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Andrographolide production in *in vitro* cultures of *Andrographis paniculata* (Burm.f.) NeesDuangporn Premjet^{1,2}, Anupan Kongbangkerd^{1,3} and Siripong Premjet^{1,3,*}¹Center of Excellence in Research for Agricultural Biotechnology, Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Phitsanulok, Thailand²Department of Agricultural Science, Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Phitsanulok, Thailand³Department of Biology, Faculty of Science, Naresuan University, Phitsanulok, Thailand

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Abstract

Fah Talai Jone, *Andrographis paniculata* (Burm.f.) Nees is a Thai medicinal plant that has been used for Covid-19 treatment as alternative medicine. Pharmacologically active diterpene lactones such as andrographolide accumulate in its leaves. This study investigated the combinatorial effects of 1-naphthaleneacetic acid (NAA) at 0, 0.5 and 1.0 mg/L and 6-benzyladenine (6-BA) (at 0, 5, and 10 mg/L) on the development and accumulation of andrographolide in *A. paniculata in vitro* using Murashige and Skoog, 1962 (MS) semi-solid medium. Results showed that lateral bud cultures had a survival rate of 33 to 73%, with good callus development when cultured on MS medium containing BA 10 mg/L and MS added with BA 10 mg/L and NAA 1.0 mg/L. Seeds germinated *in vitro* had a 100% survival rate and developed into mature plants with leaves and roots of 5-10 cm after 30 days of cultivation. Plantlets grown *in vitro* contained andrographolide at 1.14 % of dry weight, while plants grown in soil for 120 days had andrographolide content at 1.5% dry weight. However, andrographolide was not detected in the callus of treatments that contained BA, NAA alone or in combination. The *in vitro* plantlets had leaves and roots with lengths of 5-10 cm within the 30-day culture period and accumulated andrographolide at 1.14% dry weight.

Keywords: *Andrographis paniculata* (Burm.f.) Nees, Covid-19, 1-Naphthaleneacetic acid (NAA), 6-Benzyladenine (6-BA), Andrographolide**1. Introduction**

Andrographis paniculata (Burm.f.) Nees is a member of the family Acanthaceae. Historically, the plant has been used as a traditional medicine in China, Malaysia, India, Pakistan, Indonesia, the Philippines, and Thailand [1]. Other local plant names in Thailand include Fah Talai Jone, Anti-snake grass, Nam Lai Phang Phon, Mek Talai, and Fah Sathan. In Thai traditional medicine, Fah Talai Jone is classified as a bitter herb and used to treat colds and relieve flu symptoms, cough and sore throat. The herb is included in the National List of Essential Drugs, 1999 (Herbal Drug List), Ministry of Public Health [2]. During the outbreak of Covid-19 in Thailand, *A. paniculata* was considered to strengthen the immune system against viruses causing respiratory infections because of its andrographolide content [3]. Demand for *A. paniculata* has recently increased but output remains low. The planting area is around 138 rai in Nakhon Pathom, Ratchaburi and Lampang Provinces with production capacity of only 188,000 kg/year. The upstream production chain is the agricultural sector, the middle is a processing plant and the downstream is the pharmaceutical and hospital businesses. Recently, new research methods have developed high-volume, stable plant production systems through plant tissue culture giving good quality, disease-free materials for commercial production of plant pharmaceutical products. *A. paniculata* tissue cultures have been successful in India, Bangladesh, Malaysia, and Thailand [4-7]. N6-Benzyladenine (BA) and 2,4-dichlorophenoxy acetic acid (2,4-D) at low concentration promoted callus stimulation on leaves and young stem explants [7]. Benzylaminopurine (BAP) was the most effective in shoot formation from nodal explants when compared to kinetin,

thidiazuron, and 2-isopentyl adenine. Methods for culturing *A. paniculata* have been reported [8,9] but the current knowledge base is minimal for the Thai variety of *A. paniculata*. Therefore, this research studied the effects of plant growth regulators on seedling and callus formation of *A. paniculata* grown *in vitro*. Andrographolide content in this medicinal plant was also determined both *in vivo* and *in vitro*.

2. Materials and methods

2.1 Plant materials

A. paniculata seeds were collected from Phitsanulok Province. One gram of the seed was soaked in water for 6 h before sowing in peat moss. Seedlings 25-30 days old showing two true leaves were transferred into mixed soil consisting of potting soil, manure, and rice husks at ratio 1:2:2. The plants were watered daily and supplemented with 333 kg/ha of 15-15-15 chemical fertilizer twice a week. Mature plants in the greenhouse were harvested at 120 days for andrographolide analysis before the flowering stage (Figure 1A). Lateral buds cut from the second to third node of 80–90-day-old mature plants were also used as initial explants for *in vitro* cultures. The specimen was deposited at the herbarium of the Biology Department, Faculty of Science, Naresuan University Phitsanulok - Naresuan University (PNU code number 05934).

2.2 *In vitro* culture experiment

2.2.1 Seed culture

A. paniculata seeds were soaked in sterile distilled water and placed on a clean bench for 6 h. Surface sterilization was performed by soaking the seeds in 95% ethanol for 1 min, followed by immersion in sterile distilled water for 10 min. This process was repeated three times. The seeds were then placed on semi-solid Murashige and Skoog, 1962 (MS) medium containing various concentrations of BA and 1-naphthylacetic acid (NAA) (Table 1, Figure 1B) for 30 days and the results were observed and recorded. This process was conducted under a biosafety cabinet.

2.2.2 Lateral bud culture

Lateral buds collected from the greenhouse were removed from the second to third node under the shoot tip and then cut into 1.0 cm lengths. The explants were serially surface sterilized with 10, 15, 20 and 25% Clorox solution (containing 6% of active sodium hypochlorite), respectively, for 5 min at each concentration. The lateral bud explants were then moved into a sterile biosafety cabinet and rinsed with sterile distilled water three times for 5 min each time. The explants were then forced to absorb excess water and placed on top of the media at 1 piece of explant per bottle (Figure 1C). All experiments were performed with five replications of each treatment. Subcultures were performed every month, with results observed for 90 days.

2.2.3 Media preparation

Semi-solid MS (1962) medium was used for the *in vitro* culture experiments. Benzyladenine (BA) at concentrations 0, 5, and 10 mg/L was applied as a cytokinin, while NAA at concentrations 0, 0.5, and 1.0 mg/L was applied as an auxin. All nine treatments of the Murashige and Skoog, 1962 (MS) medium supplemented with combinations of different concentrations of BA and NAA are shown in Table 1.

2.2.4 Culture room condition

All the culture bottles were placed in a plant tissue culture room at $25 \pm 1^\circ\text{C}$ and humidity 80% Relative humidity (RH), illuminated by a warm white LED lamp at light intensity of the $40 \mu\text{mol}/\text{m}^2/\text{sec}$ and lighting duration 12 h/d.

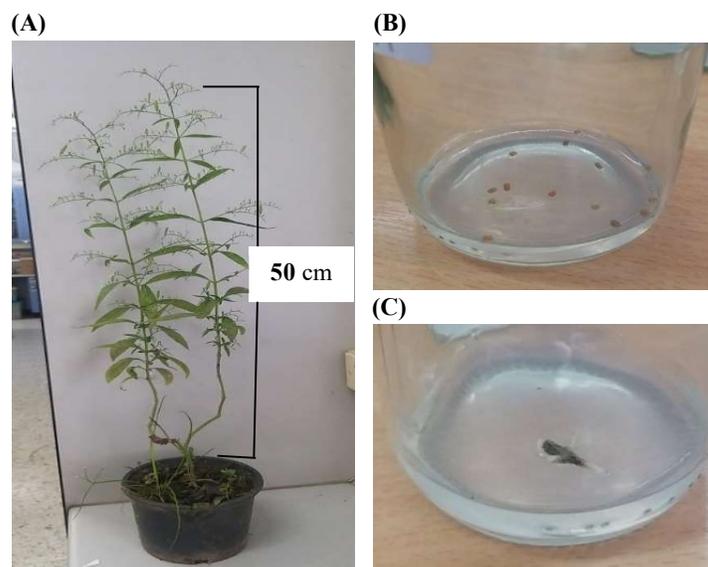


Figure 1 (A) *A. paniculata* 120 days-old plant grown in 6 inches pot, (B) initiation of seed cultures, and (C) initiation of lateral bud cultures in 4 oz glass bottles.

Table 1 *In vitro* culture experimental treatments.

Treatment	Cytokinin / Auxin
1	MS (control)
2	MS + NAA 0.5 mg/L
3	MS + NAA 1.0 mg/L
4	MS + BA 5 mg/L
5	MS + BA 5 mg/L + NAA 0.5 mg/L
6	MS + BA 5 mg/L + NAA 1.0 mg/L
7	MS + BA 10 mg/L
8	MS + BA 10 mg/L + NAA 0.5 mg/L
9	MS + BA 10 mg/L + NAA 1.0 mg/L

2.3 Fresh weight and dry weight determination

2.3.1 Fresh weight and dry weight of *A. paniculata* plants grown in the greenhouse

The above-ground parts of *A. paniculata* plants were harvested at 120 days. For dry weight determination, two replications of 1 g of fresh samples were weighed into a weighing bottle, dried at 105°C for 24 h until the weight stabilized and then stored in a desiccator.

2.3.2 Fresh weight and dry weight of *A. paniculata* *in vitro* cultures

For dry weight determination, two replications of 1 g of callus or seedling samples were weighed into a weighing bottle, dried at 105°C for 24 h until the weight stabilized and then stored in a desiccator.

2.4 Andrographolide analysis by High Performance Liquid Chromatography

Andrographolide analysis was performed and assayed using the modified method of Sharma et al., (2012) [10] with the following steps.

2.4.1 Sample preparation for extraction

Dry powder of *A. paniculata* obtained from both greenhouse cultivation and *in vitro* culture (0.5-1.0 g) was extracted with 10 ml of methanol in round bottom flasks and then refluxed in a water bath for 30 min. Each solution was filtered after cooling down. The powder was repeatedly refluxed 2-3 times to obtain a clear solution.

All methanol extracts were combined, filtered through filter paper with the final volume was adjusted to 10 ml and filtered through a 0.45 µm membrane before injection to HPLC column [10].

2.4.2 Standard andrographolide preparation

The reference standard solution of andrographolide was prepared by dissolving 10 mg andrographolide (98%, Aldrich) in 100 ml methanol to obtain a final concentration of 100 µg/ml.

2.4.3 High Performance Liquid Chromatography

The HPLC instrument used was a Shimadzu model LC 20 AD, Japan using an Inertsil ODS-3, C18 (250 mm x 4.6 mm, 5 µm) column (Brand GL Sciences Inc. Japan). An isocratic elution system was used for the separation step. The mobile phase comprised methanol 65: water 35 at flow rate 1.5 ml/min with a D2 lamp detector at 223 nm and 40°C oven temperature with 20 µl injection volume. Retention time of andrographolide was 3.7 ± 0.18 min. Andrographolide contents were calculated from linear regression of the peak area from three replicate sample injections.

2.5 Data recording and statistical analysis

Seedling development such as cotyledon, shoot, leaf, and root formation was observed and recorded every week for 90 days, with seed survival rate and seed germination percentage recorded for 30 days. Data were presented as mean \pm SE. Mean differences were analyzed by Analysis of Variance (ANOVA) and Duncan's Multiple Range Test at $p \leq 0.05$ using SPSS (IBM SPSS Statistics 20).

3. Results

3.1 *In vitro* cultures of *A. paniculata*

Two types of tissues were used in this research as seeds and lateral buds. Nine treatments of MS supplemented with various concentrations of BA and NAA were studied and compared with MS without hormones as the control treatment. Results showed that seeds germinated at 100% on the hormone-free medium after 30 days of incubation both in light and dark. In the light condition, average seedling length was 5-10 cm with 4-5 true leaves, whereas young and small germinated seedlings 0.5-1.0 cm in length and small cotyledons with no true first leaves were observed in seeds grown in the dark. The hypocotyl of seedlings grown in the dark were longer than those grown in the light (Figure 2 A-C). When BA or NAA were added to the medium, non-embryonic tissues in the seed developed into callus, while seedlings were rarely observed in all treatments containing BA and/or NAA both in light and dark condition.

Results for *in vitro* seeds cultured on medium supplemented with various BA and NAA concentrations for 30 days indicated that the highest percentage of seed germination and direct development into seedlings without callus induction was observed on medium added with 10 mg/L BA (60%) followed by medium added with 5 mg/L BA and 1.0 mg/L NAA (50%). No callus formation was observed on the control medium, and medium added with only BA. By contrast, complete and healthy callus were induced on medium added with only NAA followed by medium combination of BA and NAA, respectively (Table 2). Results revealed that the growth regulators, BA and NAA has improved seedling development but reduce seed germination to 40-50 % (Table 2). Highest growth as average callus diameter of 20.-2.5 cm, both in the dark and light regime was obtained when seeds were sowed on MS medium supplemented with 1.0 mg/L NAA. Callus cultured under darkness showed a dark brown to brownish color and most were friable, whereas mixed yellowish and greenish color of friable callus were observed in all explants cultured under trigger light (Figure 3A-D).

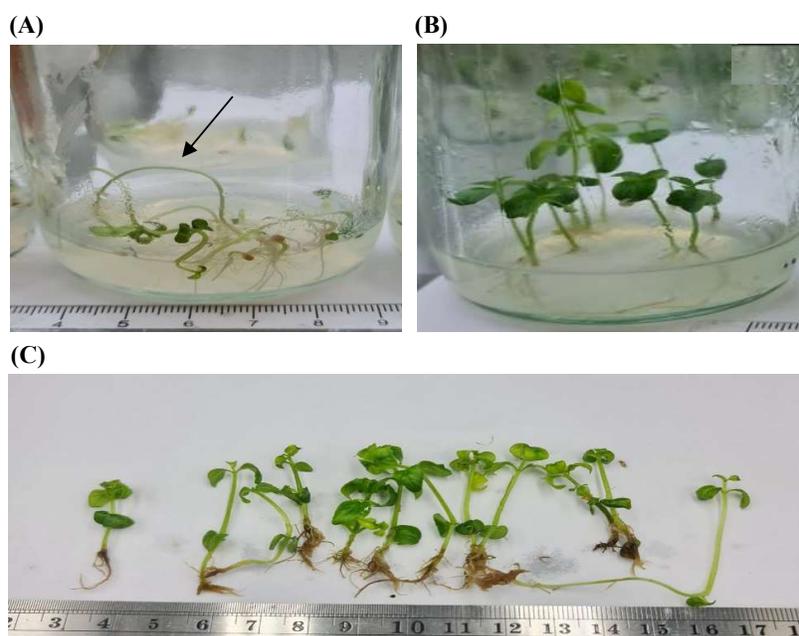


Figure 2 Morphological characteristics of *A. paniculata* germinated seeds cultured on semi-solid MS medium with no hormone and incubated under (A) dark and (B-C) light conditions showing the elongated hypocotyl of seedlings grown in the dark (arrow) for 30 days.

Table 2 Callus induction and germination rate of *A. paniculata* seeds cultured on medium augmented with different types and concentrations of BA and NAA and placed in the light and darkness for 30 days.

Treatment	Callus		Seedling formation (%)
	light	dark	
MS (control)	-	-	100
MS + NAA 0.5 mg/L	++	++++	-
MS + NAA 1.0 mg/L	+++++	++++	-
MS + BA 5 mg/L	-	-	-
MS + BA 5 mg/L + NAA 0.5 mg/L	+++	+	-
MS + BA 5 mg/L + NAA 1.0 mg/L	-	-	50
MS + BA 10 mg/L	-	-	60
MS + BA 10 mg/L + NAA 0.5 mg/L	+++	++	-
MS + BA 10 mg/L + NAA 1.0 mg/L	++++	++	-

Notes; + means diameter of callus ~ 0.5 cm, ++ means diameter callus ~ 1.0 cm, +++ means diameter callus ~ 1.5 cm, ++++ means diameter callus ~ 2.0 cm, and +++++ means diameter callus ~ 2.5 cm.

An *in vitro* node culture excised from the second to third node under the shoot tip of greenhouse mature plants was also studied. Lateral buds of the node were aseptically transferred to culture on semi-solid MS medium augmented with different concentrations of BA and NAA, and compared with MS without hormone used as the control treatment. Results showed that no new shoots sprouted from lateral buds in all medium treatments after 60 days of culture. Survival rate of lateral bud explants ranged between 9- and 73% (Table 3). Highest survival rate (73%) was observed on medium added with 1.0 mg/L NAA followed by explants cultured on medium augmented with 10 mg/L BA 0.5 mg/L NAA (33%). However, 100% of callus formation was observed on survived explants cultured on medium added with only NAA or a combination of NAA and BA, whereas medium supplemented with only BA and the control medium not only induced callus but also stimulated necrosis symptoms and eventually died. Under darkness, the largest size of callus (~2.0 cm in diameter) was obtained on medium supplemented with 0.5-1.0 mg/L NAA (Table 3 and Figure 3E). The callus was mostly friable with browning symptoms. Largest callus (~2.5 cm in diameter) among all treatments was obtained for explants cultured on medium added with 10 mg/L BA and 1.0 mg/L NAA and placed under light regime (Table 3 and Figure 3F).

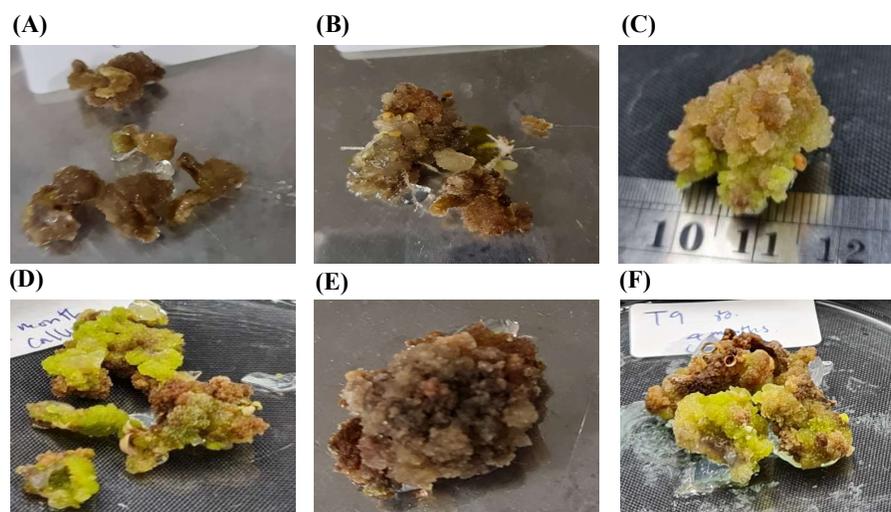


Figure 3 Morphological characteristics of *A. paniculata* callus derived from seeds cultured on semi-solid MS medium added with (A) 0.5 mg/L NAA and (B) 1.0 mg/L NAA under darkness and typical callus cultured on medium added with (C) 5.0 mg/L BA + 0.5 mg/L NAA and (D) 10.0 mg/L BA + 0.5 mg/L NAA under illumination for 30 days. Lateral buds induced (E) browning callus and (F) brownish green callus obtained from medium added with 1.0 mg/L NAA and 10.0 mg/L BA + 1.0 mg/L NAA after 90 days of culture.

Table 3 Callus induction from lateral buds of *A. paniculata* cultured on medium augmented with different and concentrations of BA and NAA and placed in light and darkness for 90 days.

Treatment	Explant survival rate (%)		Callus	
	light	dark	light	dark
MS (control)	-	-	-	-
MS + NAA 0.5 mg/L	17	11	+	++++
MS + NAA 1.0 mg/L	20	73	+	++++
MS + BA 5 mg/L	-	-	-	-
MS + BA 5 mg/L + NAA 0.5 mg/L	13	9	++	+
MS + BA 5 mg/L + NAA 1.0 mg/L	17	10	++	+
MS + BA 10 mg/L	-	-	-	-
MS + BA 10 mg/L + NAA 0.5 mg/L	33	33	++	++
MS + BA 10 mg/L + NAA 1.0 mg/L	17	17	++++	++

Notes; + means diameter of callus ~ 0.5 cm, ++ means diameter callus ~ 1.0 cm, +++ means diameter callus ~ 1.5 cm, ++++ means diameter callus ~ 2.0 cm, and +++++ means diameter callus ~ 2.5 cm.

3.2 Fresh weight and dry weight determination

Effects of auxin and cytokinin on fresh weight and dry weight of *in vitro* seedlings and callus formation were also investigated. Highest fresh weight of seedlings (4.10 ± 0.11 g) was observed on the hormone-free MS medium and classified as the best fresh weight yield group with significant differences from other treatments, followed by the second group of callus derived from lateral buds cultured on medium added with 10.0 mg/L BA and 0.5 mg/L NAA (2.96 ± 0.14 g), 10.0 mg/L BA and 0.5 mg/L NAA (2.95 ± 0.18 g), 0.5 mg/L NAA (2.86 ± 0.09 g) and 1.0 mg/L NAA (2.63 ± 0.15 g), respectively. Callus induced from lateral buds on medium added with 5 mg/L BA and 0.5 mg/L NAA gave lower fresh weight (2.33 ± 0.14 g), while medium supplemented with 0.5 mg/L NAA induced the lowest fresh weight (1.69 ± 0.08 g) of lateral buds derived callus under light regime. The highest dry weight of lateral bud-derived callus was found on medium containing 5 mg/L BA plus 0.5 mg/L NAA (0.72 ± 0.06 g) followed by explants cultured on 1.0 mg/L NAA (0.69 ± 0.04 g) with no significant difference, while lowest dry weight content from lateral bud-derived callus (0.02 ± 0.0 g) was obtained from medium added with 0.5 mg/L NAA (Table 4). The highest percentage of moisture from *in vitro* seedlings was obtained on medium with no hormone (91%) followed by *in vitro* seedlings cultured on medium added with 0.5 mg/L NAA (80%). For lateral bud-derived callus, highest moisture content was recorded on medium containing 0.5 mg/L NAA (99%), with lowest (69%) found on explants cultured on medium added with 5.0 mg/L BA and 0.5 mg/L NAA. Seedlings grown on hormone-free medium had very high moisture content and only 10% of the solid was obtained, whereas dry yield of all calluses was 1-31% of the remaining solid after drying.

Table 4 Growth of *A. paniculata* seedlings and callus cultured on different media showing fresh and dry weights.

Treatment		Fresh weight	Dry weight
Explant	Culture medium	(g)	(g)
Seedling	MS with no hormone (control)	4.10 ± 0.11 ^{a*}	0.38 ± 0.05 ^b
Seedling	MS + 0.5 mg/L NAA	2.86 ± 0.09 ^{bc}	0.34 ± 0.04 ^b
Callus	MS + 0.5 mg/L NAA	1.69 ± 0.08 ^d	0.02 ± 0.00 ^c
Callus	MS + 1.0 mg/L NAA	2.63 ± 0.15 ^b	0.69 ± 0.04 ^a
Callus	MS + 5 mg/L BA + 0.5 mg/L NAA	2.33 ± 0.14 ^c	0.72 ± 0.06 ^a
Callus	MS + 10 mg/L BA + 0.5 mg/L NAA	2.96 ± 0.14 ^b	0.35 ± 0.02 ^b
Callus	MS + 10 mg/L BA + 1.0 mg/L NAA	2.95 ± 0.18 ^b	0.33 ± 0.03 ^b

*Means with the same letter are not significantly different at $p \leq 0.05$ according to DMRT. All treatments were repeated five times, and each replicate consisted of five explants.

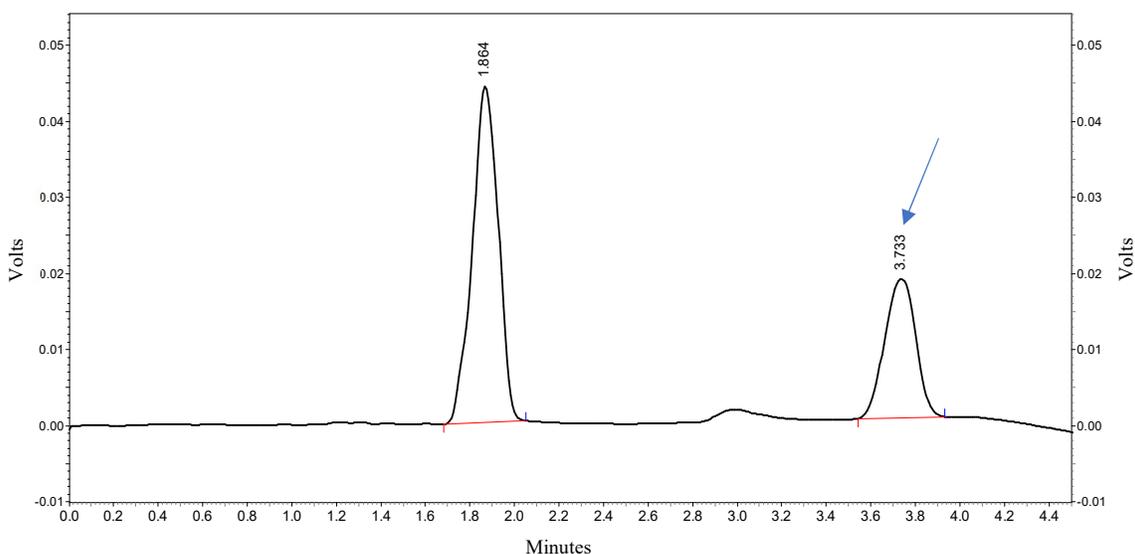
3.3 Andrographolide analysis

Andrographolide contents were analyzed in the aboveground portions of 120-day-old plants and 30-day-old seedlings and callus in *in vitro* cultures using the metabolite assay, as reported by Sharma et al. (2012). Authentic andrographolide was found to peak at 3.7 min (Figure 4), and this metabolite was detected in both 30-day-old above-ground samples and *in vitro* grown seedlings (Figure 5, 6). The andrographolide content of above-ground parts of *A. paniculata* was detected at 1.50% dry weight (Figure 5), while seedlings in the *in vitro* culture system contained 1.14% (Figure 6). However, analysis results of callus showed no andrographolide peak in all samples (Table 5).

Table 5 Andrographolide content in *A. paniculata* grown in soil, *in vitro* grown seedlings and lateral bud-derived callus analyzed by the HPLC system.

Treatment		Andrographolide	Andrographolide
Explant	Culture medium	(% dry weight)	(mg/g DW)
Aerial parts from mature plant	MS with no hormone (control)	1.50	14.59
<i>In vitro</i> seedlings	MS with no hormone (control)	1.14	11.43
	MS + 0.5 mg/L NAA	ND	-
	MS + 1.0 mg/L NAA	ND	-
Lateral bud-derived callus	MS + 5 mg/L BA + 0.5 mg/L NAA	ND	-
	MS + 10 mg/L BA + 0.5 mg/L NAA	ND	-
	MS + 10 mg/L BA + 1.0 mg/L NAA	ND	-

ND= not detected

**Figure 4** HPLC chromatogram of authentic andrographolide.

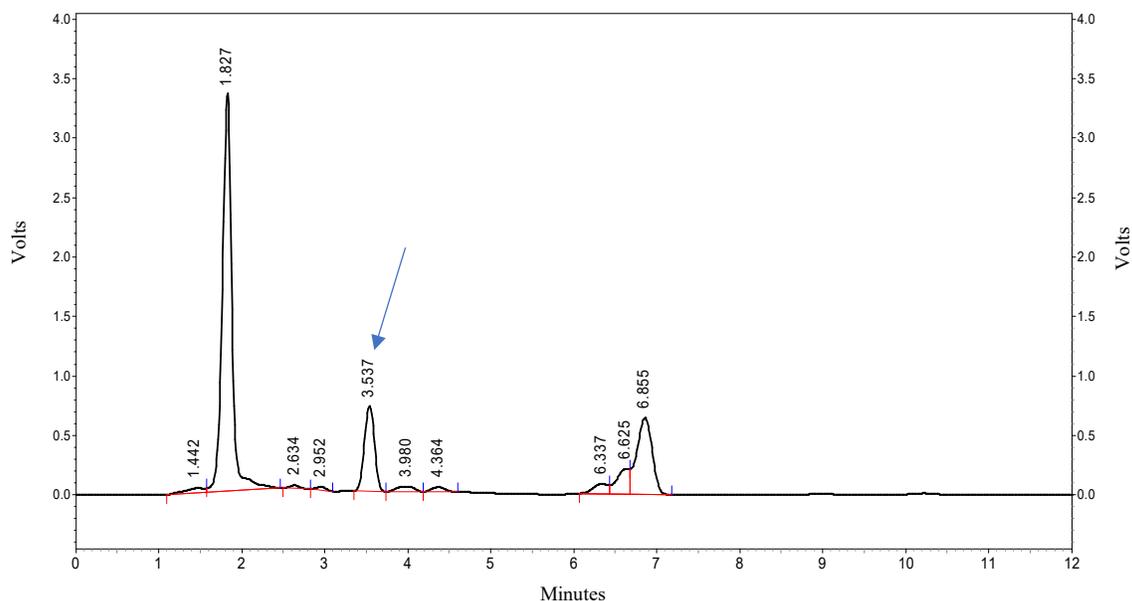


Figure 5 HPLC chromatogram of andrographolide obtained from aerial parts of *A. paniculata* grown in soil.

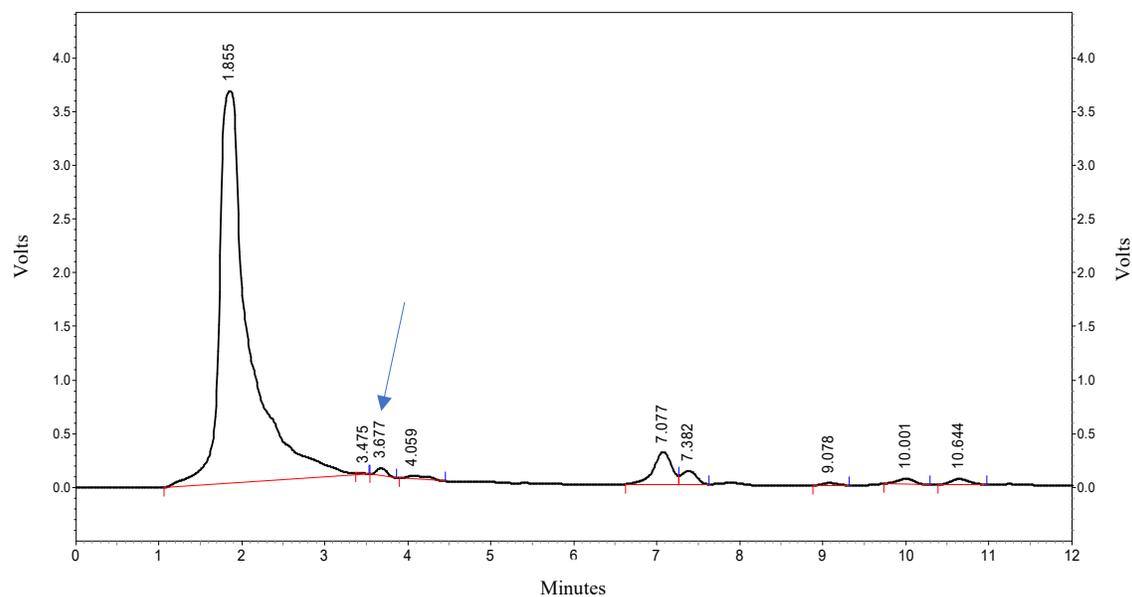


Figure 6 HPLC chromatogram of andrographolide obtained from seedlings grown *in vitro*.

4. Discussion

The *A. paniculata* tissue culture method showed that callus could be induced from lateral buds in MS added with 10 mg/L BA or MS combined with 10 mg/L BA and 1 mg/L NAA. This result concurred with Das & Bandyodnyay (2021) who found that explants from leaves, nodes and roots of *A. paniculata* cultured in MS medium containing BAP (1.0 mg/L) in combination with NAA (1.0 mg/L) were suitable for initiation of callus [2]. *A. paniculata* found in the Bababudan Hill ranges, Chikmagalur district, Karnataka, India was also subjected to sterile cultures, with nodal explants producing 14.60 shoots in MS medium containing 10 μ mol of 2-iP (2 mg/L) [4]. Sharmila et al. (2013) [5] reported that callus of *A. paniculata* induced from leaf tissues on MS medium. 0.5-1.0 mg/L produced andrographolite similar to the results of Kaewpiboon (2019) [7]. Plant varieties in each area were able to form callus at different combinations of auxin and cytokinin in media [11,12]. Zaheer et al., demonstrated that nodal explants formed strong roots in media containing NAA [13]. Explant types, and

phytohormones influenced the active compounds for diterpene lactone production in tissue cultures of cells, roots, leaves, and stems. Andrographolide and neoandrographolide were identified as the major therapeutic chemical agents, produced through the cytosolic mevalonate pathway and the alternative plastidial non-mevalonate pathway in plant especially in the leaves [14]. Leaves at the stage before flowering accumulated maximum andrographolide with leaves aged 18 weeks providing the best quality materials for the nutraceutical and pharmaceutical industries [15]. In this study, *A. paniculata* plantlets obtained in an *in vitro* system produced andrographolide at 1.14% dry weight lower amount than the intact plant (1.50% DW). The young age of the *in vitro* plantlets resulted in lower andrographolide production because the plant produces the active compound before flowering stage. A callus consists of parenchymatous cells. Callus cultures are a clump of tissues formed due to disorganized proliferation of the plant cells. Exogenous medium supplementation of auxin and cytokinin played an important role in callus cultures in both biomass and compound accumulating profiles. The absence of an andrographolide in methanolic extracts of callus cultures of *A. paniculata* possibly resulted from dedifferentiated parenchyma cells that did not obtain a suitable type of hormones to proliferate cell growth and produce andrographolide [16].

5. Conclusion

A. paniculata tissues were cultured using two types of starter tissues as lateral buds and seeds with nine formulations of BA- and NAA-added MS medium. The lateral bud explants developed into callus in MS medium augmented with 1.0 mg/L NAA, MS medium plus 10 mg/L BA, and MS added with 10 mg/L BA and 1.0 mg/L NAA. Callus was also induced from non-embryonic cells in seeds cultured in media containing BA and NAA. Complete 100% seed germination and developed seedlings were obtained within 30 days on MS medium without adding any hormones. Andrographolide was not found in the callus but was present in young plants in sterile systems at 1.14% dry weight, similar to plants grown in soil for 120 days. The 30-day-old *in vitro* seedlings produced andrographolide because the seedling at this stage were composed of true leaves as a major component of andrographolide synthesis.

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7. References

- [1] Hossain MS, Urbi Z, Sule A, Rahman KMH. *Andrographis paniculata* (Burm. f.) wall. ex Nees: A review of ethnobotany, phytochemistry, and pharmacology. *Sci World J.* 2014;2014:274905.
- [2] Faculty of Medicine Ramathibodi Hospital Mahidol University. Bioactivity of *Andrographis paniculata* (Burm. f.) wall. ex Nees [internet]. 2020 [cited 2020 Jun 19]. Available from: https://www.rama.mahidol.ac.th/altern_med/th/km/19jun2020-1729.
- [3] Sa-ngiamsuntorn K, Suksatu A, Pewkliang Y, Thongsri P, Kanjanasirirat P, Manopwisedjaroen S, et al. Anti-SARS-CoV-2 activity of *Andrographis paniculata* extract and its major component andrographolide in human lung epithelial cells and cytotoxicity evaluation in major organ cell representatives. *J Nat Prod.* 2021;84(4):1261-1270.
- [4] Das D, Bandyopadhyay M. Novel approaches towards over-production of andrographolide in *in vitro* seedling cultures of *Andrographis paniculata*. *S Afr J Bot.* 2020;128:77-86.
- [5] Sharmila R, Subburathinam KM, Sugumar P. Effect of growth regulators on andrographolide production in callus cultures of *Andrographis paniculata*. *Advanced BioTech.* 2013;12(9):17-19.
- [6] Kadapatti SS, Murthy HN. Micropropagation of *Andrographis producta* through axillary and adventitious shoot regeneration. *J Genet Eng Biotechnol.* 2022;20(1):152.
- [7] Kaewpiboon C, Boonnak N. Effect of plant growth regulators on diterpene lactone production in callus culture of *Andrographis paniculata*. *TSTJ.* 2019;28(10):1795-1801.
- [8] Prathanturug S, Schaffner W, Berger Büter K, editors. *In vitro* propagation of the Thai medicinal plant *Andrographis paniculata* Nees: Impact of different cytokinins. Proceedings of the third Asia-Pacific conference on agricultural biotechnology: Issues and choices, poster session, National Science and Technology Development Agency, Bangkok (Thailand) 2005; National Center for Genetic Engineering and Biotechnology. Bangkok (Thailand).
- [9] Purkayastha J, Sugla T, Paul A, Solleti S, Sahoo L. Rapid *in vitro* multiplication and plant regeneration from nodal explants of *Andrographis paniculata*: A valuable medicinal plant. *In Vitro Cell. Dev. Biol. - Plant.* 2008;44(5):442-447.
- [10] Sharma M, Sharma A, Tyagi S. Quantitative HPLC analysis of Andrographolide in *Andrographis paniculata* at two different stages of life cycle of plant. *Acta Chim Pharm Indica.* 2012;2(1):1-7.

- [11] Dandin VS, Murthy HN. Regeneration of *Andrographis paniculata* Nees: Analysis of genetic fidelity and andrographolide content in micropropagated plants. *Afr J Biotechnol.* 2012;11(61):12464-12471.
- [12] Scarpa GM, Prota V, Schianchi N, Manunta F. *In Vitro* Cultures for the production of secondary metabolites. *Secondary Metabolites.* Rijeka: IntechOpen; 2022.
- [13] Zaheer M, Giri CC. Enhanced diterpene lactone (andrographolide) production from elicited adventitious root cultures of *Andrographis paniculata*. *Res. Chem. Intermediat.* 2017;43(4):2433-2444.
- [14] Srivastava N, Akhila A. Biosynthesis of andrographolide in *Andrographis paniculata*. *Phytochemistry.* 2010;71(11-12):1298-1304.
- [15] Tajidin NE, Shaari K, Maulidiani M, Salleh NS, Ketaren BR, Mohamad M. Metabolite profiling of *Andrographis paniculata* (Burm. f.) Nees. young and mature leaves at different harvest ages using ¹H NMR-based metabolomics approach. *Sci Rep.* 2019;9(1):16766.
- [16] Jamil SZMR, Rohani ER, Baharum SN, Noor NM. Metabolite profiles of callus and cell suspension cultures of mangosteen. *3 Biotech.* 2018;8(8):322.