Within this context, our research group has successfully developed hydroponic cultivation of *C. asiatica* in southern Vietnam, a region where agriculture is increasingly challenged by extreme summer heat, water scarcity due to hydroelectric dam operations, and saltwater intrusion. At the initial stage, hydroponically grown *C. asiatica* demonstrated robust development, underscoring its potential to address food security needs in vulnerable agricultural landscapes. Importantly, revealing the potential of *C. asiatica* is a significant turning point through modern isolation techniques. Conventional solvent-based approaches, while widely used, are often hindered by drawbacks such as poor selectivity, long reaction times, and degradation of

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heat-sensitive compounds.[3] To overcome these limitations, advanced techniques have emerged.[6] These modern methods, such as supercritical fluid, microwave, and ultrasound, enhance efficiency and preserve valuable phytochemicals as well as reduce environmental impact, thereby elevating the merits of *C. asiatica*-derived formulations. Along with the modern analytical techniques, the precise screening of valuable phytoconstituents was facilitated.[3] Unlike Korea, where they integrated a smart farm system with the Deep Flow technique (DFT) hydroponics and collected leaves for quantifying bioactive compounds.[7] In this study, the tubers of *C. asiatica* were collected to investigate their composition and the biological activities associated with hydroponically cultivated specimens. Two extraction approaches-solvent extraction and ultrasonication- were employed to establish optimal conditions for maximising separation yield. Subsequent analyses are total polyphenol content (TPC) and total flavonoid content (TFC) determinations. In addition, antioxidant capacity was assessed through DPPH and ABTS radical species scavenging assays, while *in vitro* cell viability and anti-inflammatory evaluations were conducted to further define potential applications. This study is the first to investigate hydroponically cultivated *C. asiatica* tubers. It highlights the necessity of characterising the intrinsic properties and identifying optimal extraction parameters, thereby supporting future sustainable hydroponic-based cultivation strategies and enabling scalable commercial production across diverse plant systems, which serves food security.

EXPERIMENTAL

Experimental Chemicals and Materials

Plant Material Collection

The qualified *C. asiatica* plants, which had the green colour without any rotten leaves (Fig.-1A), were cultivated by the hydroponic scaffold under natural sunshine with daily nutrient supply. The roots appear fibrous and moderately elongated, highlighting the plant's capacity for nutrient uptake in hydroponic conditions (Fig.-1B, C, and D). The measurement provides quantitative data on root length (approximately 7-12 cm), useful for assessing growth performance. The pretreatment of *C. asiatica* tuber followed the procedure described in a previous publication.[8] After 6 months of cultivation, the pennywort tubers were carefully rinsed with tap water several times to remove unexpected contaminants. The cleaned samples (Fig.-1E) were subsequently dried under natural sunlight until fully dehydrated. Once dried, the tubers were pulverised with a mixer to yield uniform and fine particles.

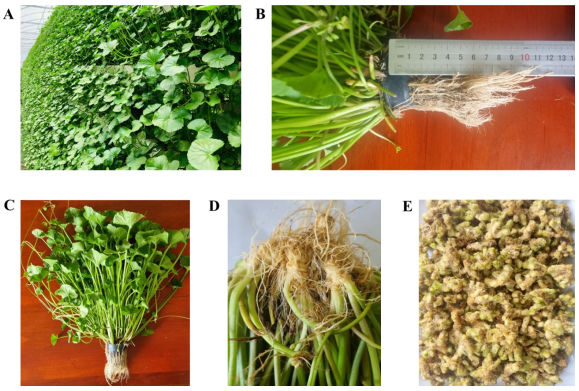

Fig.-1: (A) The Hydroponic *C. asiatica* garden, (B) Root Length Measurement with Scale Reference, (C) Freshly Harvested Plants with Intact Roots, (D) Close-Up of Root Systems with Tubers Highlighting Density and Branching, (E) Pretreated C. Asiatica Tubers

The resulting powders were stored in airtight containers under controlled conditions (low humidity, ambient temperature, and protection from light) for a maximum of one month prior to use. RAW 264.7 murine macrophage cells are a product from the American Type Culture Collection (ATCC, Manassas, VA, USA). Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), DPPH (1,1-diphenyl-2-picrylhydrazyl), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), potassium persulfate ($K_2S_2O_8$), lipopolysaccharide (LPS), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), and Griess reagent were purchased from Merck KGaA (Darmstadt, Germany) and Thermo Fisher Scientific (Waltham, MA, USA) Dimethyl sulfoxide (DMSO) and ethanol

were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ascorbic acid (5 mM in 10% DMSO) was used as a reference standard. Cardamonin was employed as a positive control in cell-based assays.

Moisture Content

2 mg of ground powder was placed in an oven at 110 ^{o}C. Every 2 hours, they were evaluated until reaching a constant weight.[9] The percentage of moisture was obtained according to the formula below:

$$M\ (\%) = \frac{G1 - G2}{G1 - G} * 100 \quad (1)$$

Herein, M: moisture content, G1: the weight of the ceramic and sample before heating (g), G2: the weight of the cup and sample after heating (g), G: the weight of the ceramic (g).

Extraction

For maceration, 20 g of dried *C. asiatica* tubers were extracted with 100 mL ethanol-water mixtures (100:0, 75:25, 50:50, v/v; solid-to-liquid ratio 1:5, w/v) under continuous vortexing for 48 h. For ultrasonication-assisted extraction (UAE), ethanol was used as the solvent in all experiments. Briefly, 20 g of dried tuber was extracted with ethanol under varying operating conditions, including ultrasonic energy, volume of ethanol, and extraction time. In both extraction methods, the resulting mixtures were subjected to vacuum filtration to collect the supernatant. The extracts were subsequently analysed for TPC and TFC. Besides, the optimised ultrasonication-assisted extract was evaporated of its solvent by a rotavapor. The obtained thick extract was stored at 4 ^{o}C for further biological assays.

Quantitative Phytochemical Analysis

Total Phenolic Content (TPC)

The Folin-Ciocalteu method was employed to define the concentration of the total phenolic compounds (TPC) with minor modification.[10] Briefly, a calibration curve for gallic acid (10–100 µg/mL) was generated by mixing 0.5 mL of each standard with 2.5 mL of 10% (v/v) Folin–Ciocalteu solution, followed by incubating for 5 minutes. Next, 2.0 mL of 7.5% Na_2CO_3 solution was added, vortexed, and placed in the dark for 30 min. Absorbance was recorded at 765 nm using a UV–Vis spectrophotometer. Diluted extracts in ethanol were treated under identical conditions. The total phenolic contents were calculated as:

$$TPC = C \times K \times V/m \quad (2)$$

Herein:
TPC: the total phenolic content (µg GA (Gallic acid equivalents)/g dried powder),
C: the concentration of diluted extracts from the standard curve (µg/mL),
K: the dilution coefficient,
V: the volume of extract used at the beginning (mL),
m: mass of the dried sample.

Total Flavonoid Content (TFC)

A calibration curve was prepared with quercetin standards (20–100 µg/mL in methanol). Each 0.5 mL aliquot was combined with 1.5 mL methanol and left for 5 min, followed by the addition of 0.1 mL of 10% $AlCl_3$. After mixing and 6 min of reaction in the dark, 0.1 mL of 1 M CH_3COOK was added, it was vortexed and placed for 45 min under light-protected conditions. During incubation, the solution gradually shifted from yellow to green. Extracts diluted in methanol were processed under the same conditions, and absorbance was recorded at 415 nm using a UV–Vis spectrophotometer.[10-11]
The total flavonoid content was calculated as:

$$TFC = C \times K \times V/m \quad (3)$$

Herein:
TFC: the total flavonoid content (µg QE (Quercetin equivalents)/g dried powder),
C: the concentration of diluted extracts from the standard curve (µg/mL),
K: the dilution coefficient,
V: the volume of extract used at the beginning (mL),
m: mass of the dried sample.

LC-MS analysis

The ethanol extract from the tubers of *C. asiatica* was screened for its composition through a UPLC-DAD-MS (Ultimate 3000 MSQ Plus, Thermo Scientific) and operated at the Institute of Advanced Technology, Vietnam Academy of Science and Technology.

Antioxidant Evaluation

The DPPH and ABTS radical scavenging assays were evaluated at the Institute of Chemistry, a member of the Vietnam Academy of Science and Technology, with the aid of a Tecan Spark UV-Vis spectrophotometer (Tecan Group Ltd., Switzerland). IC_{50} values (µg/mL, ng/mL, µM, or nM) were determined as the concentration inhibiting 50% of radicals using TableCurve AISN software. All assays were conducted in triplicate, with results expressed as mean ± standard deviation, and statistical significance set at $p \leq 0.05$.

DPPH Scavenging Ability

The DPPH radical scavenging assay was carried out as previous report with suitable adjustments.[12] DPPH was prepared in 96% ethanol and left in the dark for 12 h before use. Test extracts were diluted in pure dimethyl sulfoxide (DMSO) to yield final concentrations between 64 and 256 µg/mL. Ascorbic acid (5 mM in 10% DMSO) is a positive reference. The DPPH solution was dispensed into sample wells of a 96-well microplate, and it was placed at 37 °C for 15 min. Finally, absorbances were recorded at 515 nm.[13] The radical scavenging capacity (SC%) was calculated according to the following equation:

$$SC\ (\%) = \left(1 - \frac{OD\ [sample] - OD\ [blank]}{OD\ [negative\ control]}\right) \times 100\% \quad (4)$$

ABTS Scavenging Ability

The ABTS radical cation decolourisation assay was also examined.[14] In brief, ABTS was dissolved in ethanol and reacted with potassium persulfate (1:1, v/v) for 12 h in the dark to generate the radical cation. Test samples (64–256 µg/mL in DMSO) and ascorbic acid (5 mM in 10% DMSO) were incubated with ABTS in a 96-well plate at 37 °C. After 15 minutes, absorbances were measured at 734 nm. The radical scavenging capacity (SC%) was calculated according to the following equation:

$$SC\ (\%) = \left(1 - \frac{OD\ [sample] - OD\ [blank]}{OD\ [negative\ control]}\right) \times 100\% \quad (5)$$

Anti-inflammation Assay

The anti-inflammatory activity was assessed by measuring the inhibition of nitric oxide (NO) production in RAW264.7 macrophages stimulated with lipopolysaccharide (LPS).[15] Cells were maintained in DMEM supplemented with 10% fetal bovine serum (FBS) at 37 °C in a humidified incubator containing 5% CO_2 for 48 h. Subsequently, 2.5 x 10^5 cells/well were seeded into 96-well microplates. Following adherence, they were stimulated with lipopolysaccharide (LPS) for 24 h and treated with test samples at concentrations in the range from 64 to 256 µg/mL, with cardamonin serving as a positive control. Nitric oxide (NO) production was quantified using the Griess assay, in which cell supernatants were incubated with Griess reagent, and sodium nitrite ($NaNO_2$) standards were employed to generate a calibration curve. Their absorbances were measured at 570 nm.

In vitro Cytotoxicity

To examine the *in vitro* cytotoxicity of the test samples, RAW264.7 cells were treated under the same experimental conditions as described above. Following treatment, MTT solution (0.5 mg/mL in phosphate-buffered saline, PBS) was added to each well and incubated for 4 hours at 37 °C in a humidified atmosphere containing 5% CO_2.[15] The formed formazan crystals were dissolved in DMSO, and absorbances were measured at 540/720 nm using a Tecan Spark spectrophotometer.

RESULTS AND DISCUSSION

Critically, innovative extraction approaches have been investigated to maximise the recovery of bioactive compounds from *C. asiatica* tubers.[3] Supercritical fluid, microwave, ultrasonication, or enzyme-assisted

strategies (SFE, MAE, UAE, or EAE, respectively) are pioneers, ensuring the isolation of valuable compounds from plants by their unique principles, unlocking benefits such as enhanced selectivity, reduced extraction time, and environmentally friendly approaches.[3] Nonetheless, they still express some limitations, including equipment cost and operational complexities. In this study, the traditional ethanol maceration and ultrasonication-assisted extraction (UAE) are employed to collect the bioactive compounds from *C. asiatica* tubers. For the maceration, the dried and ground bioactive sources are immersed in a suitable solvent for a certain duration of time, followed by the evaluation of related characteristics of bioactive compounds, such as total polyphenolic content (TPC) and total flavonoid content (TFC), which may be related to antioxidant effects.[16] The introduction of appropriate solvents is the key factor to getting a high yield of extraction in terms of the relative solubility of desired compounds.[17] As shown in Fig.-2A, the effect of varying ethanol-to-water solvent ratios on the TPC and TFC profiles after 48 h of maceration is illustrated. Varying the concentration of ethanol from 50% to 100% would elevate the collected TPC and TFC. Respecting TPC, using 50% and 75% of ethanol gave a similar yield, while using 100% ethanol got the highest amount, as 1.55 mg GAE/g dried powder. The higher amount of ethanol also showed a tendency to increase the obtained TFC; using 100% ethanol as a solvent would reach 0.167 mg QE/g dried powder after 48 hours of maceration. On the other hand, the total volume of ethanol also influences the yield of extraction (Fig.-2B). The highest concentration of polyphenol content was 2.184 mg GAE/g dried powder by using 20.0 mL of ethanol, compared with 5 and 12.5 mL. Besides, the TFC profile occurred in the same tendency, in which 20 mL of solvent produced the highest concentration, as 0.202 mg QE/g dried powder. As observed, maceration is a simple and inexpensive approach to extract the thermolabile compounds from their raw material; however, this method is time-consuming and has low extraction efficiency. Thus, we headed to another approach to improve the yield, that is, ultrasonication-assisted extraction (UAE). Compared with maceration, this technique is more favourable because it uses high energy to break down the plant cell integrity to attract valuable components.[18] In ultrasonication-assisted extraction (UAE), the efficiency is primarily determined by the operational parameters applied during the process, including sonication intensity, ethanol volume, and extraction duration. Sonication energy is necessary to break down the cell wall, while the volume of ethanol is essential for enhancing the contact between the solid/solvent, and extraction time is pivotal for obtaining a high yield, but needs to be controlled to not negatively impact the quality of the expected compounds.[17]

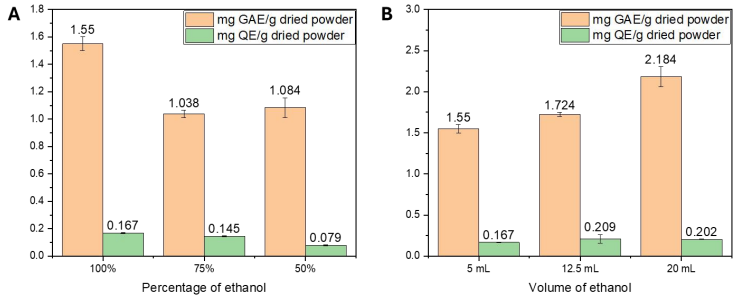

Fig.-2: TPC and TFC Values of Hydroponically Cultivated *C. asiatica* Tubers were Evaluated Using Ethanol Extraction, (A) at Different Ethanol Percentages, and (B) at 100% Ethanol.

First, the influence of applied energy was depicted in Fig.-3A, in which the increase of energy will lead to higher extraction yield in terms of TPC and TFC. Nevertheless, the efficiency was unchanged, for both TPC and TFC, if the energy was over 100, 000 J, which reflected the optimal power to fully extract the phytocompounds. Secondly, the total volume of ethanol was assessed from 5 to 20 mL. The highest TPC and TFC, in Fig.-3B, were obtained at 20 mL, in which they were 3.499 mg GAE and 0.284 mg QE per gram of dried powder. It is explained that the lower volume of ethanol caused a state of saturation and poor contact, reducing the efficiency compared with a higher input.[17] Lastly, the effect of ultrasonication time was evaluated, exhibiting a slight increase for TPC from 5-15 min and peaking at 4.496 mg GAE/g dried powder and an unchanged TFC as 0.296 mg QE/g dried powder (Fig.-3C).

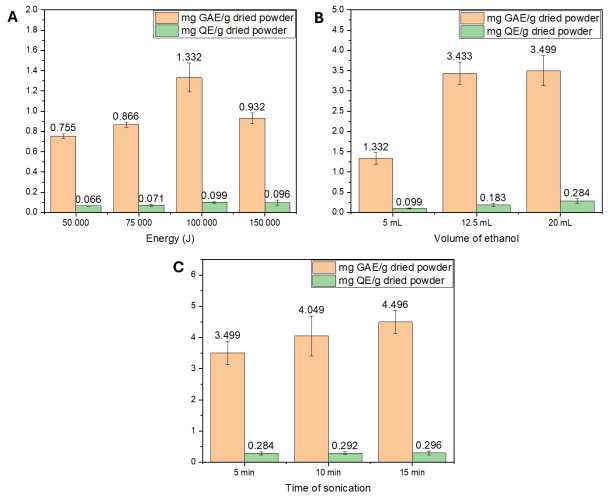

Fig.-3: The Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of Hydroponically Cultivated *C. asiatica* Tubers Were Determined using Ultrasonication Under Varying Conditions of (A) Energy Input, (B) Volume of Ethanol 100%, and (C) Extraction Time

At the optimal conditions for both maceration and ultrasonication, the obtained TFC was similar by both methods; however, the TPC would be 2-fold higher by UAE. Ultrasonication-assisted extraction relies on cavitation from ultrasound waves, which disrupts plant cell walls, increases solvent–matrix contact, and enhances diffusion, thereby improving extraction efficiency.[8] Additionally, the UAE is highly recommended for industrial production because of its high yield, low energy consumption, eco-friendliness, and scalability, although its selectivity towards expected phytocompounds is moderate.[3] Nonetheless, this model is still more favourable than supercritical fluid and microwave-assisted extraction, which are moderately to highly energy-consuming. Herein, Table-1 summarises our obtained TPC and TFC data compared with other research groups. Generally, the extraction yield was different for plants, the age and part of the plants, the solvents used, and the methods applied. Although most of the research showed the outstanding extraction from the leaves of *C. asiatica*, we still believe that the hydroponic *C. asiatica* tubers still have their potential applications through subsequent biological activities.

Table-1: The Comparison of TPC and TFC between this Study and Previously Published Articles

Sample	Method	TPC (mg GAE/g)	TFC (mg QE/g)	Ref.
C. asiatica tubers	Ultrasonication with ethanol	4.496	0.296	This study
C.asiatica leaves and shoots	Methanol extraction for 48 hours	132.6	15.1	[19]
C.asiatica leaves	Ultrasonication with methanol	129.54	308.31	[6]
C.asiatica leaves	Ethanol 70%	-	-	[20]
Leaves of *Psidium guajava*	Soxhlet	0.121	0.099	[21]
Leaves of *Basella alba*	Soxhlet	0.073	0.089	[21]
Leaves of *C. asiatica*	Soxhlet	0.061	0.087	[21]
Fruits of *Solanum torvum*	Soxhlet	0.053	0.079	[21]

C. asiatica tubers possess a diverse, powerful phytochemical profile, for instance, triterpenoid saponins, flavonoids, phenolic acids, alkaloids, phytosterols, volatile oil, and polyacetylenes.[3] Triterpenoid saponins are divided into 2 groups: asiaticoside and madecassosie. For asiaticoside ($C_{48}H_{78}O_{19}$, molecular weight is 959.12), the structure comprises triterpenoid aglycone (a pentacyclic triterpenoid, called asiatic acid,

molecular weight is 488.7) and a glucose molecule, which is attributed to promoting collagen synthesis, wound healing, antioxidant, and anti-inflammatory effects.[3] Similarly, madecassoside ($C_{48}H_{78}O_{20}$, molecular weight is 975.13) consists of a triterpenoid aglycone (a pentacyclic triterpenoid, called madecassic acid, molecular weight is 504.71) and a sugar moiety, which corresponds to collagen production, tissue repair, and anti-inflammation.[3] Regarding flavonoids, quercetin is one of the most prominent, followed by kaempferol and other flavonoids, for instance, apigenin, luteolin, and hesperidin.[3] For the extraction of pennywort tubers, to unlock the potential pharmacological activities and future cultivation, it is pivotal to find out the composition by modern techniques, such as NMR, UPLC, HPLC, LC-MS, etc. Herein, with our current facility, the LC-MS was used to reveal the major compounds that appear in the extract (Table-2). As a result of data processing, an extract of *C. asiatica* tubers putatively identified 18 metabolites. They are classified into 10 groups, including flavonoids, terpenes, and others. Asiatic acid has an m/z of 487.37 $[M–H]^-$ at 18.3 min, while madecassic acid exhibited an m/z of 503.9 $[M–H]^-$ at 23.48 min. These data are consistent with a reported article.[20]

Table-2: The Prediction of the Key Compounds of *C. asiatica* Tuber Extract

RT (min)	Dominant m/z (-)	Dominant m/z (+)	Molecular weight (g/mol)	Proposed compound
Flavonol monoglycoside				
9.02	503	200–562	-	Quercetin-hexoside (isoquercitrin/hyperoside)
10.96	-	449, 346/453	-	Quercitrin (quercetin-3-O-rhamnoside)
12.58	462.9	463.1	-	Quercetin-hexoside (isoquercitrin/hyperoside)
Flavonoid				
21.81	-	325–455/981	-	Flavonol aglycone/conjugate (Kaempferol/Quercetin derivative)
22.30	309/343/529	261/339/566	-	Flavonol aglycone/conjugate (Kaempferol derivative)
22.80	225/382/519	285/371/522	-	Flavonol aglycone (Kaempferol/Quercetin)
Flavonoid glycoside				
11.32	518.9	300–482	-	Kaempferol/Apigenin hexoside
Triterpene aglycone				
18.30	487.37	-	488.3488	Asiatic acid
23.48	503.9	944/1051	504.3451	Madecassic acid
25.00	485.1	462/659/1217	486.3451	Dehydrated Madecassic acid
Triterpene glycoside				
14.22	540-901	463–957	-	Minor triterpenoid saponin
14.86	~974	834/980	958.5119 974.5069	Asiaticoside Madecassoside
Phenolic acid ester				
12.10	515.0	478/538	516.45	Dicaffeoylquinic acid (Di-CQA isomer)
13.00	412/500	279/315	354.31	Caffeoylquinic acid (CQA isomer)
Caffeoyl/Feruloyl quinic acid family (CQA/FQA family)				
10.58	~491/572	267/393/503	-	Phenylpropanoid conjugate (CQA/FQA isomer)
Flavonol disaccharide				
7.38	~611	947/1092	610.521	Rutin
Flavonol (aglycone/conjugate)				
17.82	261/431/503	229/367/447/681		Quercetin-type aglycone or conjugate
Flavan-3-ol aglycone				
9.61	220-444	~295/285	290.271	Catechin/Epicatechin

Biological evaluation of phytocompounds plays a pivotal role in bridging natural product chemistry with modern biomedical applications. These assessments are essential for identifying and validating the pharmacological properties of plant-derived molecules and provide insights into mechanisms of action.[22] Such studies also contribute to structure–activity relationship (SAR) analyses, enabling researchers to correlate chemical features with biological efficacy and guide structural modifications to enhance therapeutic potential. Importantly, biological evaluations serve to substantiate traditional medicinal claims, thereby integrating botanical knowledge with scientific evidence.[21] Despite their promise, challenges remain, including the complexity of plant extracts, variability in bioavailability, and the need for rigorous toxicity and standardisation studies.[22] Overall, biological evaluation constitutes a critical gateway from phytochemistry to therapeutic application, ensuring that phytocompounds are systematically assessed for their potential as safe and effective agents in drug discovery and development.

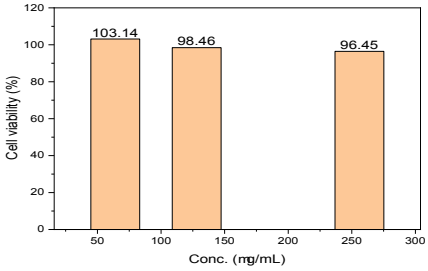

Fig.-4: Cell Viability Assessment of *C. asiatica* Tuber Extract in RAW 264.7 Cells

Back in 2024, our group did an antimicrobial assessment of *C. asiatica* tubers towards foodborne pathogens *E. coli* and *S. aureus*. The concentration of 250 mg/mL gave the antibacterial ring diameter for *E. coli* of about 7.33 ± 1.53 mm and for *S. aureus* of 8.00 ± 1.00 mm. The use of positive control of Ampicillin 100 µL exhibited the antibacterial ring diameters of *E. coli* and *S. aureus* were 14.33 ± 1.15 mm and 15.67 ± 1.53 mm, respectively.[23] Moreover, as depicted in Fig.-4, the extract of *C. asiatica* tubers shows no cytotoxicity to RAW 264.7 cells at concentrations up to 256 µg/mL. This allowed further investigation of its antioxidative activity (DPPH and ABTS scavenging) and anti-inflammatory effects in RAW 264.7 cells. Regarding the antioxidant effect, ascorbic acid expressed an IC_{50} of 17.13 µg/mL for both DPPH and ABTS scavenging. Based on Table-3, *C. asiatica* tubers exhibited IC_{50} data for DPPH and ABTS of approximately 253.45 µg/mL and 205 µg/mL, respectively. These values indicated the promise of pennywort tubers to inhibit radical species, although they exhibited much lower efficiency than those reported in other articles. For instance, the leaves and shoots of *C. asiatica* were extracted with methanol, and the inhibition concentrations for DPPH and ABTS were about 40.7 and 65.4 µg/mL, respectively.[19] If using ultrasonication with 70% ethanol for the leaves of *C. asiatica*, the IC50 of DPPH antioxidation was approximately 72.48 µg/mL,[20] while Soxhlet extraction will produce the inhibition concentration of 15.268 µg/mL.[21] We believe that antioxidation is highly dependent on the output of TFC and TPC, which may relate to antioxidant effects.[16] For anti-NO production ability, pennywort tuber extract expressed the IC_{50} at 254.4 µg/mL, indicating the anti-inflammatory activity owing to the presence of valuable bioactives.

Table-3: The Comparison of the Biological Activities of *C. asiatica* Tubers with the Other Articles

Sample	Method	Antimicrobial ring diameter	DPPH-IC_{50} (µg/mL)	ABTS-IC_{50} (µg/mL)	Ref.
C. asiatica tubers	Ultrasonication with ethanol	*E. coli* 7.3 mm *S. aureus* 8 mm	253.45	205.0	This study
C.asiatica leaves and shoots	Methanol extraction for 48 hours	-	40.7	65.4	[19]
C.asiatica leaves	Ultrasonication with methanol	-	-	-	[6]
C.asiatica leaves	Ethanol 70%	-	72.48	-	[20]
Leaves of *Psidium guajava*	Soxhlet	*E. coli* 12 mm *S. aureus* 12 mm	15.383	-	[21]
Leaves of *Basella*	Soxhlet	*E. coli* 11 mm	16.195	-	[21]

alba		*S. aureus* 10 mm			
Leaves of *C. asiatica*	Soxhlet	*E. coli* 7.5 mm *S. aureus* 7 mm	15.268	-	[21]
Fruits of *Solanum torvum*	Soxhlet	*E. coli* 7.5 mm *S. aureus* 7 mm	14.334	-	[21]

CONCLUSION

After 6 months of cultivation, hydroponically grown pennywort tubers (*C. asiatica)* were successfully grown and developed in southern Vietnam, and their diverse valuable phytochemicals were extracted by 48-hour maceration and ultrasonication with individually optimised conditions to obtain the highest yield for both TPC and TFC. The composition of the extract is made up of terpenes, flavonoids, and other compounds, comprising 18 metabolites. Subsequent biological evaluations show the antioxidation in terms of inhibiting DPPH and ABTS intermediates. Moreover, the inhibition of liposaccharide-induced-NO production was recorded at the concentration of 254.4 µg/mL. At this concentration, the extract did not create any cytotoxicity to RAW 264.7 cells, proving its safety and pharmacological efficiency in antimicrobial, antioxidant, and anti-inflammatory activities. However, further assessments should be evaluated, such as *in vivo*, to translate these powerful merits into real-life applications, for instance, wound healing, tissue repair, and anti-cancer. Anyway, this study supplied robust evidence that hydroponic pennywort tubers can serve as a key resource for sustainable food security due to their high nutritional value, and the hydroponic crops can be widely extended to other plant species.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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