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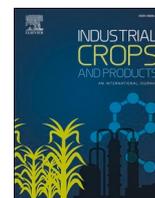
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## A comparative study of organic and conventional production methods on biomass accumulation and bioactive compounds content in *Centella asiatica* (L.) Urban in Vietnam

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### ABSTRACT

**Rationale:** *Centella asiatica* is a widely cultivated herb in South and Southeast Asia, recognized for its considerable nutritional value in these regions. In traditional ethnomedicine, this plant has been reported to possess significant medicinal properties. Triterpenoids from *C. asiatica* have emerged as key bioactive constituents with notable potential for modern therapeutic applications.

**Objectives:** We therefore aimed to evaluate cultivation practices for the production of bioactive compounds, with the goal of minimizing environmental impacts and promoting safe and sustainable agriculture.

**Materials and methods:** A comparative study was carried out in Thua Thien-Hue Province, Vietnam, to assess the efficacy of conventional versus organic production techniques in cultivation of *C. asiatica*. Fresh and dried samples from each cultivation system were harvested at intervals of approximately 4–6 weeks throughout all seasons over the two-year study period. Each batch was subsequently extracted, and the concentrations of the key bioactive triterpenoids (madecassic acid, asiatic acid, and terminolic acid) were systematically quantified to capture seasonal and temporal variations in compound accumulation.

**Results:** We found that the optimal period for obtaining the highest concentration of bioactive compounds in *C. asiatica* was from June to September, corresponding with warmer and drier climatic conditions. The content of madecassic acid was notably high in the first year, under either growing condition, surpassing the levels typically found in a broad range of *C. asiatica* accessions.

**Conclusions:** These results showed that the switch to an organic production method was not detrimental to the accumulation of valuable bioactive compounds. Implementation of these results has the potential to enhance the economic and social value of *C. asiatica* while reducing the reliance on agrochemicals, thereby minimizing their adverse effects on the environment and the health of farmers.

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## 1. Introduction

The herb *Centella asiatica* (L.) Urban (von Martius, 1879), commonly known in Vietnamese as "Rau má", belongs to the family Apiaceae. It is widely recognized and extensively distributed in South and Southeast Asia countries such as India, China, Malaysia, Vietnam, and Sri Lanka, as well as in Australia, Madagascar, and some regions of South Africa. In Vietnamese traditional medicine, *C. asiatica* is employed in the treatment of various conditions, including cirrhosis, icterus, skin diseases, tuberculosis, leprosy, dysentery, and cholera. In China, it is used for managing headaches, toothaches, the common cold, and viral hepatitis (Do, H.B, et al., 2004; Do, T.L., 2005, Tan S.C. et al., 2021; Li et al., 2024), while in Indian traditional medicine, the herb is utilized as a diuretic and tonic (Do, H.B, et al., 2004; La et al., 2005; Tan S.C. et al., 2021). These diverse pharmaceutical applications have prompted extensive scientific investigations, which have demonstrated that *C. asiatica* exhibits antimicrobial (Wei et al., 2023; Chonsut, P., et al., 2024), anti-inflammatory (Chonsut, P., et al., 2024; Mairuae et al., 2019), antioxidant (Le et al., 2025; Anbazhakan K. et al., 2023; Kandasamy et al., 2023), neuroprotective (Pham Thi Ninh et al., 2019; Farhani et al., 2023; Siddiqui and Ayyub., 2024; Saniya et al., 2025), anticancer (Ren et al., 2016; Manmuan et al., 2024; Tran, C. V. et al., 2024; Bunpo et al., 2005), hepatoprotective (Hong et al., 2021), wound-healing (Arribas-López et al., 2022), antidepressant (Zaynab et al., 2024), anti-photoaging (Yi-yun et al., 2024) and memory-enhancing (Rather et al., 2018; Firdaus et al., 2024; Rowe et al., 2024) properties. The principal bioactive constituents – asiatic acid (AA), madecassic acid (MA), terminolic acid (TA) and their glycosides (Fig. 1) – have been identified as the key compounds responsible for these pharmacological effects. These compounds are increasingly recognized for their promising potential applications in both the food industry and modern medicine (Tripathy et al., 2022; Tan et al., 2021; Min et al., 2024; Mando et al., 2024). To source a secure and sustainable supply of *C. asiatica* triterpenoids, it is imperative to understand cultivation, growth, and extraction processes, and how these relate to the three main triterpenoids and their glycosides.

One of the primary challenges in obtaining these triterpenoids is the limited understanding of their accumulation dynamics during the cultivation period of *C. asiatica*. A comprehensive analysis of these temporal variations would enable the identification of the optimal harvest time to maximize the yield of bioactive compounds. Furthermore, precise quantification of the levels and ratios of the key bioactive triterpenoids in *C. asiatica* is essential, as it underpins the formulation and production of a diverse range of food and pharmaceutical products. The production of bioactive triterpenoids in *C. asiatica* is influenced by genetic, environmental, and agronomic factors: Different *C. asiatica* accessions exhibit varying triterpenoid yields, with elite lines producing over 4 % triterpenoid content in dry weight (Kunjumon, et al., 2022). Higher light intensity has been linked to increased triterpenoid accumulation, with unshaded fields producing higher levels than those under 50 % shade (Srithongkul, et al., 2011; Priyanka, et al., 2022; Md Rayhan

et al., 2025; Bhornchai et al., 2024). Seasonal influences play a role: hot and humid conditions have been reported as optimal for triterpenoid production (Prasad, et al., 2016), while significant declines occur at temperatures below 15°C (Pipatsitee, et al., 2023). Elicitation of *Centella asiatica* leaves with methyl jasmonate and salicylic acid has been shown to significantly enhance triterpenoid accumulation, likely due to modulation of gene expression (Hathairat and Waraporn., 2020). Heavy metal accumulation in *C. asiatica* from soil contamination also has an impact: Biswas et al. (2020) reported that cadmium (Cd) and lead (Pb) levels influence triterpenoid production, with centelloside content increasing 15–20 fold at Cd concentrations of 150–200 mg/kg, an interesting result, albeit unacceptable for application due to human health and environmental considerations. The biosynthetic pathways governing triterpenoid production have been well-documented (Prasad, et al., 2019), but studies comparing agrochemical vs. organic growth methods have not emerged.

In Vietnam, *Centella asiatica* is cultivated year-round and produced in large quantities in provinces such as Thanh Hoa, Nghe An, and Hue, among others. The herb is widely distributed to cities across the country and is utilized in the preparation of vegetable dishes, teas, and soluble matcha drinks. Because of emerging applications for *C. asiatica* in the cosmetic and food industries, there is an increasing demand for high-quality *C. asiatica* that not only meets consumer standards but also exhibits elevated levels of triterpenoids. Such quality improvements would enhance the economic value of the herb and consequently increase farmers' income. To date, Vietnamese farmers have predominantly focused on the cultivation and sale of the herb, with research investigating triterpenoid content receiving limited attention. Therefore, a systematic and detailed investigation into the triterpenoid composition of *C. asiatica* is essential to provide robust scientific evidence, thereby supporting the optimized exploitation and sustainable use of this valuable local herb. *C. asiatica* has been cultivated in Quang Tho Commune, Quang Dien District, Thua Thien-Hue Province, Vietnam since 1995. The commune hosts extensive *C. asiatica* plantations, with over 250 households engaged in its cultivation across approximately 70 ha, providing a stable source of income for local farmers.

The aim of this investigation is to establish whether *C. asiatica* can be cultivated in a more environmentally sustainable manner, without compromising triterpenoid yield. Our objectives were: (1) compare conventional and organic cultivation systems in terms of bioactive compound yields, focusing on triterpenoids such as madecassic acid (MA), asiatic acid (AA), terminolic acid (TA), and their glycosides; (2) observe seasonal variations in bioactive compound accumulation to establish evidence-based recommendations for sustainable and economically optimized production. To achieve these objectives, we compared Vietnamese Good Agricultural Practices (VIETGAP) with certified organic (ORGANIC) production systems across all seasons over a two-year period (2021–2022) in Quang Tho commune. The research was conducted on *Centella asiatica* with fifteen harvests performed between 2021 and 2022, under local meteorological conditions. Emphasis was placed on monitoring the accumulation of MA due to its

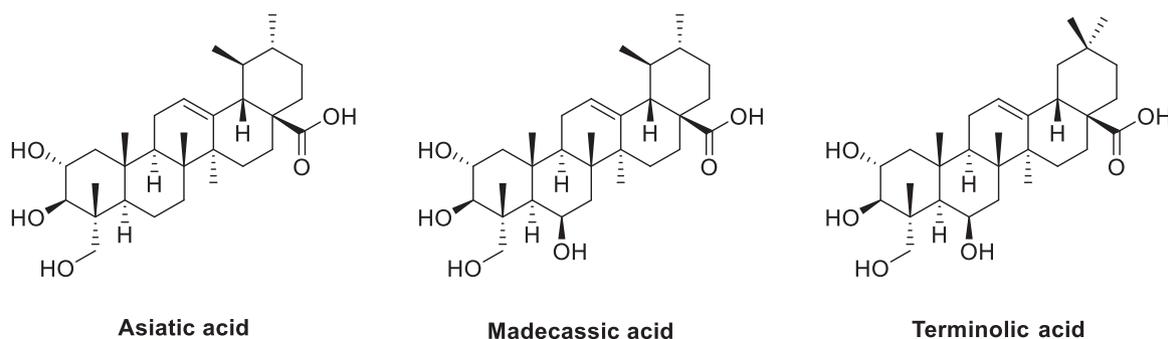


Fig. 1. Chemical structures of asiatic, madecassic and terminolic acid in *Centella asiatica*.

pharmacological relevance (Tran et al., 2018; Wei et al., 2023; Zhang et al., 2014; Han et al., 2020), alongside the quantitative evaluation of AA, TA, and their corresponding glycosides.

This research represents the first direct comparison between chemically supplemented and organic farming techniques in *C. asiatica* with respect to triterpenoid production - an essential consideration for long-term sustainability and environmental impact. By monitoring seasonal variations, this study extends and refines prior findings, providing actionable insights for producers to enhance cultivation strategies and optimize bioactive compound yields.

## 2. Materials and methods

### 2.1. VIETGAP production technique for *Centella asiatica*

#### 2.1.1. Determination of heavy metals in cultivated soils and irrigation water

Analytical equipment: Microwave digestion system (CEM, model: MARS 6), Memmert drying oven, Ohaus analytical balance (USA), Inductively Coupled Plasma Mass Spectrometer (ICP-MS, Agilent 7900), electric stove, sand bath, volumetric flasks, porcelain mortar and pestle, pipettes, Erlenmeyer flasks, and 0.45  $\mu\text{m}$  filter paper. Chemicals: Hydrochloric acid (HCl) and nitric acid ( $\text{HNO}_3$ ) of pro analysis (PA) grade (Merck). Standard solutions of each heavy metal were prepared by appropriately diluting the corresponding stock solutions (1000 ppm, Merck).

Heavy metal analysis in cultivated soils: Soil samples were collected from the surface layer (0–20 cm) in a diagonal pattern at plant sampling sites. Sampling, preservation, and treatment procedures followed Vietnamese standard TCVN 7538–2:2005 (ISO 10381–2:2002) on Soil Quality – Sampling (MONRea). Samples were air-dried, homogenized, ground, and sieved through stainless steel mesh with 2 mm aperture size. For digestion, 0.5 g of dried soil was placed in a Teflon digestion vessel, to which 9 mL of  $\text{HNO}_3$  (65 %) and 3 mL of concentrated HCl were added. Digestion was performed using microwave assistance (US EPA Method 3051 A). After digestion, the sample was cooled to room temperature, filtered using 0.45  $\mu\text{m}$  filter paper, and transferred to a volumetric flask. The total concentrations of heavy metals were quantified using ICP-MS. Data were analyzed using SPSS version 22 (IBM, UK) and OriginPro 2016.

Heavy metal analysis in irrigation water: Irrigation water samples for *Centella asiatica* were collected from the same locations as the corresponding soil samples. Each sample was stored in a 50 mL polypropylene container and transported to the laboratory for analysis. Water samples were processed and preserved in accordance with the Vietnamese standard TCVN 6663–3:2008 on Water Quality – Sampling – Part 3: Guidance on the Preservation and Handling of Water Samples (MONReb). For digestion, 100 mL of the water sample was mixed with 5 mL of  $\text{HNO}_3$  and heated on a stove to 150–200 °C until a clear solution was obtained. The solution was then cooled to 25 °C, filtered, and diluted to 100 mL with Milli-Q water. A blank sample was prepared using the same procedure. Heavy metal concentrations in the water samples were determined using ICP-MS.

#### 2.1.2. Land selection and soil preparation

A 500 m<sup>2</sup> plot of land in Quang Tho commune, Quang Dien province was selected for *Centella asiatica* cultivation in an elevated, well-drained area, ensuring optimal growing conditions. The soil chosen was free from insecticide and fungicide residues, in order to maintain environmental and product safety. Prior to planting, the land was plowed and sun-dried for 10–15 days, then finely tilled and enriched with 1050 kg of organic manure and 50 kg of phosphorus fertilizer. Raised beds were then prepared, measuring 1.2–1.5 m in width to facilitate proper drainage and plant growth.

#### 2.1.3. Planting of *Centella asiatica*

Stolons of the *C. asiatica* variety indigenous to Quang Tho were

planted in mid-September 2020, with five stolons per row, spaced 25 cm apart. Following planting, heavy irrigation was applied during dry or high-temperature conditions, and subsequent watering was conducted every two days. Under normal meteorological conditions (temperature: 30–32°C; average rainfall: <100 mm/month; no flooding), the first harvest occurred two months post-planting. Soil moisture levels were regularly monitored to optimize irrigation practices.

#### 2.1.4. Soil treatment and fertilization

To eliminate soilborne insects and larvae, 50 kg of powdered lime was evenly applied across the 500 m<sup>2</sup> plot 10–15 days before planting. One month after planting, a 15 kg N-P-K (20:20:15) fertilizer mixture was incorporated per 500 m<sup>2</sup> to support plant growth and enhance nutrient availability. This structured approach ensures optimal soil conditions, nutrient availability, and sustainable *C. asiatica* cultivation, contributing to high-quality yield and long-term agricultural viability.

### 2.2. ORGANIC production technique for *Centella asiatica*

Another 500 m<sup>2</sup> plot located in Quang Tho Commune, Quang Dien District was designated for the organic cultivation of *Centella asiatica*. The cultivation process followed the same protocols for land selection, soil preparation, planting, irrigation, and manure application as those employed in the VIETGAP production system. However, key differences exist in the use of fertilizers and pest control measures.

#### 2.2.1. Pest and weed management

In contrast to VIETGAP, the Integrated Pest Management (IPM) approach was implemented for organic *C. asiatica* cultivation. This method relies exclusively on manual removal of insects, pests, and weeds, avoiding synthetic chemicals. Only organic, bioorganic, microbial fertilizers, and certified organic insecticides were applied to maintain soil health and plant quality.

#### 2.2.2. Soil preparation and fertilization

During soil preparation, a combination of 50 kg of lime, 1000 kg of organic fertilizer, 15 kg of phosphorus, and 40 kg of mineral fertilizers was applied per 500 m<sup>2</sup> plot. The soil was left to stabilize for 10–15 days before planting.

#### 2.2.3. Growth cycle and nutrient supplementation

The first harvest was conducted 70 days after planting. To maintain soil fertility and promote continuous growth, additional fertilizers were applied at specific intervals: 3–4 days post-harvest: 100 kg of organic fertilizer, 7.5 kg of phosphorus, and 20 kg of mineral fertilizers were distributed over 500 m<sup>2</sup>. 20 days post-harvest: A second round of 7.5 kg of phosphorus and 20 kg of mineral fertilizers was applied over the same area.

### 2.3. Harvesting

The harvest interval for the VIETGAP product was typically 4–5 weeks, whereas that for the ORGANIC product extended to 6–7 weeks due to the comparatively slower growth rate under organic cultivation conditions. Over the 26-month study, 15 harvests were conducted under both cultivation conditions to assess the dry weight percentage relative to fresh weight (Fig. 5a, Table 3a) and the ethanol/water raw extract yield from dry weight (Fig. 5b). To ensure consistency, leaves used for analysis were manually selected based on the predefined criteria of being neither too old nor too young, free from visible insect or pest damage, and exhibiting an optimal leaf shape indicative of healthy growth.

### 2.4. Extraction

Dried samples of *Centella asiatica*, cultivated using either the

VIETGAP or ORGANIC production technique, were processed under controlled conditions. The harvested samples were oven-dried at 45–50°C until a moisture content of 8–10 % was achieved, then finely ground into powder. For extraction, 130 g of the powdered material was subjected to maceration with an 80:20 (v/v) ethanol/water mixture (3 × 250 mL) at room temperature for 24 h per cycle. The resulting extract was concentrated under reduced pressure, yielding 22.0 g of crude extract. This extract was subsequently dissolved in 200 mL of ethanol/water (1:1, v/v), followed by the addition of 60 mL of 20 % NaOH. The mixture was heated at 70°C for 4 h to facilitate hydrolysis. After hydrolysis, the solution was acidified to approximately pH 4.0 using 5 % HCl and allowed to stand overnight. The resulting precipitate (14.2 g) was collected, dried, and analyzed. Both the crude extract and the precipitated solid were subjected to high-performance liquid chromatography (HPLC) for the quantification of madecassic acid (MA), asiatic acid (AA), and terminolic acid (TA).

### 2.5. Quantitative determination of madecassic, asiatic and terminolic acid by HPLC

Quantitative analysis of triterpenoid contents was performed on an Agilent 1290 HPLC system consisting of an auto-sampler and a binary pump system (Agilent Corporation, CA, USA) coupled with a UV–Vis detector. HPLC grade reagents, acetonitrile, and methanol were purchased from Burdick & Jackson (Morristown, NJ, USA). All other chemicals were of analytical grade unless otherwise noted. Double-distilled water was prepared using a Millipore system and filtered through a 0.22 µm Millipore aqueous phase filter before HPLC analysis.

The mobile phase consisted of a gradient elution with solvent A (0.02 % formic acid and 0.225 % β-cyclodextrin in water) and solvent B (methanol). The proportion of solvent B was 70 % for 15 min, then maintained at 100 % solvent B for another 10 min. The mobile phase flow rate was 0.5 mL/min.

An Agilent Poroshell EC C-18 column (4.6 mm × 100 mm, 5 µm) maintained at a controlled temperature of 25°C was used. Optical absorption of the eluate was monitored at 205 and 210 nm. Data were processed using Openlab software (Agilent).

#### 2.5.1. Optimisation of extraction efficiency of madecassic, asiatic, and terminolic acid from the extract

Extraction solvents (50 %, 75 %, and 96 % ethanol in water, and 50 %, 75 %, and 100 % methanol in water), extraction time (10, 30, 60, 90, and 120 min), and extraction methods (shake, reflux, and ultrasonication) were evaluated by measuring the level of bioactive components obtained under each set of conditions.

#### 2.5.2. Validation

Calibration curves were fit to the equation  $y = ax + b$  using a linear regression in which  $y$  and  $x$  were the HPLC analyte peak area relative to that of the internal standard (IS) and compound concentration, respectively. To determine the limits of detection (LOD) and limits of quantitation (LOQ), standard stock solutions were serially diluted in methanol and injected into the HPLC system for analysis. LOD was defined as the concentration at which the least intense detectable peak in the chromatogram had a signal-to-noise (S/N) ratio of three. LOQ was defined as the lowest quantitative level with an S/N ratio of 10. Intra-day precision and accuracy were examined by analysing the standard solutions five times within a single day. Inter-day precision and accuracy were determined by measuring samples on five different days. Recovery tests were performed to evaluate the accuracy of the method by spiking known quantities of mixed standards into samples with a known quantity of each standard. The spiked samples were then extracted and analysed immediately. The added standards were prepared at three different concentrations and each concentration was analysed five times. Stability of the standard methanol solutions was evaluated at 15, 20, 25, 30, 35, and 40°C after aging for 30 and 10 days, respectively.

#### 2.5.3. Optimisation of chromatographic conditions

HPLC conditions were optimized to obtain the shortest possible run time while maintaining the baseline resolution of adjacent peaks. Eluents of solvent A with various acidic modifiers (acetic acid, formic acid, and phosphoric acid) at different concentrations (0.10 %, 0.20 %, and 0.50 %) and β-cyclodextrin (0.112, 0.225, 0.45, 0.9 % β-cyclodextrin) were evaluated. The addition of 0.2 % formic acid and 0.225 % β-cyclodextrin to solvent A section. Absorbance of the eluate at 205 and 210 nm yielded the highest S/N ratio for compounds. The highest chromatographic peak resolution with minimal peak tailing was observed with the column held at 25°C.

## 3. Results

### 3.1. Planting, harvesting and biomass yield of *C. asiatica*

Both VIETGAP and ORGANIC *C. asiatica* were cultivated in adjacent fields under identical meteorological conditions with full sunlight exposure (Fig. 2). Bearing in mind the findings of Biswas et al. (2020), we monitored soil for heavy metal contaminants. As shown in Table 1, the concentrations of four toxic metals (lead (Pb), mercury (Hg), arsenic (As), and cadmium (Cd)) were found to be well below the Vietnamese limits for heavy metals in irrigation water (National Technical Regulation QCVN 08-MT:2015/BTNMT on surface water quality and TCVN 6773:2000 on water quality – Water used for irrigation) (MONREC). These standards provide permissible limits for heavy metals such as lead, mercury, cadmium, arsenic, chromium, and other metals in water intended for irrigation purposes, in order to ensure the safety of crops and protect human health. Similarly, Table 2 presents data confirming that the levels of arsenic (As), cadmium (Cd), lead (Pb), chromium (Cr), and copper (Cu) in the soil were within safe limits according to Vietnamese standards for heavy metals in agricultural soil (QCVN 03-MT:2015/BTNMT and TCVN 7209:2002) (MONRED). These results were analyzed by the Quality Assurance and Testing Center II (Vietnam), confirming compliance with safety regulations.

ORGANIC *C. asiatica* exhibited slower growth rates, requiring 2–3 additional weeks for biomass accumulation compared to the VIETGAP crops. A notable visual distinction was observed in leaf coloration: ORGANIC leaves displayed a more vivid green hue (Fig. 3a), while VIETGAP leaves appeared slightly paler (Fig. 3b).

Over a 26-month period (October 2020 – November 2022), *C. asiatica* was harvested 15 times under both VIETGAP and ORGANIC cultivation conditions—eight harvests in 2021 and seven in 2022 for VIETGAP, and seven in 2021 and eight in 2022 for ORGANIC. The dry-to-fresh weight ratio of *C. asiatica* varied between 9 % and 14 % (w/w) per harvest (Fig. 4a). The variation in biomass yield across the years was largely influenced by climatic factors such as sunlight, rainfall, and temperature. Notably, higher dry-to-fresh weight ratios (14–15 %) were recorded during the summer months (March – August) compared to lower values in spring (January – February) and winter (November – December). The reduced sunlight and increased rainfall during winter, combined with occasional flooding in Quang Tho commune, created suboptimal growth conditions (average temperatures 27–29°C). Consequently, *C. asiatica* exhibited slower growth rates during the colder months, leading to extended intervals between harvests.

The ethanol/water raw extract yield (relative to dry weight) ranged from 8 % to 12 % (w/w) in summer and autumn, reaching as high as 15–16 % under optimal conditions (Fig. 4b). These fluctuations were attributed to environmental factors affecting biomass accumulation between harvests.

### 3.2. Extraction and hydrolysis

To determine the triterpenoid content in *C. asiatica*, extraction of the compounds is necessary. Various extraction and isolation methods for bioactive triterpenoids from *C. asiatica* have been reported, with solvent



**Fig. 2.** (a) ORGANIC *C. asiatica* field. (b) Harvesting of *C. asiatica* in the ORGANIC field. (c) VIETGAP *C. asiatica* field. (d) Preparation of fertilizer for ORGANIC production of *C. asiatica*.

**Table 1**

The concentration of heavy metals (mg/L) in irrigation water from Quang Tho commune, Thua Thien Hue province, Vietnam.

Metal	Found values (mg/L)	Vietnamese standard (mg/L)
Lead (Pb)	< 0.01	≤ 0.05
Mercury (Hg)	< 0.001	≤ 0.001
Arsenic (As)	< 0.0015	≤ 0.05
Cadmium (Cd)	< 0.002	≤ 0.01

**Table 2**

The concentration of heavy metals (mg/kg) in the soil from Quang Tho commune, Thua Thien Hue province, Vietnam.

Metal	Found values (mg/kg)	Vietnamese standard (mg/kg)
Arsenic (As)	12.3	≤ 15
Cadmium (Cd)	0.3	≤ 1.5
Lead (Pb)	32.2	≤ 70
Zinc (Zn)	66.5	≤ 200
Chromium (Cr)	30.2	≤ 150
Copper (Cu)	33.6	≤ 100

selection playing a crucial role.

In this work, we optimized the extraction protocol, testing a range of solvent mixtures and extraction conditions, with an ethanol/water mixture (80:20, v/v) identified as the most effective for extracting triterpenoids from dried *C. asiatica* leaves. A two-step extraction strategy was implemented: dried *C. asiatica* samples were extracted using 80 % ethanol to obtain a total ethanol extract, which was subsequently fractionated. The first fraction was directly analyzed via high performance liquid chromatography (HPLC) to quantify the free triterpenic acids (AA, MA, and TA). The second fraction underwent hydrolysis with 20 % NaOH in an ethanol/water mixture (1:1, v/v) at 70°C for 4 h to remove

glycoside units, thereby converting all bound forms into their free acid forms for quantification. After hydrolysis, the solution was acidified to induce precipitation of free triterpenic acids or to facilitate extraction with ethyl acetate (Jung et al., 2007).

### 3.3. Quantitative determination of madecassic, asiatic and terminolic acid content by HPLC

In this study, a small amount of  $\beta$ -cyclodextrin was added to the mobile phase for HPLC analysis using a UV detector at 205 and 210 nm. This approach was selected due to its straightforward application and ability to achieve high-resolution separation of the three target triterpenoids (Fig. 7). Among the various reported methods for analyzing triterpenoid content in *C. asiatica*, the incorporation of  $\beta$ -cyclodextrin in the mobile phase has proven to be highly effective (Pan et al., 2007; Kai et al., 2008).  $\beta$ -Cyclodextrin interacts with the polycyclic carbon skeleton of the triterpenoids, facilitating their separation—particularly for the isomeric pairs madecassic acid/terminolic acid and madecassoside/terminoloside.

To optimize separation efficiency, solvent A was tested with different acidic modifiers, including acetic acid, formic acid, and phosphoric acid, at concentrations of 0.10 %, 0.20 %, and 0.50 %, respectively. Additionally,  $\beta$ -cyclodextrin concentrations of 0.112 %, 0.225 %, 0.45 %, and 0.9 % were evaluated. The optimal mobile phase composition was determined to be 0.2 % formic acid and 0.225 %  $\beta$ -cyclodextrin in solvent A, which provided excellent resolution, peak symmetry, and shape. Further optimization involved testing various gradient elution profiles, with the best-performing composition selected. The highest signal-to-noise (S/N) ratio was observed at detection wavelengths of 205 and 210 nm. Chromatographic resolution was further enhanced by maintaining the column temperature at 25°C, which minimized peak tailing and ensured reliable quantification.

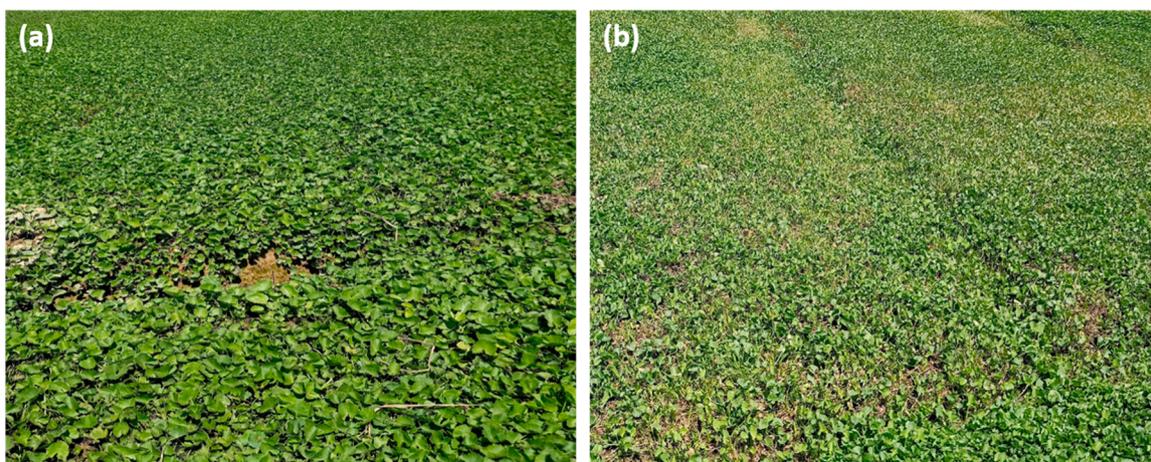


Fig. 3. (a) ORGANIC *C. asiatica* and (b) VIETGAP *C. asiatica* showing leaf colour.

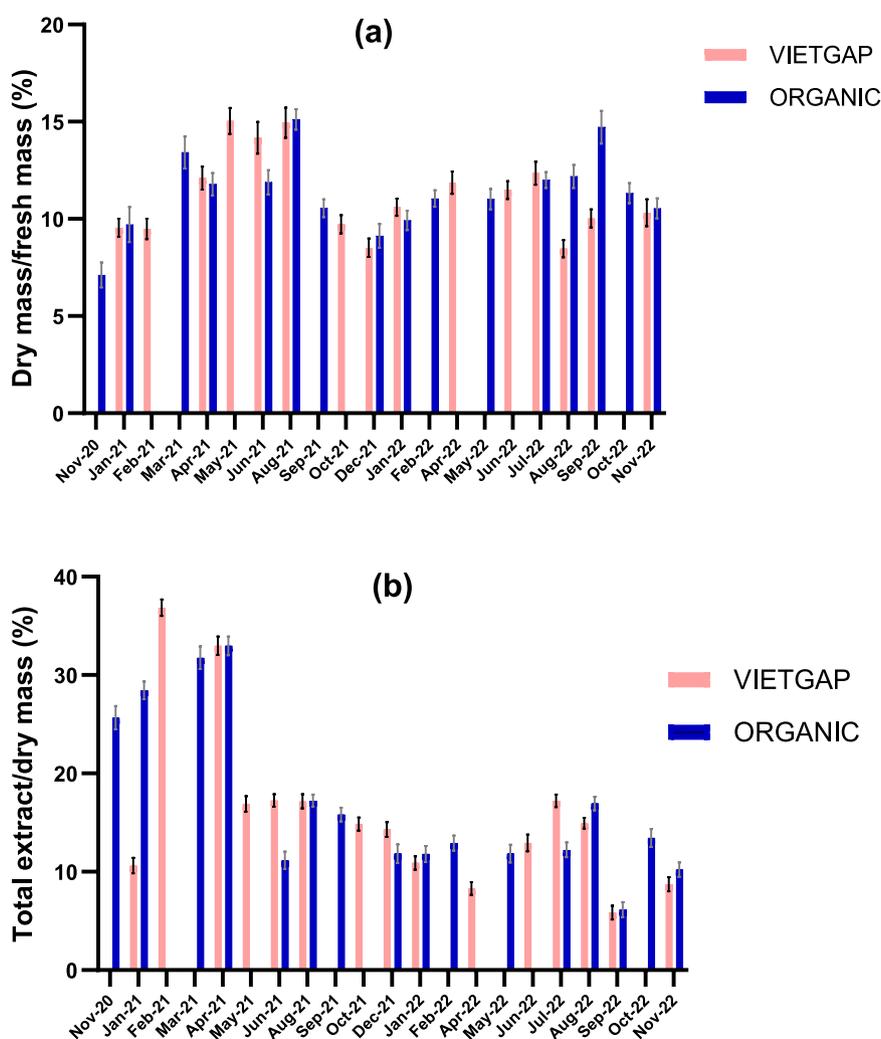


Fig. 4. (a) Comparison in weight of dry *C. asiatica* with fresh *C. asiatica* for VIETGAP and ORGANIC samples during the different harvests from November 2020 to November 2022. (b) The yields of the extracts from the dry *C. asiatica* for VIETGAP and ORGANIC samples. Each bar represents the mean  $\pm$  SD of three replicates.

3.4. Accumulation states and contents of triterpenic acids in VIETGAP and ORGANIC *C. asiatica*

In this study, the accumulation of MA was of primary interest due to its significant bioactivity (Tran et al., 2018; Wei et al., 2023; Zhang

et al., 2014; Han et al., 2020). As illustrated in Fig. 5, the MA content gradually increased from January 2021, reaching its peak in June 2021, before slowly declining until December 2021. A similar trend was observed in the second year, with MA levels rising from January 2022, peaking in August 2022, and then gradually decreasing until November

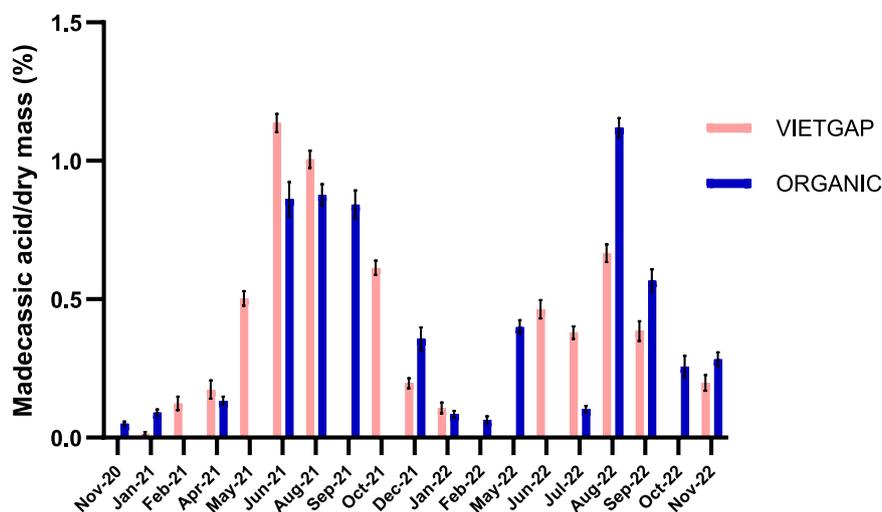


Fig. 5. The percentage of madecassic acid in dry *C. asiatica* samples over the course of the year cultivated under both ORGANIC and VIETGAP conditions. Each bar represents the mean  $\pm$  SD of three replicates.

2022. These results suggest that the optimal harvesting period for *C. asiatica* with high MA content, suitable for pharmaceutical applications, is between May and October, regardless of the cultivation method. As shown in Fig. 5 and Table 3, the MA content in *C. asiatica* from both cultivation systems exhibited a slight decline in 2022 compared with 2021, even during the peak harvesting period from June to September when triterpenoid accumulation was typically highest. This reduction may be attributed to plant aging, as the *C. asiatica* plants had undergone nearly two years of continuous growth and repeated harvesting from the same rootstocks. Specifically, in the VIETGAP production, the MA contents recorded in June, August, and October 2021 were  $11.40 \pm 0.96$ ,  $10.00 \pm 0.68$ , and  $6.00 \pm 0.93$  mg/g, respectively. These figures compare favourably with many reported accessions (Kunjumon, et al., 2022). In contrast, the corresponding values in 2022 for June, July, August, and September were  $4.36 \pm 0.01$ ,  $12.24 \pm 0.22$ ,  $6.55 \pm 0.58$ , and  $4.38 \pm 0.05$  mg/g. A comparable trend was observed in the ORGANIC cultivation system, where the MA contents in June, August, and September 2021 were  $8.80 \pm 0.09$ ,  $8.60 \pm 0.08$ , and  $8.30$

$\pm 0.03$  mg/g, respectively, while in 2022, these values decreased to  $2.55 \pm 0.02$ ,  $11.19 \pm 0.95$ , and  $3.71 \pm 0.05$  mg/g (Table 3).

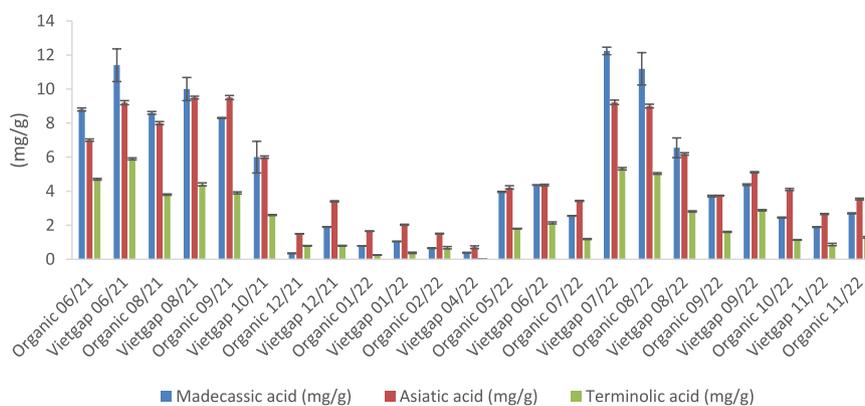
Fig. 6 and Table 4 present the quantified levels of all three triterpenoids (AA, MA, and TA) in VIETGAP and ORGANIC *C. asiatica* samples, based on HPLC analysis of ethanol extracts without alkaline hydrolysis. Specifically, in June 2021, the MA content surpassed that of AA and TA in both cultivation methods, measuring 8.8, 7.0, and 4.7 mg/g (ORGANIC) and 11.4, 9.2, and 5.9 mg/g (VIETGAP), respectively. In August, slight declines in MA (10 mg/g) and TA (4.4 mg/g) were observed for VIETGAP, while AA increased slightly to 9.5 mg/g. From October 2021 to April 2022, the content of all three acids declined significantly, likely due to low temperatures and high rainfall. All triterpenic acid levels peaked in June, coinciding with the highest local temperatures.

Comparing the relative levels of the different triterpenoids, during the rainy season in October, MA and AA levels declined and equalized in VIETGAP samples. Seasonal variations played a significant role in

Table 3

Content of MA/AA/TA in the EtOH extract before hydrolysis. Values are mean  $\pm$  SD of three replications.

Production technique/Date of harvest	Temperature		Mean Rainfall (mm month <sup>-1</sup> )	Content of triterpenic acid			
	Max <sup>o</sup> C	Min <sup>o</sup> C		Madecassic acid (mg/g)	Asiatic acid (mg/g)	Terminolic acid (mg/g)	Total acids (mg/g)
Organic 06/21	40.5	24.5	32	8.80 $\pm$ 0.09	7.00 $\pm$ 0.09	4.70 $\pm$ 0.05	20.5
Vietgap 06/21				11.40 $\pm$ 0.96	9.20 $\pm$ 0.12	5.90 $\pm$ 0.06	26.5
Organic 08/21	40.2	23.7	53	8.60 $\pm$ 0.08	8.00 $\pm$ 0.10	3.80 $\pm$ 0.04	20.4
Vietgap 08/21				10.0 $\pm$ 0.68	9.50 $\pm$ 0.09	4.40 $\pm$ 0.09	23.9
Organic 09/21	34.9	23.1	536	8.30 $\pm$ 0.03	9.50 $\pm$ 0.12	3.90 $\pm$ 0.06	21.7
Vietgap 10/21	33.4	20.0	1438	6.00 $\pm$ 0.93	6.00 $\pm$ 0.08	2.60 $\pm$ 0.03	14.6
Organic 12/21	27.2	15.6	490	0.35 $\pm$ 0.02	1.50 $\pm$ 0.02	0.80 $\pm$ 0.01	2.65
Vietgap 12/21				1.90 $\pm$ 0.02	3.40 $\pm$ 0.05	0.80 $\pm$ 0.02	6.1
Organic 01/22	29.2	16.5	96	0.79 $\pm$ 0.01	1.65 $\pm$ 0.03	0.25 $\pm$ 0.01	2.69
Vietgap 01/22				1.05 $\pm$ 0.02	2.03 $\pm$ 0.04	0.38 $\pm$ 0.04	3.46
Organic 02/22	29.0	14.0	71	0.65 $\pm$ 0.01	1.51 $\pm$ 0.03	0.68 $\pm$ 0.07	2.84
Vietgap 04/22	36.5	16.5	381	0.39 $\pm$ 0.02	0.71 $\pm$ 0.09	0.02 $\pm$ 0.01	1.12
Organic 05/22	37.0	19.0	157.3	3.96 $\pm$ 0.03	4.22 $\pm$ 0.11	1.8 $\pm$ 0.02	9.98
Vietgap 06/22	38.0	25.0	34	4.36 $\pm$ 0.01	4.36 $\pm$ 0.07	2.14 $\pm$ 0.06	10.86
Organic 07/22	37.4	24.1	61	2.55 $\pm$ 0.02	3.43 $\pm$ 0.04	1.19 $\pm$ 0.03	7.17
Vietgap 07/22				12.24 $\pm$ 0.22	9.22 $\pm$ 0.18	5.32 $\pm$ 0.07	26.78
Organic 08/22	36.0	24.0	157.6	11.19 $\pm$ 0.95	9.00 $\pm$ 0.19	5.04 $\pm$ 0.05	25.23
Vietgap 08/22				6.55 $\pm$ 0.58	6.19 $\pm$ 0.08	2.81 $\pm$ 0.04	15.55
Organic 09/22	35.0	23.0	1012	3.71 $\pm$ 0.05	3.74 $\pm$ 0.04	1.61 $\pm$ 0.03	9.06
Vietgap 09/22				4.38 $\pm$ 0.05	5.11 $\pm$ 0.06	2.88 $\pm$ 0.04	12.37
Organic 10/22	31.9	19.1	1367	2.45 $\pm$ 0.03	4.10 $\pm$ 0.07	1.14 $\pm$ 0.02	7.69
Vietgap 11/22	32.0	18.0	226	1.89 $\pm$ 0.02	2.66 $\pm$ 0.04	0.87 $\pm$ 0.07	5.42
Organic 11/22				2.70 $\pm$ 0.03	3.54 $\pm$ 0.06	1.29 $\pm$ 0.05	7.53



**Fig. 6.** Content of three triterpenic acids (mg/g) in dry *C. asiatica* cultivated under VIETGAP (VG) and ORGANIC (ORG) conditions. Each bar represents the mean  $\pm$  SD of three replicates.

**Table 4**

Content of MA/AA/TA in the EtOH extract (mg/g) before and after hydrolysis. Values are mean  $\pm$  SD of three replications.

Production technique/Date of harvest	Content of triterpenic acid							
	Madecassic acid (mg/g)		Asiatic acid (mg/g)		Terminolic acid (mg/g)		Total acids (mg/g)	
	Before hydrolysis	After hydrolysis	Before hydrolysis	After hydrolysis	Before hydrolysis	After hydrolysis	Before hydrolysis	After hydrolysis
Organic 01/22	0.79 $\pm$ 0.01	3.14 $\pm$ 0.04	1.65 $\pm$ 0.03	4.64 $\pm$ 0.06	0.25 $\pm$ 0.01	1.78 $\pm$ 0.02	2.69	9.56
Vietgap 01/22	1.05 $\pm$ 0.02	5.54 $\pm$ 0.07	2.03 $\pm$ 0.04	8.86 $\pm$ 0.09	0.38 $\pm$ 0.04	3.24 $\pm$ 0.05	3.46	17.64
Organic 02/22	0.65 $\pm$ 0.01	1.63 $\pm$ 0.04	1.51 $\pm$ 0.03	2.41 $\pm$ 0.06	0.68 $\pm$ 0.07	1.17 $\pm$ 0.02	2.84	5.21
Vietgap 04/22	0.39 $\pm$ 0.02	0.50 $\pm$ 0.01	0.71 $\pm$ 0.09	0.92 $\pm$ 0.02	0.02 $\pm$ 0.01	0.09 $\pm$ 0.01	1.12	1.51
Organic 05/22	3.96 $\pm$ 0.03	4.70 $\pm$ 0.05	4.22 $\pm$ 0.11	4.44 $\pm$ 0.0 = 4	1.8 $\pm$ 0.02	1.99 $\pm$ 0.02	9.98	11.13
Vietgap 06/22	4.36 $\pm$ 0.01	8.58 $\pm$ 0.09	4.36 $\pm$ 0.07	9.61 $\pm$ 0.15	2.14 $\pm$ 0.06	5.22 $\pm$ 0.05	10.86	23.41
Organic 07/22	2.55 $\pm$ 0.02	4.00 $\pm$ 0.03	3.43 $\pm$ 0.04	4.58 $\pm$ 0.07	1.19 $\pm$ 0.03	1.74 $\pm$ 0.02	7.17	10.32
Vietgap 07/22	12.24 $\pm$ 0.22	14.23 $\pm$ 0.21	9.22 $\pm$ 0.18	11.60 $\pm$ 0.15	5.32 $\pm$ 0.07	6.89 $\pm$ 0.08	26.78	32.72
Organic 08/22	11.19 $\pm$ 0.95	13.46 $\pm$ 0.19	9.00 $\pm$ 0.19	10.54 $\pm$ 0.17	5.04 $\pm$ 0.05	6.15 $\pm$ 0.06	25.23	30.15
Vietgap 08/22	6.55 $\pm$ 0.58	18.09 $\pm$ 0.34	6.19 $\pm$ 0.08	10.68 $\pm$ 0.13	2.81 $\pm$ 0.04	6.91 $\pm$ 0.07	15.55	35.68
Organic 09/22	3.71 $\pm$ 0.05	17.56 $\pm$ 0.82	3.74 $\pm$ 0.04	10.44 $\pm$ 0.20	1.61 $\pm$ 0.03	6.99 $\pm$ 0.09	9.06	34.99
Vietgap 09/22	4.38 $\pm$ 0.05	5.33 $\pm$ 0.06	5.11 $\pm$ 0.06	5.34 $\pm$ 0.07	2.88 $\pm$ 0.04	2.98 $\pm$ 0.03	12.37	13.65
Organic 10/22	2.45 $\pm$ 0.03	7.13 $\pm$ 0.07	4.10 $\pm$ 0.07	11.41 $\pm$ 0.14	1.14 $\pm$ 0.02	4.16 $\pm$ 0.04	7.69	22.7
Vietgap 11/22	1.89 $\pm$ 0.02	4.22 $\pm$ 0.02	2.66 $\pm$ 0.04	5.26 $\pm$ 0.06	0.87 $\pm$ 0.07	2.08 $\pm$ 0.02	5.42	11.56
Organic 11/22	2.70 $\pm$ 0.03	6.10 $\pm$ 0.06	3.54 $\pm$ 0.06	7.83 $\pm$ 0.08	1.29 $\pm$ 0.05	3.20 $\pm$ 0.04	7.53	17.13

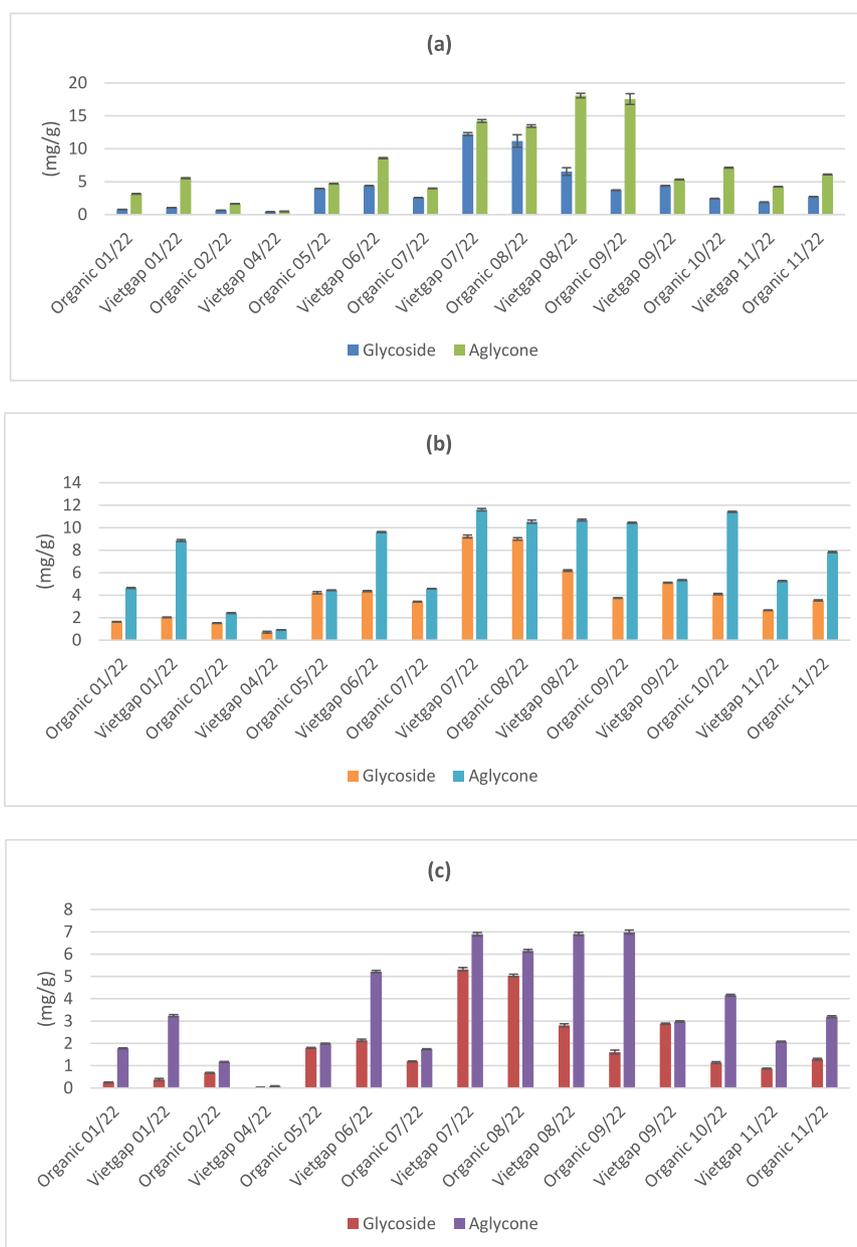
triterpenic acid accumulation. From December 2021 to January 2022, during cooler temperatures, AA exhibited the highest concentration, approximately two- to fourfold higher than MA and TA, respectively, maintaining this dominance until May before decreasing below MA levels from June to August. These patterns were consistent with observations from the first year. Adverse weather conditions between February and May 2022, including flooding, strong storms, and prolonged rainfall in Thua Thien-Hue province, resulted in no harvests during this period. By July and August 2022, MA content again surpassed AA levels, whereas TA remained consistently lower.

We performed experiments to compare free triterpenic acids with their glycosides by analyzing ethanol extracts before and after alkaline hydrolysis. Fig. 7 and Table 4 illustrate the quantified levels of triterpenic acids before and after hydrolysis in VIETGAP and ORGANIC samples, based on HPLC analysis. The results indicate that hydrolysis significantly increased triterpenic acid content, ranging from 1.2- to 8.5-fold compared to unhydrolyzed extracts. Specifically, for ORGANIC samples harvested in January, MA, AA, and TA levels increased approximately 4.0-, 2.8-, and 7.1-fold, respectively. In VIETGAP samples from the same period, increases of 5.3-, 4.3-, and 8.5-fold were observed. However, these values declined sharply in subsequent months, with reductions ranging from 2.5- to 1.1-fold between February and September. Interestingly, from October to November, these ratios increased again, ranging from 2.2- to 4.78-fold in January before gradually declining until August, then rising again between September and December.

The triterpenoid content before hydrolysis represents the natural abundance of free triterpenic acids in *C. asiatica*, while post-hydrolysis levels indicate their total quantities, including glycosidic forms. The difference between these values reflects the glycoside fraction. MA predominantly existed in glycoside form in January, August, September, October, and November 2022 for both VIETGAP and ORGANIC samples. AA glycosides were most prevalent in January for both cultivation methods and in September and October for ORGANIC samples only. Similarly, TA glycosides were dominant in January for both methods and in September and October exclusively for ORGANIC samples. The combined totals of free and glycosidic triterpenoids measured here are high compared to other accessions, and approach those of 'elite' examples (Kunjumon, et al., 2022).

#### 4. Discussion

*Centella asiatica* is an important crop for many communities. In the commune where this study was conducted,  $\sim$ 70 ha of *C. asiatica* are cultivated yielding an average annual production of around 2800 metric tons of fresh biomass, which is sold as fresh vegetables, teas, and matcha powder, supporting 300 households (Báo., 2024). Long term sustainability is a vital concern for both the economic and environmental health of the community, and reducing agrochemicals is an important part of this process. There are concerns that overuse or poor regulation of agrochemicals is one of the causes of the high liver cancer burden in Vietnam (Bray et al., 2024; Cordier et al., 1993). Since madecassic acid,



**Fig. 7.** The contents of madecassic (a), asiatic (b), and terminolic acid (c) (mg/g; in dry *C. asiatica* weight) after hydrolysis (total bar height), separated into the aglycone (i.e. content prior to hydrolysis, lighter colour) and glycone (additional quantity obtained after hydrolysis, darker colour) presented for January to November of 2022. Each bar represents the mean  $\pm$  SD of three replicates.

from *C. asiatica*, could be the starting point for new therapeutics against liver cancer (Tran et al., 2018; Tran et al., 2024), its production should not exacerbate the same problem. The primary importance of this work is demonstration that if *C. asiatica* is grown for the production of madecassic acid, or its other terpenoids, this agrochemical use can be omitted, without harm to the chemical yield of the compounds, ensuring that sustainable production is feasible. Since *C. asiatica* is used in many other products, such as skin creams (Tripathy et al., 2022; Tan et al., 2021, Min et al., 2024, Mando et al., 2024), this potential for sustainable economic growth already exists regardless of whether any hypothetical medicine is ever approved.

Regarding practical aspects of extraction, methanol has been claimed to be the most effective solvent for maceration extraction at room temperature (Ahyar et al., 2024), while ethanol has been widely used across multiple extraction techniques, including heat-, ultrasound-, and microwave-assisted extraction. Ethanol/water systems (50–80 %

ethanol, v/v) have been demonstrated to efficiently extract terpenoids and their glycosides (Teerin et al., 2025). Extraction efficiency is also influenced by parameters such as temperature and extraction duration (Okseini et al., 2023). The choice of method depends on whether aglycones (free acids) or glycosides are desired for downstream applications. Our standardized extraction and analytical approach have enabled accurate quantification of *C. asiatica* triterpenoids.

In comparison to the findings of others regarding the optimal growth conditions of *C. asiatica* relating to triterpenoid production, our results align with previous studies on biomass and triterpenoid content, which reported significant seasonal variations in biomass and centelloside accumulation, with generally warm and humid climates favouring higher triterpenoid content (Prasad et al., 2016; Rohini and Smitha, 2022). Our results also revealed that glycoside forms of the triterpenoids dominated in *C. asiatica* under both VIETGAP and ORGANIC cultivation systems in January, September, and October, coinciding with periods

when the average temperature decreased following the hot summer months in Thua Thien Hue province, reaching minimum values of approximately 16.5 °C (Table 3). This finding suggests that cooler climatic conditions favour glycoside biosynthesis in *C. asiatica* over formation of aglycones. These results are consistent with previous studies (Plengmuankhae, W et al., 2015) reporting that low-temperature treatment of leaves after harvest significantly enhances glycoside accumulation, underscoring the influence of temperature on triterpenoid metabolism in *C. asiatica*.

Our findings provide further insights for optimizing harvest schedules to maximize triterpenoid yield, through data on the impact of cultivation techniques on secondary metabolite production. This contributes to the optimization of bioactive compound isolation for pharmaceutical, nutraceutical, or cosmetic applications, enhancing the potential of *C. asiatica* as a valuable medicinal plant with minimal negative environmental impact.

## 5. Conclusion

A study on the cultivation of *Centella asiatica* using VIETGAP and ORGANIC farming methods was conducted in central Vietnam over a 26-month period, encompassing 15 harvests per method. We found no significant differences in the dry mass-to-fresh mass ratios, or the total extract percent between VIETGAP and ORGANIC samples. Seasonal fluctuations were observed in biomass and extract yields, with higher dry weight percentages recorded during the summer months. The three major triterpenoids (madecassic acid, asiatic acid, and terminolic acid) were predominantly present in glycoside form during autumn and winter across both cultivation systems.

These results not only provide scientific evidence for optimizing the harvest period of *C. asiatica*, particularly for pharmaceutical and cosmetic applications, but also carry practical implications for growers and policymakers. For producers, the findings may serve as a guideline for scheduling harvests during triterpenoid-rich seasons and integrating soil and nutrient management strategies to enhance bioactive compound accumulation. For policymakers, our study demonstrated parity in yield and bioactive content between ORGANIC and VIETGAP systems, supporting the promotion of organic certification schemes as a viable, environmentally responsible alternative. Collectively, this research offers a science-based foundation for integrating organic practices with nutrient management policies to sustain high triterpenoid productivity while advancing eco-friendly and health-conscious cultivation of *C. asiatica*.

## CRedit authorship contribution statement

**Tran Loc Van:** Investigation. **Tran Chien Van:** Supervision, Funding acquisition. **Nguyen Luu Thi:** Investigation. **Huynh Ni Thi Ngoc:** Investigation. **Nguyen Dung Thi:** Investigation. **Be Yen Thi Hoang:** Investigation. **Nguyen Tri Luong:** Investigation. **Serpell Christopher J.:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Tran Thao Thi Phuong:** Writing – original draft, Supervision, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Michelle D. Garrett:** Writing – review & editing, Project administration, Funding acquisition. **Nguyen Nhiem Xuan:** Formal analysis. **Tran Van Sung:** Writing – original draft, Supervision, Funding acquisition, Conceptualization. **Pham Ninh Thi:** Investigation.

## Declaration of Competing Interest

The authors report no declarations of interest.

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## Data availability

Data will be made available on request.

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