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Evaluation of Myo-inositol Influence on Andrographolide Production in *Andrographis paniculata*

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ABSTRACT
Andrographis paniculata is widely recognized for its medicinal properties, especially andrographolide. This secondary metabolite has the potential to be antimicrobial, anti-inflammatory, and antidiabetic. This study aimed to enhance secondary metabolite production through tissue culture techniques by incorporating Myo-inositol into media formulations. The experimental setup involved germinating *A. paniculata* seeds under controlled conditions at 15°C with continuous illumination. Two media formulations were conducted: one supplemented with Myo-inositol and the other as a control. After germination and growth, the leaf, stem, and root tissues were harvested and macerated with methanol. Andrographolide concentrations were quantified using High-Performance Liquid Chromatography (HPLC), and statistical analysis was conducted using an independent samples t-test. The results revealed that the leaves contained the highest andrographolide concentration among the tissues analyzed. The andrographolide content in media without Myo-inositol was 4.072% and 3.052% with Myo-inositol. However, supplementing Myo-inositol in the media did not result in a statistically significant increase in andrographolide concentration compared to the control. These findings suggest that while the leaf tissue is a valuable source of andrographolide, the role of Myo-inositol in enhancing its production requires further investigation.

Keywords: *Andrographis paniculata*; andrographolide; Myo-inositol; tissue culture; HPLC.

INTRODUCTION
Plants have long been recognized as valuable source of pharmaceutical compound and medicines. Their complex composition of bioactive compounds synergistically interacting to yield a greater overall effect in disease prevention and treatment (1). Plant derived molecules play a crucial role in the pharmaceutical and biotechnology industries, serving as key components in drug development, antioxidant, food additives, biofuels, and industrial applications. To maximize the potential and applicability of these biomolecules for commercialization, various plant biotechnological and genetic approaches have been employed (2).

The discovery of plant-derived natural products has attracted considerable scientific interest due to their potential pharmaceutical applications. Advances in screening methodologies and analytical techniques have facilitated the identification and optimization of bioactive compound. Additionally, synthetic modification strategies have enhanced the pharmacological properties of these natural leads, supporting their development into effective therapeutic agents (3).

Plant tissue culture provides a sustainable and controlled environment for producing secondary metabolites, independent of geographical and climatic limitations (4). This approach ensures a continuous supply of bioactive compounds to meet the growing demand in the pharmaceutical industry (5). Optimizing culture media formulations is essential to enhancing secondary metabolite yields. One strategy involves supplementing tissue

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culture media with Myo-inositol, which acts as a precursor for cell wall components and plays a crucial role in cell growth, division, and shoot regeneration (6).

Myo-inositol and its metabolic derivatives such as pinitol, galactinol, and raffinose contribute to plant growth and development and are also involved in stress adaptation. The supply of Myo-inositol has been shown to alleviate the detrimental effects of salt stress by maintaining osmotic balance and regulating ion homeostasis. Specifically, Myo-inositol reduces the Na^+/K^+ ratio in roots while increasing it in shoots, thereby enhancing plant resilience under saline conditions (7). Myo-inositol is synthesized from glucose-6-phosphate by key enzymes, including Myo-inositol monophosphatase (IMP) and Myo-inositol phosphate synthase (INPS). INPS functions as the rate-limiting enzyme and its upregulation enhances stress tolerance in response to drought, salinity, and cold stress (8,9).

Among medicinal plants, *Andrographis paniculata* (Burm. F) Ness, a member of the Acanthaceae family, is widely used in medicinal herb across China and Southest Asia (10). This plant is particularly valued for its secondary metabolite content, with andrographolide being the primary bioactive compound. Andrographolide is a main bioactive diterpene lactone exhibits various pharmacological activities such as antimicrobial, anti-inflammatory, and antidiabetic effects (11). Notably, *A. paniculata* demontrates significant antimicrobial activity, particularly against gram-positive bacteria such as *Staphylococcus aureus*, although its compound are ineffective against *Candida albicans* (4).

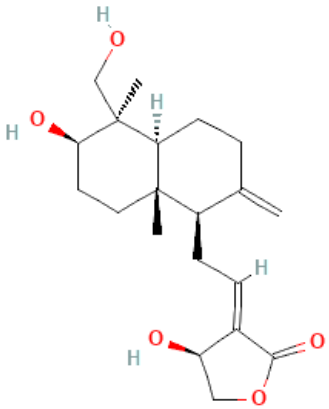

Figure 1. The 2D Chemical Structure of Andrographolide
PubChem: CID 5318517

Additionally, *A. paniculata* plays a role in glucose metabolism and utilization by enhancing insulin sensitivity, upregulating glycolytic enzymes and increasing GLUT4 protein expression. These properties suggest its potential in preventing insulin resistance and managing diabetes (12). Moreover, andrographolide possesses potent anti-inflammatory activity by inhibiting pro-inflammatory cytokines such as IL-6, TNF-α, and IFN-γ, which are key mediator in inflammatory disease and cytokine storms (13). Its antioxidant properties further contribute to liver health by reducing oxidative stress, a common factor in liver damage.

Given the pharmacological importance of *A. paniculata*, several efforts have been made to enhance its secondary metabolite content using plant tissue culture techniques. One such strategy involves the supplementation of Myo-inositol in tissue culture media to assess its potential role in enhancing andrographolide production. However, the impact of Myo-inositol on secondary metabolite biosynthesis in *A. paniculata* remains unclear. Therefore, this study aims to investigate the influence of Myo-inositol supplementation on andrographolide production by cultivating *A. paniculata* under controlled conditions and comparing media with and without myo-inositol. The findings are expected to provide insights into the role of

Myo-inositol in secondary metabolite biosynthesis and its potential application in large-scale pharmaceutical production.

METHODS

Tool

The equipment employed in this study included a Laminar Air Flow (Biosafety Cabinet ESCO Class II). A pH meter (Ohaus) was used to adjust the pH of the culture medium. An autoclave (GEA) was used for strerilizing media and instruments such as cultures bottles, surgical forceps, scalpels (No 11), beaker glass (pyrex), Erlenmeyer (pyrex), petri dish line with sterile filter paper (Whatman No 1), at pressure 15 atm and at 121^{0}C for 15 min prior to use. Microtubes (Eppendorf 1,5 mL) and tissue grinder pestle (AXVSTE) were used for extraction. Syringes (Microlab) and membrane filters (0,45-μm pore size;Millipore) were used for filtration of the mobile phase and the samples extracs. High-Performance Liquid Chromatography (HPLC) analysis was performed using an Agilent Technologies 1260 Infinity II system (Agilent, USA).

Materials

The materials used in this study included *Andrographis paniculata* seeds collected from Subang, West Java, Indonesia. Murashige and Skoog (MS) medium (HiMedia), supplemented with sucrose, Myo-inositol (HiMedia), agar (HiMedia). For seed surface sterilization, ethanol 70% and sodium hypochlorite were used, while sterile distilled water used for rinsing and medium preparation. Andrographolide Standar (TCI) was used for calibration curve preparation. Methanol HPLC grade (Merck) was used as an extraction solvent.

Detailed Procedure

Explant Surface Sterilization

The seed preparation involved soaking the seeds in water for 24 hours. Floating seeds were discarded, while the submerged seeds were subjected to surface sterilization and subsequently planted in the growth medium. The seeds were sterilized by treating them with 70% ethanol for 2 minutes and 5,25% sodium hypochlorite (NaOCl) for 20 minutes, followed by three rinses with sterile water (14).

Growing Conditions

The sterilized seeds were then grown on MS medium with a pH of 5,7, supplemented with 30% sucrose, and a gelling agent. The medium was divided into two types: one without added vitamins (M1) and another consisting of MS medium supplemented with Myo-inositol (M2) 0.5 mg/L. The medium was sterilized using an autoclave at 121°C for 15 minutes. The cultures were incubated for 12 weeks at 15°C under continuous light in the incubation room.

Extraction of Andrographolide

The plantlets were harvested 12 weeks after inoculation and separated into three groups: leaves, stems, and roots. Each group was placed in sterile Petri dishes lined with sterile filter paper and dried using a blower in a Laminar Air Flow (LAF) cabinet. The dried samples were weighed to obtain a mass of 1–3 grams. The samples were then transferred to pre-weighed microtubes and ground with 1 mL of methanol solvent (15). The samples were macerated for 3 days, and the extracts were filtered using a 0.45 µm filter.

Chromatographic Parameters

Quantitative analysis of andrographolide in *A. paniculata* was performed following the protocol by Sharma (9) with modification using High-Performance Liquid Chromatography (HPLC). The analysis was conducted with an Agilent Technologies Series 1260 Infinity II system equipped with an automatic injector, a column oven, and a

UV detector. The column used was ZOBRAX Eclipse Plus C18 (150 mm × 4.6 mm i.d., 5 µm particle size). The injection volume was 5 µL, and the flow rate was 1 mL/min, with a retention time of 5 minutes. The separation was carried out using a ZOBRAX Eclipse Plus C18 reverse-phase column and a mobile phase of methanol-water (6.5:3.5 v/v), with detection at 224 nm (16). The concentration of andrographolide was calculated using a calibration curve prepared from andrographolide standards (Tokyo Chemical Industry). The calibration curve was constructed with five concentrations: 10 ppm, 20 ppm, 40 ppm, 80 ppm, and 100 ppm.

Data Analysis

The experiment followed a randomized design and was analyzed using a t-Test: Two-Sample Assuming Equal Variances with a significance level of $p < 0.05$.

RESULT AND DISCUSSION

This study investigated the influence of Myo-inositol on andrographolide production in *Andrographis paniculata* cultures. Andrographolide is a key secondary metabolite with significant pharmacological properties, holds considerable therapeutic value. This research aimed to determine whether Myo-inositol acts as a precursor or enhancer in the andrographolide biosynthetic pathway. While Myo-inositol is primarily involved in metabolic processes related to apical hook and hypocotyl growth, particularly under dark conditions, this study explored its possible role in promoting andrographolide production. It was hypothesized that Myo-inositol, potentially in combination with ZPT and elicitors (17), might enhance andrographolide synthesis.

A. paniculata cultures were grown under controlled conditions at temperature 15°C under continuous light in Murashige Skoog (MS) media without (M1) and with Myo-inositol (M2). The cultures were supplemented with 0.5 mg/L of Myo-inositol. Andrographolide levels were quantified using HPLC, and the resulting data were statistically analyzed to determine the significance of any observed trends. This study has the potential to optimize andrographolide production for therapeutic applications and contribute to advancements in plant-based biotechnology by elucidating the role of Myo-inositol in this important metabolic pathway.

Plant Culture of *A. paniculata*

During the 4-week incubation period at 15°C under continuous light, *A. paniculata* seeds grown without Myo-inositol (M1) exhibited better and faster growth compared to those grown with Myo-inositol (M2). This can be seen in the growth of shoots and roots, as well as in the quality and number of leaves. Figure 2 presents a visual comparison of the growth performance between cultures grown without Myo-inositol (M1) and with Myo-inositol (M2). Based on these observations, several factors may contribute to the suboptimal effect of Myo-inositol, including its involvement in primary metabolic pathways and differences in genetic regulation. According to previous studies, Myo-inositol plays a crucial role in various primary metabolic processes, including serving as a precursor in the biosynthesis of phospholipids and inositol phosphates (18). As a result, Myo-inositol is predominantly utilized in these pathways, limiting its availability for secondary metabolism, such as andrographolide biosynthesis. Furthermore, Myo-inositol has not been shown to significantly affect the expression of genes related to andrographolide biosynthesis compared to methyl jasmonate (19).

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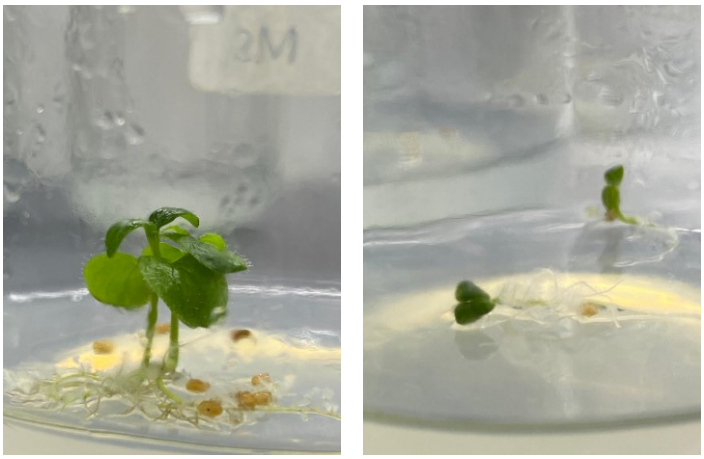

A. Without Myo-inositol (M1) B. with Myo-inositol (M2)

Figure 2. The effect on Myo-inositol at *A. paniculata* growth. After 4 weeks of incubation the seeds at 15^{o}C and 24-hours of light using media without (A) and with Myo-inositol (B).

After 12 weeks of incubation as shown in Figure 3, the *A. paniculata* plant cultures were harvested, and the leaves, stems, and roots were carefully collected to evaluate andrographolide production. Each plant part was processed individually to ensure accurate quantification of secondary metabolite distribution. The collected samples were subjected to maceration using methanol, an effective solvent for extracting bioactive compounds.

Figure 3. After a 12-week incubation period, each part of *Andrographis paniculata* was harvested for analysis.

The methanol extracts were then analyzed using High-Performance Liquid Chromatography (HPLC), a highly precise technique for identifying and quantifying andrographolide. This analysis provided detailed insights into the andrographolide concentration in each plant part, allowing a comprehensive comparison between

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treatments with and without Myo-inositol. Through this approach, the influence of Myo-inositol's on secondary metabolite biosynthesis and its distribution across different plant tissues could be effectively evaluated.

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Andrographolide Content Analysis

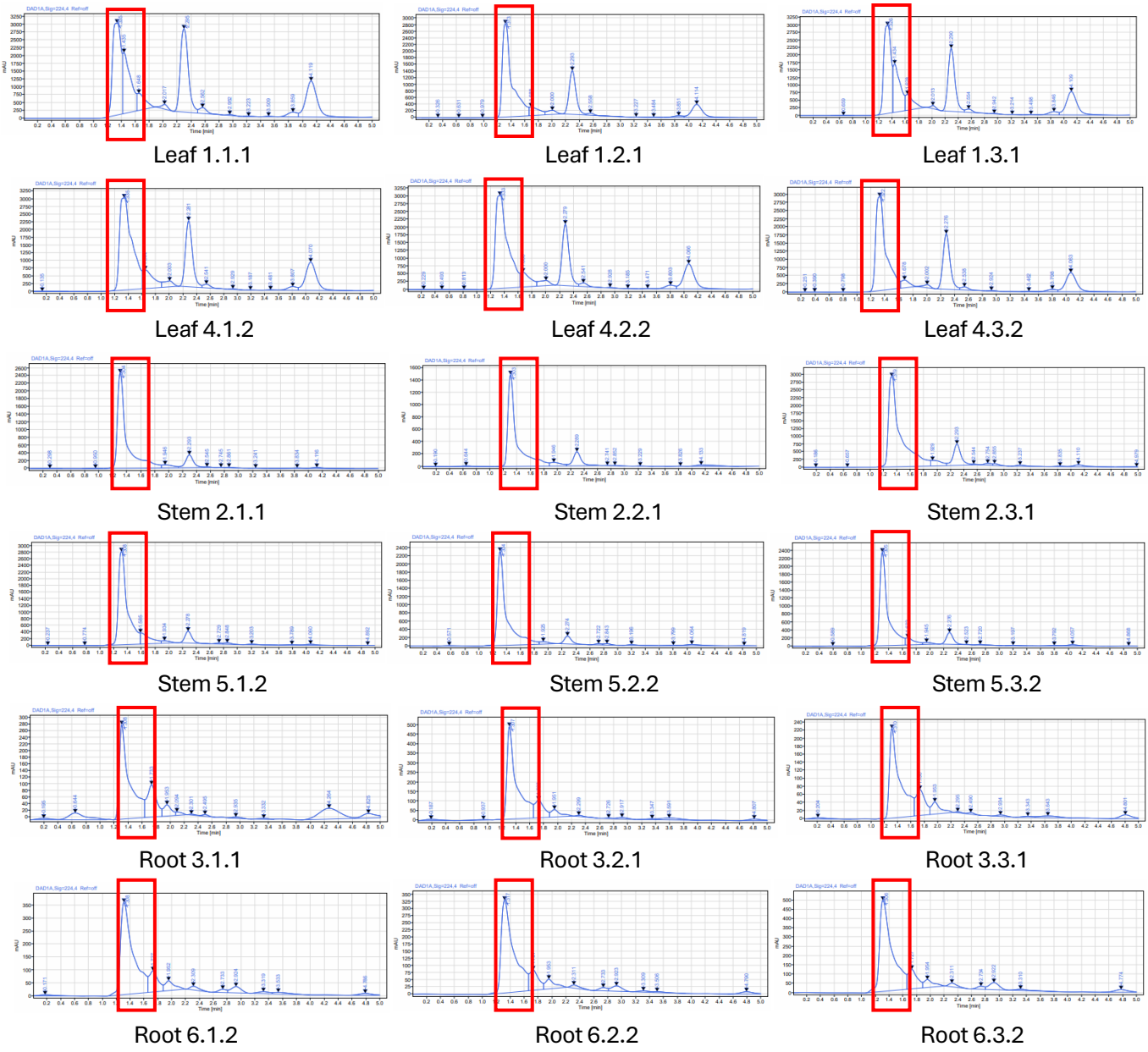

Figure 4. The Chromatogram of Andrographolide Content Analysis from Leaf, Stem, and Root of *A. paniculata* Cultures.

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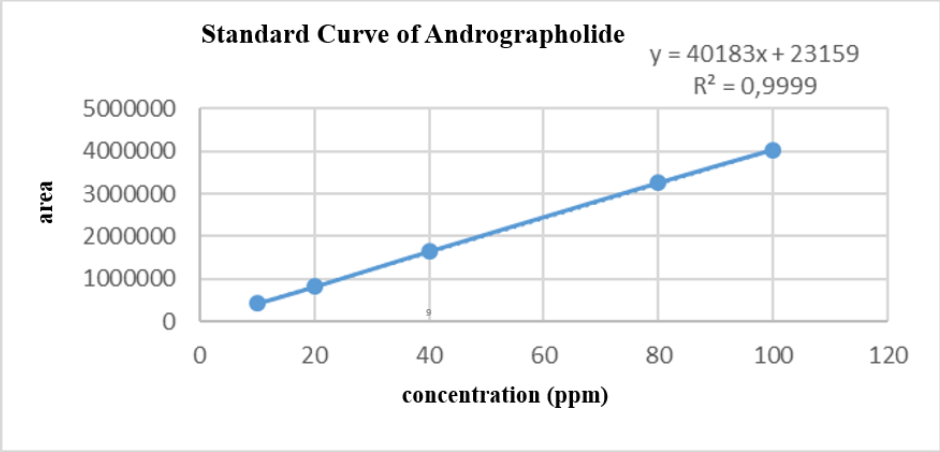

Figure 5. The Standard Curve of Andrographolide

Table 1. The Evaluation of Andrographolid Contents

	Sample	AUC	Weight (mg)	Concentration (ppm)	Rate	Average (%)
Without Myo-inositol (M1)	Leaf 1.1.1	21274.85	485.20	15.19	3.130	4.072
	Leaf 1.2.1	9386.87	115.90	6.55	5.648	
	Leaf 1.3.1	15252.82	314.40	10.81	3.438	
	Stem 2.1.1	2709.12	246.40	1.69	0.687	0.925
	Stem 2.2.1	1926.70	107.90	1.12	1.041	
	Stem 2.3.1	5595.85	362.30	3.79	1.046	
	Root 3.1.1	17.87	103.70	-0.26	-0.255	Detected, but not quantified
	Root 3.2.1	55.83	67.20	-0.24	-0.352	
	Root 3.3.1	18.02	89.80	-0.26	-0.294	
With Myo-inositol (M2)	Leaf 4.1.2	15495.41	349.80	10.99	3.141	3.052
	Leaf 4.2.2	14130.05	357.10	9.99	2.798	
	Leaf 4.3.2	12187.07	266.80	8.58	3.216	
	Stem 5.1.2	2551.76	179.60	1.58	0.878	0.737
	Stem 5.2.2	1426.88	147.30	0.76	0.516	
	Stem 5.3.2	2103.12	153.20	1.25	0.817	
	Root 6. 1.2	129.93	54.20	-0.18	-0.337	Detected, but not quantified
	Root 6. 2.2	101.99	66.40	-0.20	-0.306	
	Root 6. 2.3	169.44	103.80	-0.15	-0.149	

The results of the HPLC analysis revealed distinct differences in andrographolide concentrations across the plant parts of *Andrographis paniculata* under the two treatments. Based on data from Tabel 1, in the leaves, the concentration of andrographolide was higher in the absence of Myo-inositol (4.072%) compared to the presence of Myo-inositol (3.052%). Similarly, in the stems, andrographolide concentration was greater without Myo-inositol (0.925%) than with Myo-inositol (0.737%).

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Based on these results, the leaves consistently exhibit a high concentration of andrographolide. This is in line with the study by Sharma, which reported that leaves contain the highest andrographolide content compared to other parts of *Andrographis paniculata*, with a concentration of approximately 2–3% of dry weight. Interestingly, andrographolide was detected in the roots, but its concentration could not be measured due to its low levels. Adventitious root cultures of *Andrographis paniculata* grown in modified MS medium produced higher andrographolide levels compared to those grown in standard MS medium (21). The same study also mentioned that medium modifications involving the addition of vitamins such as Myo-inositol may contribute to root growth but do not specifically enhance andrographolide biosynthesis. Therefore, further research is needed on medium modifications and the use of elicitors that contribute to increased andrographolide levels, particularly those that can enhance the expression of genes in the mevalonate (MVA) pathway and the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway.

Statistical Analysis

Statistical analysis showed a p-value of 0.136 in Table 2. The p-value indicates the probability of observing the results obtained if there was actually no significant effect of Myo-inositol. Since this value is greater than the generally accepted threshold of 0.05 for statistical significance, the results indicate that there was no statistically significant difference in andrographolide production between the treatment groups. In other words, the observed variation in andrographolide levels cannot be confidently attributed to the effects of Myo-inositol, as it may be due to random variability or other uncontrolled factors in the experimental setup. Although the data do not support a significant effect of Myo-inositol on andrographolide production under the conditions tested, this finding highlights the need for further exploration. This includes optimizing experimental parameters such as Myo-inositol concentration, incubation time, or media composition, and investigating alternative mechanisms that may influence andrographolide biosynthesis, such as the role of other signaling molecules or the expression of key biosynthetic genes.

Table 2. t-Test: Two-Sample Assuming Equal Variances

	With Myo-inositol	Without Myo-inositol
Mean	3.052	4.072
Variance	0.050	1.886
Observations	3.000	3.000
Pooled Variance	0.968	
Hypothesized Mean Difference	0.000	
df	4.000	
t Stat	-1.270	
P(T<=t) one-tail	0.136	
t Critical one-tail	2.132	
P(T<=t) two-tail	0.273	
t Critical two-tail	2.776	

CONCLUSION

Andrographis paniculata seeds exhibited better growth when cultured in media without the addition of Myo-inositol compared to media with myo-inositol. The leaves of the plant were identified as the primary site of andrographolide production, showing significantly higher concentrations than other parts. In media without Myo-inositol, the andrographolide content reached 4.072%, while in media with myo-inositol, it was slightly lower at 3.052%. However, a comparison between MS media with and without Myo-inositol showed no significant difference in overall andrographolide production. Future research should focus on optimizing in vitro growth conditions, exploring alternative additives, employing genetic engineering approaches, and conducting long-term cultivation studies to enhance andrographolide production in *A. paniculata*.

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REFERENCES

1. Kilic M. The Healing Power of Plants for Health. In: Y. Waisundara V, editor. Medicinal Plants - Harnessing the Healing Power of Plants [Internet]. IntechOpen; 2024 [cited 2025 Jan 28]. Available from: https://www.intechopen.com/chapters/1192958
2. Mishra A, Shaik HA, Sinha RK, Shah BR. Andrographolide: A Herbal-Chemosynthetic Approach for Enhancing Immunity, Combating Viral Infections, and Its Implication on Human Health. Molecules. 2021 Nov 21;26(22):7036.
3. Chaachouay N, Zidane L. Plant-Derived Natural Products: A Source for Drug Discovery and Development. Drugs Drug Candidates. 2024 Feb 19;3(1):184–207.
4. Tripathi MK, Mishra N, Tiwari S, Shyam C, Singh S, Ahuja A. Plant Tissue Culture Technology: Sustainable Option for Mining High Value Pharmaceutical Compounds. Int J Curr Microbiol Appl Sci. 2019 Feb 20;8(02):1002–10.
5. Prashant SP, Bhawana M. An update on biotechnological intervention mediated by plant tissue culture to boost secondary metabolite production in medicinal and aromatic plants. Physiol Plant. 2024 Jul;176(4):e14400.
6. Fazili MA, Bashir I, Ahmad M, Yaqoob U, Geelani SN. In vitro strategies for the enhancement of secondary metabolite production in plants: a review. Bull Natl Res Cent. 2022 Feb 19;46(1):35.
7. Li C, Tien H, Wen M, Yen HE. MYO -inositol transport and metabolism participate in salt tolerance of halophyte ice plant seedlings. Physiol Plant. 2021 Jul;172(3):1619–29.
8. Gangwar H, Kumari P, Gahlaut V, Kumar S, Jaiswal V. Identification and comprehensive analysis of MIPSs in Rosaceae and their expression under abiotic stresses in rose (Rosa chinensis). Front Plant Sci. 2022 Nov 3;13:1021297.
9. Sharma S, Sharma YP, Bhardwaj C. HPLC quantification of andrographolide in different parts of *Andrographis paniculata* (Burm.f.) Wall. ex Nees.
10. Jiang M, Sheng F, Zhang Z, Ma X, Gao T, Fu C, et al. *Andrographis paniculata* (Burm.f.) Nees and its major constituent andrographolide as potential antiviral agents. J Ethnopharmacol. 2021 May;272:113954.
11. Liu L, Yan Y, Zheng L, Jia H, Han G. Synthesis and structure anti-inflammatory activity relationships studies of andrographolide derivatives. Nat Prod Res. 2020 Mar 18;34(6):782–9.
12. Saka WA, Oyekunle OS, Akhigbe TM, Oladipo OO, Ajayi MB, Adekola AT, et al. *Andrographis paniculata* improves glucose regulation by enhancing insulin sensitivity and upregulating GLUT 4 expression in Wistar rats. Front Nutr. 2024 Oct 31;11:1416641.

13. Low M, Suresh H, Zhou X, Bhuyan DJ, Alsherbiny MA, Khoo C, et al. The wide spectrum anti-inflammatory activity of andrographolide in comparison to NSAIDs: A promising therapeutic compound against the cytokine storm. Ali A, editor. PLOS ONE. 2024 Jul 17;19(7):e0299965.
14. Sy K, Kristianti T, Suhandono S, Elfahmi E. The transformation of dbr2 and p19 Genes into Artemisia annua L. by Agrobacterium tumefaciens Mediation: Metabolites Analysis.
15. Ahamad J, Ameen MSM, Anwer ET, Uthirapathy S, Porwal O. Qualitative and Quantitative Standardization of *Andrographis paniculata* by TLC Technique and UV Method. Adv Med Dent Health Sci. 2019 Oct 9;2(3):29–32.
16. Du Q, Jerz G, Winterhalter P. Separation of andrographolide and neoandrographolide from the leaves of *Andrographis paniculata* using high-speed counter-current chromatography. J Chromatogr A. 2003 Jan;984(1):147–51.
17. Sharma N, Chaudhary C, Khurana P. Wheat Myo-inositol phosphate synthase influences plant growth and stress responses via ethylene mediated signaling. Sci Rep. 2020 Jul 1;10(1):10766.
18. Siracusa L, Napoli E, Ruberto G. Novel Chemical and Biological Insights of Inositol Derivatives in Mediterranean Plants. Molecules. 2022 Feb 24;27(5):1525.
19. Sharma SN, Jha Z, Sinha RK, Geda AK. Jasmonate-induced biosynthesis of andrographolide in *Andrographis paniculata*. Physiol Plant. 2015 Feb;153(2):221–9.
20. Indrati O, Martien R, Rohman A, Nugroho AK. EMPLOYMENT OF ATR-FTIR AND HPLC-UV METHOD FOR DETECTION AND QUANTIFICATION OF ANDROGRAPHOLIDE. Int J Appl Pharm. 2018 Nov 22;10(6):135.
21. Sharma SN, Jha Z, Sinha RK. Establishment of In Vitro Adventitious Root Cultures and Analysis of Andrographolide in *Andrographis Paniculata*. Nat Prod Commun. 2013 Aug;8(8):1934578X1300800803.
22. National Center for Biotechnology Information (2025). PubChem Compound Summary for CID 5318517, Andrographolide. Retrieved May 22, 2025 from https://pubchem.ncbi.nlm.nih.gov/compound/Andrographolide