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Influence of Varieties and Elicitors on Biomass and Bioactive Compound Yield of *Centella Asiatica* Growing in Pathumthani

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Abstract

Centella Asiatica is a medicinal plant distributed throughout tropical and sub-tropical areas. It has been used extensively for the treatment of nervous disorders and skin diseases due to its contents of bioactive compounds such as asiaticoside, madecassoside, flavonoids, etc. The fact that there is a huge demand in the pharmaceutical and cosmetic preparation markets led to our interest in increasing Centella biomass and bioactive constituents per planting area. This study focused on using different elicitors (salicylic acid, yeast extract and malt extract) for the enhancement of biomass and content of two main bioactive components (asiaticoside and madecassoside) in five accessions of Centella (Nakhonpathom, Prachinburi, Rayong, Ubonratchathani and Nakhonsrithammarat) growing in Khlong Luang, Pathum Thani. The findings exhibited that the accession Nakhonpathom provided the highest biomass yield and accumulation of two bioactive compounds (madecassoside and asiaticoside) when 1 mg/L salicylic acid was applied two times at 7-day intervals and 7 days ahead of harvesting.

Disciplinary: Plant Sciences, Herbal Medicine.

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1 Introduction

Centella Asiatica, or Asiatic pennywort, is a herb that is listed on the Thai Ministry of Public Health's National Herbal Drug List. All parts of the plant are used as medicines and other health products. In traditional medicine practice, it has been used to treat internal contusions, fortify the

heart, as an analeptic, to treat exhaustion and fatigue, promote micturition, promote kidney function, and treat skin disease and enhance wound healing (Mingmuang, *et al*, 2020). For beauty treatments, *Centella* can be an ingredient in products including skin creams, facial cleansing gels, shampoo, soap, and toner. The phytochemicals naturally found in *Centella* are said to help reduce black spots and scars, promote the growth of new skin cells and tissues, and nourish hair (Panya, 2018). The important bioactive constituents of *Centella* are mainly in the triterpenoid glycoside group, including asiaticoside, madecassoside, asiatic acid and madecassic acid (Tongekkaew, 2013).

For *Centella* cultivation, the main emphasis has been on yield and growers tend to select accessions that are high-yielding in their area. However, the high-yielding accessions may not have the highest content of bioactive components, and in fact, the opposite may be true. A study that collected and tested growing several accessions of *Centella* from different parts of Thailand and quantified the asiaticoside content in each by Piriyapatarakij (2008) found that the 4 accessions that contained the most asiaticoside were Nakhonsrithammarat, Prachinburi, Rayong, and Ubon Ratchathani. When grown in the hot season, Nakhonsrithammarat and Ubonratchathani accessions contained the most asiaticoside, and when grown in the rainy season, Rayong and Ubonratchathani contained the most asiaticoside.

Elicitors are substances that stimulate plants' defense mechanisms by stressing them and thereby cause them to synthesize more secondary metabolites (Yodyothi, 2014; Kundu et al., 2016). There have been several studies about the use of different kinds of elicitors to increase secondary metabolites in *Centella*. For example, exposure to 100 µM salicylic acid for 10 days stimulated the expression of *CabAS* (the gene responsible for triterpenoid synthesis) in *Centella* cells by three times compared to control (Loc et al., 2016). It was also reported that the use of methyl jasmonate (MJ) and yeast extract as elicitors could stimulate asiaticoside production by 1.5 and 1.4 times (Kim et al., 2004). You can see that besides choosing the most appropriate accession of *Centella* and cultivating it in the proper season, the use of elicitors is another way to maximize the accumulation of important secondary metabolites in *Centella*. This can be a way to produce high-quality raw materials for the herbal medication and cosmetics industries.

2 Research Problem & Research Objective

For the production of raw materials for herbal products, it is important to have both high yields and good quality. That means you should choose a variety of accession of plants that is suitable for the growing area, use appropriate production technology to maintain safety and achieve high harvest volume per unit area, and also find ways to make sure the plants contain high volumes of active substances that are desired. This research is about using safe and easily available elicitors on different accessions of *Centella* in order to recommend a production system that can supply high-quality raw materials for the production of health and beauty products.

This study emphasizes the results of 3 kinds of elicitors on the yield and secondary metabolite content of 5 accessions of *Centella Asiatica*.

3 Materials and Methods

The experiment was performed from June to August 2021 at the shade net house test field of the Thailand Institute of Scientific and Technological Research (TISTR) in Khlong Ha Sub-district, Khlong Luang District, Pathum Thani Province. The experiment was a 5 x 4 factorial experiment in CRD with 3 repetitions. The factors were factor a: *Centella* accession, consisting of Nakhonsrithammarat (NS), Prachinburi (P), Rayong (R), Nakhonpathom (NP) and Ubonratchathani (U), and factor b: elicitor type and concentration, consisting of no elicitor or control (C), salicylic acid (SA), yeast extract (YE) and malt extract (ME) at the concentrations of 1 mg, 1 g and 10 g per liter of water.

Planting vessels measuring 20 x 25 x 15 cm. were filled with a 1:1 mixture of peat moss and coarse sand. Four *Centella* stolon sections were planted in each vessel and the vessels were arranged in the shade net house. Basic compound formula chemical fertilizer (16-16-16) was applied at the rate of 1 g per vessel each week. The planting vessels were watered once a day. Thirty days after planting, the elicitor formulas were applied by spraying to totally cover the above-ground parts of the plants. This was followed 7 days later by second spraying. Five days after the second elicitor application, plant growth data were collected by measuring the leaf width, leaf length, number of leaves in each vessel, and total leaf area per vessel. Seven days after the second elicitor application, the entire above-ground parts of the plants were harvested by cutting the stems about 1 cm above the soil surface. Then the plant's fresh weight and dry weight per vessel were measured. The dried plants were ground to powder and subject to chemical distillation using a column separation method modified from Dorni et al. (2017). The amounts of asiaticoside (AS) and madecassoside (MD) were analyzed by HPLC. An HPLC column reverse phased C18 method was used with the mobile phase consisting of methanol:acetonitrile:H_2O (1:1:1) at the rate of 1 ml/minute. The wavelength of 210 nm was used to detect the amount of AS and MD in each sample.

4 Results

4.1 *Centella* Growth Rate and Yield

4.1.1 High-Yield and Fastest-Growing Accessions

Ubon Ratchathani accession had large leaves and the largest leaf area per vessel at 41.12 cm^2. The second largest was Nakhonpathom accession, followed by Nakhonsrithammarat, Rayong and Prachinburi, in that order. Rayong produced more leaves than other accessions, with an average of 93.5 leaves per vessel.

Data on plant yield showed that at harvest, Nakhonpathom accession had the highest fresh weight accumulation at 136.16 g per vessel, followed by Ubonratchathani and Nakhonsrithammarat at 127.99 g and 126.97 g per vessel, while Rayong averaged 119.20 g per vessel, and Prachinburi had the lowest fresh weight at 101.04 g per vessel. This was also reflected in the data for average dry weight per vessel, where Nakhonpathom and Ubonratchathani had the highest dry weight, ranging from 10.3-10.8 g per vessel, while all the other accessions had an average dry weight of less than 10

g per vessel. Prachinburi accession had the lowest plant dry weight. You can see that Nakhonpathom's average fresh and dry weights were 1.3 and 1.8 times those of Prachinburi, which was the lowest performing in terms of yield (Table 1).

4.1.2 Elicitor Effects on *Centella* Growth and Yield

The application of elicitors had an effect on *Centella* leaf generation. Spray application of SA solution at a concentration of 1 mg/L for 2 times, 7 days apart, resulted in the greatest number of leaves when *Centella* was harvested 7 days after the second spray application. The plants in this treatment group had the most leaves per unit area at 86 leaves/500 cm^2, which was 1.2 times more than the control plants (Table 1).

4.1.3 Combined Influence of Accession and Elicitor on the Growth and Yield Per Unit Area of *Centella*

Comparing all the accessions tested, Nakhonpathom had the overall best growth and yield. The application of elicitors tended to have no effect or in some cases tended to restrict the growth of *Centella,* with the exception of SA at the concentration of 1 mg/L, which resulted in a slight (0.1 times) increase in leaf area in Nakhonpathom accession. However, when applied at the higher concentration of 1 g/L, almost all the elicitors, except for ME, resulted in a decrease of fresh weight by 0.03-0.18 times. Similarly, in Rayong and Nakhonsrithammarat we recorded only an increase in the number of leaves or leaf areas when elicitors were applied.

In Ubon Ratchathani accession, the application of YE at the concentration of 1 g/L resulted in increased growth and 0.12 times greater yield. In Prachinburi, which was the lowest-yielding accession overall, the use of all 3 kinds of elicitors helped increase growth and yield about 0.08-0.24 times. SA at a concentration of 1 mg/L had the greatest positive effect on the growth and yield of Prachinburi (Tables 1 and 2).

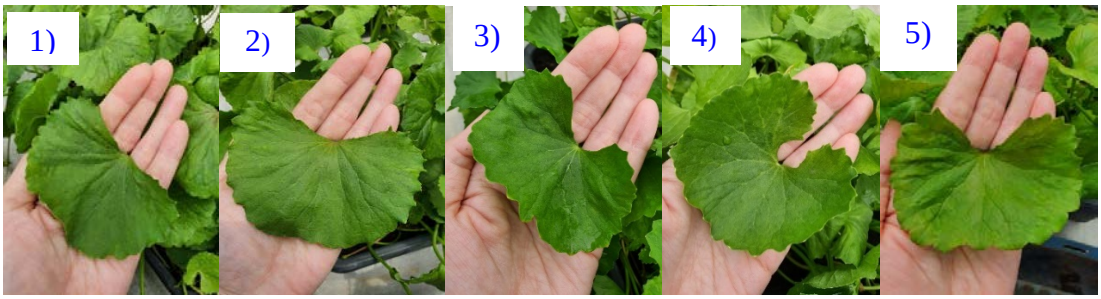

Figure 1: Fully grown leaves of the tested accessions of *Centella Asiatica* (1) Rayong (2) Ubonratchathani (3) Prachinburi (4) Nakhonsrithammarat and (5) Nakhonpathom.

Table 1: Total leaf area, number of leaves, fresh weight, dry weight, and amount of bioactive compounds madecassoside and asiaticoside in 5 accessions of *Centella Asiatica* grown with 3 different kinds of elicitors applied (and control)

Treatment	Per 500 cm^2 growing area				MD content (mg/g dry weight)	AS content (mg/g dry weight)
	Total leaf area (cm^2)	Number of leaves	Fresh weight (g)	Dry weight (g)		
accession (a)						
a1 = NP	34.70^{b}	77.7^{b}	136.16^{a}	10.796^{a}	3.22^{ab}	2.40^{b}
a2 = P	22.74^{d}	78.2^{b}	101.04^{c}	5.945^{c}	3.45^{ab}	3.98^{a}
a3 = R	24.69^{d}	93.5^{a}	119.20^{b}	8.969^{ab}	4.00^{a}	3.60^{a}
a4 = U	41.12^{a}	68.0^{b}	127.99^{ab}	10.278^{a}	2.52^{b}	3.13^{ab}
a5 = NS	29.47^{c}	70.5^{b}	126.97^{ab}	7.936^{ab}	2.73^{b}	2.20^{b}
elicitor (b)						
b1 = no elicitor	30.06	72.7^{b}	123.89	9.160	2.81	2.74
b2 = ME 1 g/l	29.95	76.4^{ab}	119.29	8.513	2.89	2.95
b3 = SA 1 mg/l	31.84	86.3^{a}	126.80	9.641	3.55	3.27
b4 = YE 1 g/l	30.30	74.9^{b}	119.11	7.825	3.47	3.29
a1b1	34.57^{bcd}	71.3^{bcd}	142.77^{a}	12.033^{a}	2.25^{cd}	1.88efg
a1b2	33.72^{bcde}	79.3^{bcd}	142.62^{a}	11.583^{abc}	1.59^{d}	1.57fg
a1b3	37.96^{abc}	86.7^{bcd}	138.57^{ab}	11.690^{ab}	5.98^{a}	4.17ab
a1b4	32.47^{bcdefg}	73.3^{bcd}	120.70^{abcde}	7.877^{cdef}	3.06^{bcd}	2.00defg
a2b1	20.01^{i}	73.3^{bcd}	91.29^{e}	4.837^{f}	3.07^{bcd}	3.75bcde
a2b2	21.85^{hi}	78.0^{bcd}	98.21^{de}	6.183^{ef}	3.07^{bcd}	3.84bcd
a2b3	23.20^{hi}	90.7^{ab}	113.68^{abcde}	7.927^{bcdef}	2.46^{cd}	2.41bcdefg
a2b4	25.90^{fghi}	70.7^{bcd}	100.96^{cde}	4.833^{f}	5.15^{ab}	5.90a
a3b1	25.54^{ghi}	88.0^{bc}	128.81^{abcd}	10.273^{abcd}	4.09^{abc}	3.50bcde
a3b2	26.89^{efghi}	85.3^{bcd}	109.59^{abcde}	7.457^{def}	3.83^{abcd}	3.41bcdef
a3b3	23.23^{hi}	110.7^{a}	130.56^{abcd}	10.753^{abcd}	4.29^{abc}	3.91bc
a3b4	23.09^{hi}	90.0^{ab}	107.84^{bcde}	7.393^{def}	3.80^{abcd}	3.58bcde
a4b1	41.75^{a}	65.3^{d}	124.01^{abcde}	10.160^{abcd}	2.18^{cd}	2.27cdefg
a4b2	41.77^{a}	66.7^{cd}	123.45^{abcde}	9.730^{abcde}	2.47^{cd}	3.00bcdefg
a4b3	42.08^{a}	70.7^{bcd}	125.83^{abcd}	9.733^{abcde}	2.57^{cd}	3.73bcde
a4b4	38.87^{ab}	69.3^{bcd}	138.66^{ab}	11.490^{abc}	2.87^{bcd}	3.50bcde
a5b1	28.45^{defgh}	65.3^{d}	132.56^{abc}	8.497^{abcdef}	2.48^{cd}	2.30bcdefg
a5b2	25.51^{ghi}	72.7^{bcd}	122.56^{abcde}	7.613^{def}	3.52^{bcd}	2.91bcdefg
a5b3	32.73^{bcdef}	72.7^{bcd}	125.38^{abcd}	8.100^{bcdef}	2.45^{cd}	2.12cdefg
a5b4	31.17^{cdefg}	71.3^{bcd}	127.38^{abcd}	7.533^{def}	2.49^{cd}	1.46g
F-test (a x b)	*	*	*	*	*	*
C.V. (%)	14.18	17.49	16.65	26.18	43.28	37.30

NP= Nakhonpathom accession; P= Prachinburi accession; R= Rayong accession; U= Ubonratchathani accession; NS= Nakhonsrithammarat accession

ns = no statistically significant difference, * = different to a statistically significant degree at $P<=0.05$

Table 2: Comparison of the effects of different elicitors on growth and yield of the tested accessions of *Centella Asiatica* when grown in a shade net house during the rainy season in Pathum Thani Province (from data in Table 1)

accession	Leaf area				Number of leaves				Biomass accumulation				Dry weight				best
	C	M	S	Y	C	M	S	Y	C	M	S	Y	C	M	S	Y	
NP	-	↓	↑	↓	-	-	-	-	-	-	↓	↓	-	↓	↓	↓	C*
P	-	↑	↑	↑	-	-	↑	-	-	↑	↑	↑	-	↑	↑	-	S
R	-	↑	↓	↓	-	↓	↑	↑	-	↓	-	↓	-	↓	-	↓	C
U	-	-	-	-	-	↑	↑	↑	-	-	↑	↑	-	↓	↓	↑	Y
NS	-	↓	↑	↑	-	↑	↑	↑	-	↓	↓	↓	-	↓	↓	↓	C

C = control (no elicitor); M= malt extract; S= salicylic acid; Y= yeast extract

- no statistically significant difference from control; * highest yield

↑ more than control; ↑ greatest increase; ↓ less than control; ↓ least compared to control

4.2 Bioactive Compound Accumulation

4.2.1 Accessions with High Levels of Bioactive Compounds

Rayong was the accession that had the highest levels of both bioactive compounds tested for - madecassoside (MD) and asiaticoside (AS) - at 4.00 and 3.60 mg/g of dry weight. The second highest producer was Prachinburi accession at 3.45 and 3.98 mg/g of dry weight. The amounts of MD and AS detected in Nakhonpathom accession and Ubonratchathani accession were 3.22 and 2.40 mg/g dry weight and 2.52 and 3.13 mg/g of dry weight, respectively, while the lowest amount of bioactive compounds (2.73 and 2.20 mg/g of dry weight) were found in Nakhonsrithammarat accession (Table 1).

4.2.2 Effect of Elicitors on the Accumulation of Bioactive Compounds

Application of all the elicitors that we tested resulted in greater accumulation of both MD and AS, with average levels of 2.89-3.55 mg/g dry weight for MD and 2.95-3.29 mg/g dry weight for AS. Increases in bioactive compounds tended to be the highest when SA was applied, but there was no statistically significant difference compared to the control (Table 1).

4.2.3 Combined Influence of Accession and Elicitor on Bioactive Compound Accumulation

Of the 5 accessions and 3 elicitors we tested, Nakhonpathom contained the most MD (5.98 mg/g dry weight) when treated with salicylic acid (SA) and Prachinburi contained the most AS (5.90 mg/g dry weight) when treated with yeast extract (YE).

Looking at each accession, Nakhonpathom responded the best when sprayed with SA, producing 5.98 mg/g dry weight MD and 4.17 mg/g dry weight AS, while Prachinburi, Rayong, Ubonratchathani and Nakornsrithammarat contained the most bioactive compounds when sprayed with YE, SA, YE and ME, respectively (5.15 mg/g dry weight MD and 5.90 mg/g dry weight SA, 4.29 mg/g dry weight MD and 3.91 mg/g dry weight SA, 2.57 mg/g dry weight MD and 3.73 mg/g dry weight SA, and 3.52 mg/g dry weight MD and 2.91 mg/g dry weight SA, respectively) (Tables 1 and 3).

Table 3: Comparison of the effect of different elicitors on the accumulation of asiaticoside and madecassoside in 5 different accessions of *Centella Asiatica* grown in a shade net house during the rainy season in Pathum Thani Province (from data in Table 1)

Accession	madecassoside				asiaticoside				recommendation	
	C	M	S	Y	C	M	S	Y	MD	AS
NP	-	↓	⇑	↑	-	↓	⇑	↑	S*	S
P	-	-	↓	↑	-	-	↓	↑	Y	Y*
R	-	↓	-	↓	-	↓	↑	-	C/S	S
U	-	-	-	↑	-	↑	⇑	⇑	Y	S/Y
NS	-	↑	-	-	-	-	↓	⇓	M	C/M

C= control (no elicitor); M= malt extract; S= salicylic acid; Y= yeast extract; MD= madecassoside; AS= asiaticoside
- no statistically significant difference from control; * highest amount of bioactive compound
↑ more than control; ⇑ the most; ↓ less than control; ⇓ the least compared to control

5 Discussion

5.1 Influence of Single Factors and Combined Factors on Fresh Weight Yield

Considering only the fresh weight of the above-soil portion of *Centella* plants grown in the rainy season (June – August) in Khlong Luang District, Pathum Thani Province, Nakhonpathom accession had the highest fresh weight per unit area, followed by Ubonratchathani and Nakhonsrithammarat accessions, which had about 6-7% less fresh weight than Nakhonpathom accession.

Our results are different from those of the Chachoengsao Agricultural Occupation Promotion and Development Center which tested Rayong, Chachoengsao, Nakhonpathom, Nongbualampoo and Nakhonsrithammarat accessions in Phanomsarakham District, Chachoengsao Province. They reported that Nongbualampoo and Rayong accessions were the most suitable for growing in their area. This may be related to the environmental, climatic and soil conditions as well as different fertilizer applications) Khumkan, S. 2019(. When Jai-aye)2013) tested growing 8 accessions in a shade net house with 60% shading, the highest yield was obtained from Trat accession, followed by Chiangrai, Phayao, Phetburi, Nakhonpathom, Ratchaburi, Rayong and Chantaburi accessions, in that order.

5.1.1 Influence of Elicitors

For general *Centella* cultivation when growers are not selecting a specific accession for fresh harvest, it is not necessary to use any elicitor because none of the elicitors had a significant effect on the fresh weight of the above-ground portion of *Centella* in this experiment.

5.1.2 Influence of Accession Combined with Elicitor

For the greatest fresh weight harvest per unit area, Nakhonpathom accession is recommended and there is no need to use an elicitor. Alternatively, if you grow Ubonratchathani accession and spray ME at the concentration of 1 g/L two times, 7 days apart, and wait 7 days after the second spraying to harvest, you can expect an approximately 12% increase in fresh weight. This is in agreement with the report of Kundu et al. (2016) who found that adding 1 mg/L of ME along with 6-benzyl amino purine (BAP) to the nutrient medium resulted in the greatest number of leaves when 5 accessions of *Centella Asiatica* were raised in tissue culture, and the overall growth was 2-4 times greater when compared to adding SA or JA (jasmonic acid) together with BAP to the medium.

5.2 Influence of Single Factors and Combined Factors on Bioactive Compound Content

The most important secondary metabolites in *Cerntella* for making herbal medicine and cosmetics products are madecassoside (MD) and asiaticoside (AS). MD is the most prevalent triterpene found in *Centella* extract. It is used to delay skin aging and promote wound healing as well as fight infection and it has anti-psoriasis effects because of its lipid oxidation prevention

properties and its ability to promote collagen synthesis and stimulate blood vessel formation)Tan et al., 2021). Another of the important phytochemicals found in *Centella,* AS, has antioxidant properties that make it quite effective in reducing the effects of harmful UV-A radiation that causes cell degradation. AS works together with MD and Asiatic acid to stimulate the formation of type 1 collagen, the most common type of collagen in the human body that promotes flexibility and is key in wound healing) Kwon et al., 2014).

5.2.1 Influence of Accession

Our results showed that the different accessions of *Centella* produced different amounts of phytochemicals. The highest content of MD and AS were found in Rayong accession, followed by Prachinburi, Nakhonpathom, Ubonratchathani and Nakhonsritthammarat, in that order. The average amounts of MD and AS found in Rayong were 32% and 38% higher than in Nakhonsrithammarat. This is consistent with the work of Jai-aye (2013), who reported that the Rayong accession contained more asiaticoside compared to the other accessions studied.

5.2.2 Influence of Elicitors

The application of elicitors tends to promote the accumulation of bioactive compounds in *Centella*. In our experiment, SA at the concentration of 1 mg/L had the greatest effect. This is similar to the findings of Loc et al. (2017) who found that treatment with SA 100 µM helped stimulate madecassoside synthesis in *Centella* cells.

5.2.3 Combined Influence of Accession and Elicitor

When elicitors were sprayed on the different *Centella* accessions to stimulate a greater accumulation of bioactive compounds, Nakhonpathom accession had the greatest MD and AS content (5.98 and 4.17 mg/g dry weight(when treated with SA. The second greatest secondary metabolite content was observed in Prachinburi accession at 5.15 and 5.90 mg/n dry weight when treated with YE. These figures were 17-28% and 6-34% greater than the bioactive compound content measurements from Rayong accession when treated with SA. Before this, the use of SA as a chemical elicitor and YE as an abiotic elicitor to stimulate the production of centellosides (madecassoside and asiaticoside) in *Centella* has been previously reported by several researchers including Kim et al. (2014), Loc and Giang (2012), Giang et al. (2016) and Loc et al. (2017).

We can conclude that to harvest the most of the 2 bioactive compounds of interest (MD and AS) when growing *Centella* in Pathum Thani Province, or other parts of central Thailand, growers should choose Nakhonpathom accession, because it can yield higher fresh weight and dry weight per unit area than Prachinburi accession when treated with SA spray at the concentration of 1 mg/L. Two applications should be done, spaced one week apart, and the *Centella* can be harvested one week after the second spraying.

Additional studies should be done using varying concentrations and durations of elicitor application, and growing the plants at different times of the year.

6 Availability of Data and Material

Data can be made available by contacting the corresponding author.

7 References

Stockhausen L, Turale S. An explorative study of Australian nursing scholars and contemporary scholarship. J Nurs Scholarsh [Internet]. 2013 Mar [cited 2019 Feb 19];43(1):89-96.

Tongekkaew, J. Centella: a very useful herb. Ubon Ratchathani University Journal of Science and Technology. 2013;15(3): 70-75. (in Thai)

Mingmuang, J., Cheunnangchi, W., Sakpetch, A., Niamsakul, S. and Ontong, S.,. Development of a method of triterpene analysis in *Centella* using UPLC. Thai and Alternative Medicine Journal. 2020;18(2): 270-286. (in Thai)

Jai-aye, P. Research and development of Centella production technology. Research report, Department of Agriculture. 2013. 29 p. (in Thai)

Yodyothi, Y. Induction of disease resistance and expression of *PR-1* gene expression in *Hevea brasiliensis* using different elicitors. Master's degree thesis. Prince of Songkhla University. 2014. (in Thai)

Khumkan, S. Research on 5 centella accessions to promote commercial cultivation. Kom Chad Leuk newspaper, online version of 29 July, 2019 (general news column) 2019. www.komchadluek.net/news/381404 (in Thai)

Piriyapatarakij, A. *Cited in* Jai-aye, P. 2013 Research and development of Centella production technology. Research report, Department of Agriculture. 2008. 29 p. (in Thai)

Panya, I. Development of a serum from centella extract. Master's Degree thesis. Srinakharinwirot University. 2018. (in Thai)

Dorni, A.I.C., Peter, G., Jude, S., Arundhathy, C.A., Jacob, J., Amalraj, A., Pius, A., and Gopi, S. UPLC-Q-ToF-MS-guided enrichment and purification of triterpenoids from *Centella asistica* (L.) extract with microporous resin. Journal of Liquid Chromatography & Related Technologies. 2017;40(1): 13-25.

Kim O.T., Kim M.Y., Hong M.H., Ahn J.C., and Hwang, B. Stimulation of asiaticoside accumulation in the whole plant cultures of *Centella Asiatica* (L.) urban by elicitors. Plant Cell Rep. 2014;23(5):339-44.

Kundu, K., Roy, A., Saxena, G., Kumar, L., and Bharadvaja, N. Effect of different carbon sources and elicitors on shoot multiplication in accessions of C*entella asistica*. Medical & Aromatic Plants. 2016;5(4). DOI: 10.4172/2167-0412.1000251.

Kwon, K.J., Bae, S., Kim, K., An, I.S., Ahn, K.J., An, S., and Cha, H.J. Asiaticoside, a component of *Centella Asiatica*, inhibits melanogenesis in B16F10 mouse melanoma. Molecular Medicine Reports. 2014;10: 503-507.

Loc, N.H. and Giang, N.T. Effects of elicitors on the enhancement of asiaticoside biosynthesis in cell cultures of centella (*Centella Asiatica* L. Urban). Chemical Papers. 2012;66: 642-648.

Loc, N.H., Giang, N.T., and Huy, N.D. Effect of salicylic acid on expression level of genes related with isoprenoid pathway in centella (*Centella Asiatica* (L.) Urban) cells. 3 Biotech. 2016;6:86-93.

Loc, N.H., Giang, N.T., Huy, N.D., and Lan, T.T.P. Accumulation of madecassoside- a major component of centelloside- in centella (*Centella Asiatica* (L.) Urban) cells elicited by salicylic acid. Periodicum Biologorum. 2017;119(1): 81-85.

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