Crimson Publishers
Wings to the Research

Case Report

High Active Component Lines of *Centella Asiatica* (L.)

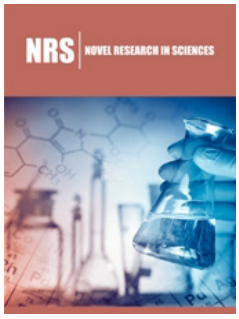
Pranom Chaiai[1]*, Ungkana Poungngenmak[1], Kesorn Chamchuen[2] and Watchara Suwannaart[3]

[1]Department of Agriculture, Office of Agricultural Research and Development, Thailand

[2]Department of Agriculture, Phichit Research and Development Center, Thailand

[3]Department of Agriculture, Office of Agricultural Research and Development, Thailand

ISSN: 2688-836X



***Corresponding author:** Pranom Chaiai, Office of Agricultural Research and Development, Thailand

Submission: July 15, 2022

Published: July 29, 2022

Volume 11 - Issue 4

How to cite this article: Pranom Chaiai*, Ungkana Poungngenmak, Kesorn Chamchuen, Watchara Suwannaart. High Active Component Lines of *Centella Asiatica* (L.). Nov Res Sci. 11(4). NRS. 000766. 2022.
DOI: 10.31031/NRS.2022.11.000766

Abstract

Centella asiatica (L.) is commonly ingested and turned into a variety of products because it is rich in antioxidants and has a wide range of medicinal properties. The asiaticoside substance was used to compare various active ingredient-rich Asiatic pennywort clones. In a greenhouse with 50% shading, five rayong lines, a commercial nakhon pathom line, and regional lines grown in the provinces of Phrae, Phichit, and Chainat were propagated. Weed control and consistent watering three times per week were maintained, and the plants were ready for harvesting 90 days after planting. The observed plant was divided into three groups based on DNA analysis

a) Group 1 included Rayong and Nakhon Pathom lines,

b) Group 2 included Phichit and Nonthaburi lines, and

c) Group 3 included Phrae lines.

Vegetative growth parameters were discovered to differ depending on cultivation site and plant line. The Phrae trials produced fresh yields of 725-754kg/rai (1 rai=0.16 hectare) and dried weights of 73-81kg/rai with no significant difference, whereas Rayong line gave the highest asiaticoside level of 44 %w/w and the local phrae line performed the lowest level of 0.27%w/w. The Phichit trial delivered a fresh yield of 805-1,012kg/rai and a dried weight of 70-86kg/rai, with the lowest asiaticoside content ranging from 0.1-0.12% w/w. The experiment in Chainat produced the highest fresh yields of 1,203-1,433kg/rai and dried weights of 179-315kg/rai, with the Nakhon Prathom line outweighing the other two lines in dried powder weight. According to all available information, the growing location of Asiatic pennywort may influence the asiaticoside production. This study, however, only included data from the cool growing season; additional trials in other seasons will be required to identify the best Asiatic pennywort plant line/lines with desirable asiaticoside content that are suitable for all cultivation climates.

Keywords: *Centella asiatica*; Vegetative growth; Asiaticoside

Abbreviations: TISTR: Thailand Institute of Scientific and Technological Research; RAPD: Random Amplified Polymorphic DNA; DMRT: Duncan's New Multiple Range Test; RYG: Rayong; NPT: Nakhon Prathom; PCT: Phichit; NBI: Nonthaburi; PRE: Phrae

Introduction

Asiatic pennywort is a valuable herbal plant that can be used medicinally in its entirety. Its leaves contain anti-inflammatory triterpene centellosides phytochemical compounds like asiaticoside, madecassic acid, madecassoside, and asiatic acid, which have been shown to be anti-inflammatory [1], promote wound healing, decrease blood loss, and have a seductive effect [2]. The cost of producing a commercial Asiatic plant was reported to be 5,340THB/rai (854.40 baht/hectare) with a total yield of 5,000kg/rai (800kg/hectare), which was traded at 7 baht/kg, and the farmer could earn 29,660 baht/rai (4,745.60 baht/hectare) (Department of Agricultural Extension, 2019). Furthermore, the Kanchanaburi Research and

Development Center classified this plant as having 25-50 percent residue. To achieve the highest quality and quantity of Asiatic pennywort production, it is critical to use a good cultivar with high active ingredient properties and an appropriate chemical-free production technology. Low quality and insufficient produce, as well as a scarcity of excellent production practices, necessitate additional research into finding Asiatic pennywort varieties that provide beneficial ingredients for the cosmetic, medicinal, and healthy beverage industries.

Chaiai et al. [3] searched the upper north for highly productive 800-1,789kg/rai or 128-286kg/hectare asiatic pennywort plants with high active substances. The trat clone was found to be the most productive, followed by lines from Chiang Rai, Phayao, Petchaburi, Nakhon Pathom, Ratchaburi, Rayong, and Chanthaburi. Rayong lines had the highest average asiaticoside concentration of 0.59 percent w/w, followed by Phayao, Ratchaburi, Trat, Chiang Rai, Petchaburi, Chanthaburi, and Nakhon Pathom. However, the high-active level varies among the clones studied. In this study, high-active ingredient lines were examined in the provinces of Phrae, Phichit, and Chainat in order to select the best lines for commercial cultivation. The collection of 16 *C. asiatica* lines took place at the Thailand Institute of Scientific and Technological Research (TISTR) in the province of Pathum Thani. The four clones with the highest concentrations of the asiaticoside compound were Nakhon Sri Thammarat, Prachinburi, Rayong, and Ubon Ratchathani The asiaticoside content was also significantly influenced by the favorable climate, with the highest asiaticoside production produced by the Nakhon Sri Thammarat and Ubon Ratchathani lines growing in the summer, while the Rayong and Ubon Ratchathani lines produced more beneficial substance during the rainy season [4].

Materials and Methods

A randomized complete block design was used in a field experiment conducted during the cool season to compare three Asiatic pennywort lines: Rayong, Nakhon Pathom (commercial line), and a local line (control treatment) growing in three separate field trials: Phrae, Phichit, and Chainat, each with seven replications. The local lines investigated in Phrae, Phichit, and Chainat were Phrae, Phichit, and Nonthaburi, respectively. Plowing and 14 days of sun drying prepared the grounds for cultivation. The single planting bed included 21 beds overall, each measuring 2x3m and 15cm high, with a row spacing of 80cm. On top of the bed, manure was applied, and a greenhouse with 50% shading that measured 15m wide x 30m long x 2.5m high was constructed. Asexual propagation using healthy runners at the four-leaf stage was prepared and planted in a bed with a growing space of 10x10cm. After a month of an hour of sprinkler watering in the morning and afternoon, the plant was only watered once a day in the morning until harvesting. The presence of moisture in the dirt indicated that there was sufficient water. Using DOA management techniques (2013), 3-4kg/rai (0.48-0.64kg/hectare) of 25-7-7 fertilizer and 3 kg/rai (0.48kg/hectare) of 46-0-0 fertilizer were applied at 15-20 days and 30-40 days after planting, respectively. To combat the common cutworm epidemic, a bio agent of Bacillus thuringiensis 60ml/20L of water mixed with surfactant was sprayed after 3PM. At 30 and 60 days after planting, the growth rate was measured along with the quantity of runners, plants per runner, length of the runners, number of leaves, and size of the leaves.

The genetic diversity of all Asiatic pennywort plants studied was investigated in the laboratory. Young leaves were collected at random and tested using Random Amplified Polymorphic DNA (RAPD) with12 primers. 90 days after planting, whole plants were removed from the soil with a shovel in a one square meter/bed, dried leaves were removed, and water cleaned, and set out to air dry before being weighed. A kilogram of fresh plants was randomly selected, the roots were removed and finely cut, and the plants were dried in a hot-air oven set to 55°C for 48 hours. After recording a dried weight, the sample was ground into powder, and 20g of the powder was analyzed for asiaticoside compounds using the method described by [5]. In a statistical study, the Duncan's New Multiple Range Test (DMRT) was used to compare the means of all parameters.

Results and Discussion

Molecular analysis

The molecular analysis of the five *C. asiatica* observations was illustrated using the city block distance. Rayong and the commercial line Nakhon Pathom were combined, while the lines of Phichit and Nonthaburi, which were grown locally in Phichit and Chainat, were separated. Furthermore, with only one member, the clade formed the third group, the Phrae line (Figure 1); [6].

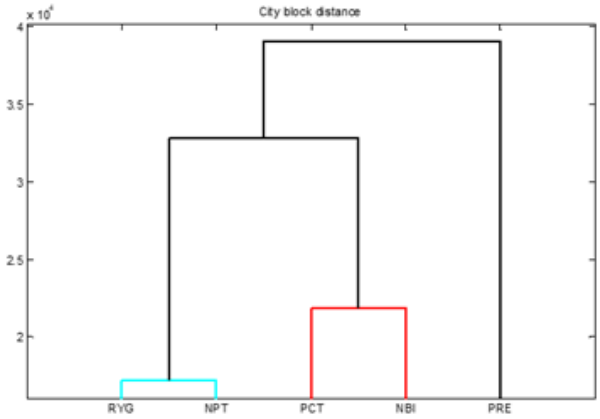


Figure 1: Phylogenetic tree of the five asiatic pennywort lines including RYG=Rayong; NPT=Nakhon Prathom; PCT=Phichit; NBI=Nonthaburi; PRE= Phrae.

Growth rate

There were no discernible differences in stolons quantity among Asiatic pennywort lines grown in three different locations. The number of stolon in the Phrae, Phichit, and Chainat experiments ranged from 1.71-1.89, 2.06-2.51, and 1.81-1.83, respectively. On the other hand, the Phichit trial produced slightly more stolons than the other growing sites. Cultivation of Asiatic pennywort in Phrae

revealed that all lines produced the same number of plants per stolon, which ranged from 6.43-6.79 on average. The same pattern was seen in Chainat, where *C. asiatica* lines gave the number of plants per stolon with an average of 3.17-3.43 plants (Table 1). The Phichit line, which grows locally in Phichit, had the lowest plants per stolon, ranging from 3.39-4.56.

Growing Asiatic pennywort in Phrae offered the best growth in terms of number of plants per stolon at 6.43-6.79 plants, while Phichit trials supplied a minimum range of 3.17-3.43 plants/stolon, with no statistical difference between the three lines planted in both areas (Table 1). The average stolon length measured from Phrae was 45.35-60.07cm, with the local line indicating the shortest stolon of 45.35cm (Table 1). The Phrae and Phichit lines had the same stolon length pattern, but the Phichit line produced fewer plants per stolon (3.39-4.56 plants), followed by the Chainat line (3.17-3.43 plants). The Phrae trial produced leafy Asiatic pennyworts, followed by the Chainat trial, at 14.4-15.8 and 7.24-7.70 per plant, respectively (Table 2), with no statistical difference in leaf quantity among lines in either location. Rayong and Nakhon Prathom lines produced fewer leaves than the Phichit line, which produced the most abundant leaves (6.51 on average) in the Phichit experiment. Based on the data collected, it appeared that different locations may influence the quantity of leaves. Except for the Phrae and Phichit sites, all experiments in Chainat had the same pattern of leaf width (4.95-5.93cm) and length (3.65-3.94cm), with no statistical differences between the three lines observed. The Nakhon Prathom line has the largest leaf, measuring 3.69 and 5.93cm wide and 5.78 and 3.73cm long, owing to the size of the plant leaf growing in Phrae and Phichit (Table 2).

Table 1: The vegetative growth rate of Asiatic pennyworts grown in three locations: Phrae, Phichit, and Chainat.

	Number of Stolons			Stolon Length (cm)			Number of Plants per Stolon		
Line	**Phrae**	**Phichit**	**Chainat**	**Phrae**	**Phichit**	**Chainat**	**Phrae**	**Phichit**	**Chainat**
Rayong	1.81 a	2.06 a	1.83 a	51.94 ab	50.00 ab	31.41 b	6.79 a	4.56 a	3.17 a
Nakhon Prathom	1.89 a	2.51 a	1.81 a	60.07 a	59.70 a	33.94 b	6.43 a	4.17 ab	3.34 a
Phrae	1.71 a	-	-	45.35 b	-	-	6.47 a	-	-
Phichit	-	2.34 a	-	-	45.60 b	-	-	3.39 b	-
Nonthaburi	-	-	1.99 a	-	-	46.22 a	-	-	3.43 a
CV (%)	15.8	15.3	1.8	20.8	18.5	13.1	25.5	18.1	13.1

Source: Means followed by the same letter within a column are not significantly different according to the Duncan's New Multiple Range Test (DMRT).

Table 2: Asiatic pennywort leaf growth in three locations: Phrae, Phichit, and Chainat.

	Number of Leaves per Plant			Leaf Width (cm)			Leaf Length (cm)		
Line	**Phrae**	**Phichit**	**Chainat**	**Phrae**	**Phichit**	**Chainat**	**Phrae**	**Phichit**	**Chainat**
Rayong	15.8 a	4.82 b	7.43 a	3.53 a	4.95 b	4.89 a	4.82 b	3.27 b	3.65 a
Nakhon Prathom	14.4 a	4.86 b	7.70 a	3.69 a	5.93 a	4.90 a	5.78 a	3.73 a	3.68 a
Phrae	14.9 a	-	-	2.79 b	-	-	4.07 c	-	-
Phichit	-	6.51 a	-	-	5.41 b	-	-	3.45 b	-
Nonthaburi	-	-	7.24 a	-	-	5.55 a	-	-	3.94 a
CV (%)	20.3	24.1	12.7	10.8	7.3	15.2	8.3	6.1	16.8

Yield and asiaticoside content

In every location studied, the lines of Rayong, Nakhon Prathom, and all three local lines, including Phrae, Phichit, and Nonthaburi, demonstrated no significant difference in fresh yield. Growing Asiatic pennywort in Chainat netted a maximum fresh yield of 1,202.79-1,433.40kg/rai, which is quite in contrast to its vegetative growth data. Chainat had the fewest plants per runner and the shortest runner length, while the number of runners is not significantly different between the two locations. Furthermore, based on leaf data, all of its parameters fall in the middle range between the maximum Phrae and the minimum Phichit. As a result, the plant grown in this area may produce the thickest leaf. The Phichit site provided fresh produce at a rate of 805-1,012kg/rai, which was higher than the Phrae site's rate of 724.57-754.29kg/rai (Table 3). The dried weight result is consistent with the fresh yield data, which displayed Chainat as the growing location with the highest dried yield. However, Nakhon Prathom delivered the highest dried produce at 315.35 kg/rai, while Rayong line delivered the lowest at 179.22kg/rai (Table 3). In contrast, all observed lines in the other two sites revealed no significant difference in weight of dried yield. The Phichit site achieved 70.60-85.60kg/rai of dried weight, while the Phrae site obtained a minimum of 73.36-81.20kg/rai of dried weight.

Table 3: Fresh yield and dried weight of asiatic pennyworts grown in three locations: Phrae, Phichit, and Chainat.

Line	Fresh Yield (kg/rai)			Dried Weight (kg/rai)		
	Phrae	Phichit	Chainat	Phrae	Phichit	Chainat
Rayong	724.57 a	902 a	1,202.79 a	75.12 a	82.60 a	179.22 b
Nakhon Prathom	731.43 a	1,012 a	1,433.40 a	81.20 a	85.60 a	315.35 a
Phrae	754.29 a	-	-	73.6 a	-	-
Phichit	-	805 a	-	-	70.60 a	-
Nonthaburi	-	-	1,283.05 a	-	-	230.95 b
CV (%)	34.7	35	19.7	34.3	12.8	19

Source: Means followed by the same letter within a column are not significantly different according to the Duncan's New Multiple Range Test (DMRT). -1 hectare=0.16 rai.

The growing sites with the highest concentrations of asiaticoside compound, according to (Table 4), were Phrae and Chainat. There was a significant difference in active content between the three lines in Phare. Rayong and Nakhon Prathom had the highest levels of asiaticoside, at 0.44 and 0.35 percent w/w, respectively, while the local line Phrae had the lowest level, at 0.27 percent w/w. The Chainat trial yielded no statistically significant levels of 0.32-0.49 percent w/w (Table 4). The high active compound extracted from Rayong and Nakhon Prathom planted in both Phrae and Chainat provided a variable asiaticoside production when compared to those reported in our study in 2013, where Rayong was previously disclosed to be vying with Nakhon Prathom. Furthermore, Nonthaburi was a new line of Asiatic pennywort with a high active level of asiaticoside property. Our 2013 study discovered that the Rayong line produced the most asiaticoside (0.59 percent w/w), but it was different in this study because the useful substance may link to the growing site rather than the line. All *C. asiatica* lines grown in Phrae and Chainat produced asiaticoside levels that were very close to those previously discovered (0.59 percent w/w), as shown by the data. If the location is the primary factor influencing asiaticoside production, additional data such as climate information, soil analysis prior to the start of the experiment, and above and below ground pest records may be taken into account for a more accurate result.

Table 4: Asiaticoside compound of asiatic pennyworts grown in three locations: Phrae, Phichit, and Chainart.

Line	Asiaticoside content (%w/w)		
	Phrae	Phichit	Chainat
Rayong	0.44 a	0.10 a	0.49 a
Nakhon Prathom	0.35 ab	0.12 a	0.32 a
Phrae	0.27 b	-	-
Phichit	-	0.12 a	-
Nonthaburi	-	-	0.48 a
CV (%)	18.1	26	49.4

Source: Means followed by the same letter within a column are not significantly different according to the Duncan's New Multiple Range Test (DMRT).

Conclusion

C. asiatica lines with high asiaticoside production properties were evaluated in this study. Cultivating areas were found to affect the amount of useful substance extracted from the lines of Rayong, Nakhon Prathom, Phrae, Phichit, and Nonthaburi. The data showed that Phrae and Chainat were the best locations to plant Asiatic pennywort in order to extract the most asiaticoside. More research may be needed, however, to identify appropriate materials that provide a valuable Asiatic pennywort-based high-active compound for consumption and industrial purposes.

Acknowledgement

The authors would like to express gratitude to the Asiatic pennywort farmer's group in Nakhon Prathom, Phrae, Phichit, and Chainat for their outstanding cooperation throughout this research project. We would like to extend our appreciation to the supervisors and staff at the Science and Technology Service Center, Faculty of Science, Chiang Mai University, for DNA analysis, as well as the Center of Analysis for Product Quality (Division of Natural Products), Faculty of Pharmacy, Mahidol University, for analytical work on high active compounds.

References

1. Vogel HG, De Souza NJ, D'sa A (1990) Effect of terpenoids isolated from *Centella asiatica* on granuloma tissue. Ac Ther 16(4): 285-298.
2. Ramaswamy AS, Periasamy SM, Basu NK (1970) Pharmacological studies on *Centella asiatica*. J Res Indian Med 4: 160-175.
3. Chaiai P, Suriyapromchai P, Sangsoy W, Manudsom K, Meesuk S (2010) *Centella asiatica* (L.) cultivation and collection in various growing sites. Department of Agriculture Bangkok, pp. 217-260.
4. Piriyapattarakit A, Tangkitchot P, Chaloemklin P (2009) Asiatic pennywort production in an organic agriculture system. Agri Sci J 40(3): 205-208.
5. Monton C, Luprasong C, Suksaeree J, Songsak T (2018) Validated high performance liquid chromatography for simultaneous determination of stability of madecassoside and asiaticoside in film forming polymeric dispersions. Rev Bra de Farm 28(3): 289-293.
6. Department of Agricultural Extension (2019) Plant production status.