Research Article

Effect of Different Growing Media And Media Water Status On Main Bioactive Compound Of Hempedu Bumi (*Andrographis paniculata*)

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Abstract | *Andrographis paniculata* also known as Hempedu Bumi or King of Bitters, is a medicinal plant belongs to the Acanthaceae family and is valued for its bioactive compounds with significant pharmacological benefits. Hence, the objective of this research was to determine andrographolide content of *A. paniculata* based on different growing media and media water status. The experiment was conducted in a greenhouse at the Institute of Sustainable Agrotechnology (INSAT), Universiti Malaysia Perlis (UniMAP), following a randomized complete block design (RCBD) with two factors, comprising nine treatment combinations and three replications. Three types of growing media (compost, topsoil, and sand) were mixed in different ratios (1:2:3, 2:2:1, and 3:2:1), and subjected to three levels of soil water potential: -20 kPa (high), -60 kPa (medium), and -100 kPa (low). Plants were harvested at 120 Days After Sowing (DAS) at physiological maturity. Andrographolide content in the leaf, stem, and root tissues was quantified using high-performance liquid chromatography (HPLC). The results revealed that the combination of compost, topsoil, and sand in a 3:2:1 ratio under high soil water potential significantly increased andrographolide accumulation compared to other treatments. The andrographolide content varied significantly across plant parts. In the leaves, the highest concentration (5.239 mg/g) was 3.0-fold higher than the lowest (1.739 mg/g). In the stem, the maximum (2.284 mg/g) was 6.2-fold greater than the minimum (0.368 mg/g), while in the root, the highest value (0.822 mg/g) was 10-fold higher than the lowest (0.082 mg/g). Overall, the highest andrographolide content was observed in the leaf compared to stem and root. These findings suggest that using a growing medium composed of compost, topsoil, and sand at a 3:2:1 ratio, along with sufficient water availability, is optimal for enhancing andrographolide production in *A. paniculata* cultivated under greenhouse conditions.

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Introduction

Medicinal plants represent an invaluable component of global biodiversity and have long served as essential resources in healthcare systems. As noted by Srivastava (2000), medicinal plants are commonly employed in the treatment and prevention of various diseases and are widely recognized for their health-promoting properties. In recent years, this importance has been reaffirmed by research showing that over 50% of the world's population still rely on plant-derived traditional medicines, and that more than 50% of modern medicines have origins in natural sources such as plants, fungi, or other organisms (World Health Organization, 2022; El-Saadony *et al*., 2025). Modern interdisciplinary studies that combine evolutionary ecology, molecular biology, ethnopharmacology and biochemistry are revealing new pharmacological leads from medicinal plants, emphasizing not only their historic role but their continuing potential for discovering novel therapeutics (Davis and Choisy, 2024; Tlhapi *et al*., 2024; El-Saadony *et al*., 2025). In many regions, particularly in developing countries, medicinal plants remain the most accessible and affordable source of primary healthcare, underscoring their enduring socio-economic and cultural significance. Furthermore, the pharmacological potential of these plants extends beyond traditional remedies, serving as a critical foundation for contemporary drug discovery and development, especially in addressing emerging health challenges such as antimicrobial resistance and chronic diseases. The World Health Organization (WHO, 2022) similarly defines medicinal plants as those possessing one or more organs that contain bioactive compounds with therapeutic potential, including metabolites that contribute to drug development. The contribution of medicinal plants to human well-being is profound, having constituted the primary form of medical treatment for centuries prior to the emergence of synthetic pharmaceuticals. Their role continues today, not only in rural and indigenous communities but also increasingly in integrative medicine, due to their affordability, accessibility, and the lower side-effect profiles of many plant-derived remedies compared to some synthetic drugs (Ansari *et al*., 2025). Among these, *Andrographis paniculata* has garnered increasing scientific interest in recent years due to its notable pharmacological and therapeutic properties (Gowda *et al*., 2023; Adiguna *et al*., 2023). *A. paniculata* is a prominent medicinal species within the Acanthaceae family. It is an erect annual plant, typically growing to heights of 30–110 cm, with a stout, dark-green stem that is quadrangular in cross-section, often exhibiting longitudinal furrows or slight wings along its angles. The leaves are lanceolate and borne on petioles, while the flowers are relatively small, often white or with pale hues, arranged in racemes or panicles. Its fruiting bodies are elongated linear capsules which dehisce to release numerous tiny, yellow-brown seeds (Intharuksa *et al*., 2022; Talei *et al*., 2025; Prabhakornritta *et al*., 2025). *A. paniculata* locally known as *Hempedu Bumi* in Malaysia, is regarded as a native plant with substantial commercial potential, primarily attributed to its intense bitterness. As demand for bitter herbal tonics and immune-supporting botanicals grows both domestically and internationally, the economic prospects for *A. paniculata* cultivation, extraction, and standardized formulation increase significantly (Suhaini *et al*., 2020; Intharuksa *et al*., 2022). This plant has been widely utilized in Ayurvedic, Chinese, and Unani medicinal systems for the treatment of numerous ailments. It also possesses a long-standing history in traditional medicine, where it has been employed for various therapeutic purposes, including the treatment of the common cold, digestive disorders, upper respiratory tract infections, influenza, and jaundice. Furthermore, its preparations are often regarded as liver tonics and as agents that purify or "detoxify" the blood (Intharuksa *et al*., 2022; Prabhakornritta *et al*., 2025; Singh *et al*., 2025).

This medicinal plant produces a wide range of bioactive compounds. The aerial parts of *A. paniculata* are the most commonly used, as they contain a range of bioactive compounds, including diterpene glycosides, diterpenoids, flavonoids, lactones, and flavonoid glycosides (Zeng *et al*., 2022; Sharangi *et al*., 2025). Modern medical research has identified andrographolide as the primary bioactive compound in *A. paniculata*, noted for its broad spectrum of therapeutic properties and significant pharmacological relevance (Mussard *et al*., 2020; Li *et al*., 2025). Andrographolide, a strongly bitter-tasting labdane diterpenoid derived from *A. paniculata*, is a key component with at least 26 Ayurvedic formulations used for the treatment of liver disorders. The therapeutic potential of *A. paniculata* has facilitated the development of numerous pharmaceutical products, with 29 formulations currently approved for use in mainland China (Gowda *et al*., 2023; Li *et al*., 2025). For example, andrographolide sulfonate formulations has widespread clinical usage for inflammatory, infectious, and respiratory indications, and are among the most commercially successful *Andrographis*/andrographolide-based products in the Chinese market (Li *et al*., 2025).

Andrographolides demonstrate significant anticancer and immunomodulatory activities, indicating strong potential for development as chemotherapeutic agents (Gowda *et al*., 2023; Pandey and Rao, 2018). Andrographolide is colorless, bitter crystalline compound with molecular formula $C_{20}H_{30}O_5$. It can be easily dissolved in methanol, ethanol, pyridine, acetic acid, and acetone but slightly dissolved in ether and water (Kumoro and Hassan, 2006; Luo *et al*., 2025). The melting point of this compound is 228^{0}C – 230^{0}C and the ultraviolet spectrum in ethanol, λmax is 223 nm (Kumoro and Hassan, 2006). Andrographolides are safe, non-toxic and strong natural antioxidant in comparison with other phyto-antioxidants (Niranjan *et al*., 2010; Kumoro and Hassan, 2006).

Growing media serve several essential functions, including providing physical support for plants, retaining and supplying water and nutrients, and facilitating adequate oxygen availability to the roots. An effective growing medium should possess the appropriate physical and chemical properties necessary to support plant growth and ensure optimal conditions for plant production, particularly in containerized systems (Abha Manohar *et al*., 2022; Kumaresan *et al*., 2023). Numerous studies have demonstrated that the growing medium used in greenhouse cultivation plays a crucial role in influencing the production of bioactive constituents in plants. Various types of growing media have been assessed for their suitability in greenhouse systems, each exhibiting distinct physical and chemical properties that can influence bioactive compounds in plants. Compost is an example of an organic amendment that not only contributes organic matter but also enhances the nutrient-holding capacity of the soil (Kauser *et al*., 2025). Several studies have reported promising outcomes regarding the quality enhancement of medicinal herbs through the application of compost as a source of organic nutrients (Kazimierczak *et al*., 2015; Basak and Gajbhiye, 2018; Qudoos *et al*., 2024). Recent evidence highlights that the choice of growing medium in greenhouse cultivation significantly influences the accumulation of secondary metabolites and bioactive constituents in medicinal plants. For instance, substrate composition and nutrient availability have been shown to modulate the production of phenolic acids, flavonoids, and terpenoids in *Ocimum basilicum* and *Rosmarinus officinalis*, thereby directly affecting their pharmacological potential (Bustamante *et al*., 2020; Burdina and Priss *et al*., 2016). Moreover, studies on *Mentha arvensis* have shown that compost-based growing media positively affect chlorophyll content, menthol accumulation, and overall phytochemical richness (Sharma *et al*., 2023).

One of the most important environmental factors limiting crop production is the availability of soil water. Both too much and too little water can cause stress in plants. Crop growth can be hampered and nutrients can leach when soil water potential (SWP) is too high. Because of limited oxygen diffusion in the soil, root growth and function may be impeded by high SWP levels (Hodnett *et al*., 1990; Ganesan *et al*., 2024). In general, an ideal soil water potential promotes water and nutrient uptake and supports the physiological functions required for plant growth. Low soil water potential, on the other hand, can have detrimental effects on crop development. For example, it can increase stomatal resistance, which lowers photosynthesis, and soil mechanical resistance, which limits root penetration. A recent study by Gasiewska *et al*. (2025) shows that *Plantago lanceolata* and *Tanacetum parthenium* under water limitation had reduced biomass, but certain secondary metabolites increased under moderate water limitation though the response varied among specific metabolites.

To date, there are limited studies on the growth performance and phytochemical variations in *A. paniculata* as influenced by growing media and media water status. This gap in knowledge formed the basis for the present study. Thus, this research focuses on the effect of growing media and media water status on accumulation of bioactive compounds in *A. paniculata*. The findings of this study will contribute to identifying the optimal growing media and media water status required to achieve maximum andrographolide content under greenhouse conditions.

Material and Methods

Planting material

Seeds of *A. paniculata* were obtained from the

Agrotechnology Research Station, Universiti Malaysia Perlis (UniMAP), located in Sg. Chuchuh, Perlis. The seeds were germinated in seedling trays filled with a standard nursery substrate consisting of peatmoss to ensure uniform emergence. After the seedlings reached the 4–6 leaf stage, uniform plants were transplanted into experimental polybags. Each plant was grown in a polybag with dimensions of approximately 10″ × 12″ (25 × 30 cm). Each polybag was filled with approximately 10 kg of growing media prepared according to the designated ratios of compost, topsoil, and sand for the respective treatments.

Experimental condition

The experiment was conducted under greenhouse conditions at UniMAP, where the average temperature ranged between 28–33°C, relative humidity was maintained between 65–80%, and natural daylight provided an average light intensity of 1200–1500 µmol m^{-2} s^{-1}. These controlled environmental conditions ensured optimal growth and minimized external variability during the experimental period.

Table 1: *Growing media applied in this experiment.*

Treatment	Growing media composition		
	Compost	Topsoil	Sand
Mix 1	1	2	3
Mix 2	2	2	1
Mix 3	3	2	1

Table 2: *Media water status applied in this experiment.*

Treatment	Media water status (kPa)
Low	-100
Medium	-60
High	-20

Experimental designs

The study employed a two-factor factorial design arranged in a randomized complete block design (RCBD) in order to minimize the effects of environmental variability and ensure the reliability of treatment comparisons. The experiment consisting of two factors which were growing media and media water status as outlined in Table 1. Each factor comprised three levels, resulting in a total of nine treatment combinations. To ensure statistical reliability and reduce experimental error, each treatment was replicated three times across blocks. Additionally, three levels of media water status (high, medium, and low) were imposed. Irrigation was administered when the soil water potential reached specific threshold values, as detailed in Table 2. Soil water potential was continuously monitored using a gypsum block sensor, which provided reliable measurements of moisture availability within the root zone. This approach ensured that irrigation was administered accurately according to the target water status treatments (Marković *et al*., 2024).

Analysis of growing media

Mechanical analysis was conducted using standard sieve analysis to determine the particle size distribution of the growing media. Mechanical analysis of the growing media was performed using the sieve method to determine particle size distribution. Soil samples were air-dried, gently disaggregated, and passed through a series of standard sieves with finer mesh sizes (ranging from 4.75 mm to 0.075 mm) mounted on a mechanical shaker. The proportion of soil retained on each sieve was weighed and expressed as a percentage of the total sample allowing the determination of particle size fractions and texture classification (Blake and Hartge, 1986; Ren *et al*., 2025). The core method was used to measure bulk density. Water was allowed to permeate the media for two days in order to saturate the core rings. The samples were oven-dried for 24 hours at 105°C after the weights were recorded and bulk density was calculated as the ratio of oven-dry mass to core volume (Yahya *et al*., 2009; Gyawali *et al*., 2025). Chemical analyses included the determination of organic carbon content via the Walkley and Black dichromate oxidation method, in which 1 g of air-dried soil was digested with potassium dichromate and concentrated sulfuric acid, and the excess dichromate was back-titrated with ferrous ammonium sulfate (Walkley and Black, 1934; Olayinka *et al*., 1998). Total nitrogen was quantified through Kjeldahl digestion procedure, in which soil samples were digested with concentrated sulfuric acid in the presence of catalysts to convert organic and inorganic nitrogen compounds into ammonium (NH_4^+). The digest was subsequently analyzed by distillation and titration to quantify the ammonium content, providing an estimate of the total nitrogen concentration in the soil sample (Bremner and Mulvaney, 1982; Rahimian and Khoshgoftarmanesh, 2025). Cation Exchange Capacity (CEC) was assessed using the ammonium acetate method to evaluate the media's capacity to retain and exchange essential nutrients. CEC was determined using the ammonium acetate method at pH 7. Soil samples (<2 mm) were saturated with 1 M NH_4OAc, washed with ethanol to remove excess ions, and subsequently leached with 1 M KCl to displace adsorbed NH_4^+. The displaced ammonium was quantified by distillation and titration method (Schollenberger and Simon, 1945; Nel *et al*., 2023). Soil pH was measured in a 1:2.5 soil-to-water suspension using a calibrated pH meter, while electrical conductivity (EC) was determined using a conductivity meter. The physical and chemical characteristics of the growing media are summarized in Table 3 and Table 4.

Table 3: *Soil physical analyses of growing media*

Growing media	Particle size (%)				Bulk density (g/cm^{-3})
	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	
Mix 1	42.88	41.68	10.30	5.14	1.20
Mix 2	30.68	39.00	22.08	8.24	1.08
Mix 3	23.16	36.14	27.58	13.12	0.91

Table 4: *Soil chemical analyses of growing media*

Growing media	pH	EC (dSm^{-1})	Organic carbon (%)	Total N (%)
Mix 1	7.4	0.28	1.86	0.09
Mix 2	7.19	0.47	4.97	0.18
Mix 3	6.80	0.61	7.09	0.27

Harvesting

Harvesting of *A. paniculata* plants was conducted manually at 120 days after sowing (DAS). Mature plants were carefully uprooted from the pots in the greenhouse and immediately rinsed with tap water to remove any adhering soil particles and debris. Excess surface moisture was gently blotted using paper towels. The plants were then separated into roots, stems, and leaves.

Grinding

All dried plant parts (leaves, stems, and roots) were ground into a fine powder using a mechanical blender followed by a homogenizer. The powdered samples were stored in airtight plastic containers at room temperature until further analysis.

Determination of andrographolide content

The extraction and quantification of andrographolides from the methanolic extracts were performed using High Performance Liquid Chromatography (HPLC). A reverse phase C-18 Thermo Hypersil GOLD column (250 nm x 4.6 mm, 5 µm particle diameter) with the mobile phase methanol and water (65:35v/v) provide good separation of andrographolide from the sample extract with the flow rate 1.0 ml/min, and an injection volume of 20 µL. Plant samples were analyzed in triplicate to ensure reproducibility and reliability of results. Detection of andrographolide was conducted at a wavelength of 223 nm, which corresponds to the compound's maximum absorbance. The andrographolide content in the samples was determined by comparing the area under the curve (AUC) of the standard andrographolide peak with the corresponding peak in the sample chromatogram (Figure 1). Quality control was maintained by running standard checks and blank samples between injections to avoid carryover and ensure instrument accuracy (Sharma *et al*., 2012; Singh *et al*., 2025).

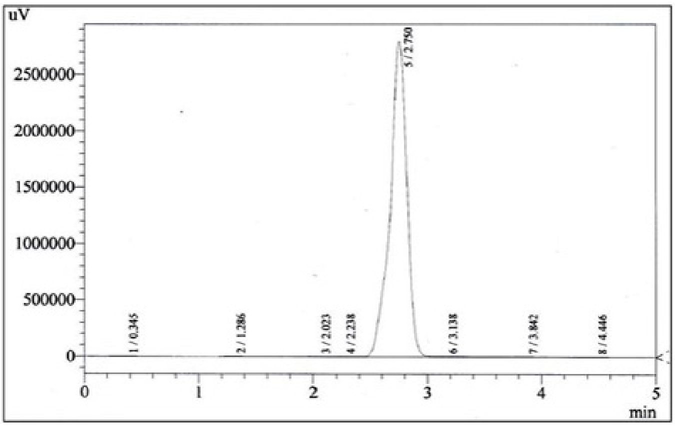

Figure 1: *HPLC chromatogram of andrographolide standard*

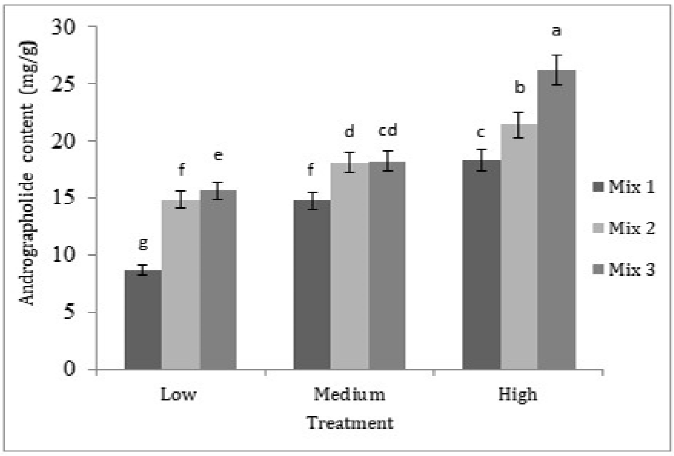

Figure 2: *Andrographolide content in leaves influenced by different growing media and media water status (means and standard error bars).*

Statistical analysis

All data were averaged on a plot basis prior to statistical evaluation. Statistical analyses were performed using JMP Pro 11 (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) was conducted to determine the significance of treatment effects at a confidence level of α = 0.05. When ANOVA indicated significant treatment effects, Tukey's Honest Significant Difference (HSD) test was employed for multiple comparison procedures to distinguish differences among least square means at $p < 0.05$.

Results and Discussion

Andrographolide content in leaves

The statistical analysis of andrographolide content in leaves was illustrated in Figure 2. A significant effect of the treatment combinations on andrographolide yield in the leaves was observed. The results reveal the highest andrographolide content observed in plants cultivated in Mix 3 under high soil water potential with a mean value of 5.239 mg/g. In contrast, Mix 1 consistently recorded the lowest andrographolide content across all water regimes, particularly under low moisture conditions (1.739 mg/g). Thus, the highest andrographolide content in the leaves was 3.0-fold greater than the lowest recorded content.

Plants grown in Mix 3 under high soil water potential resulted in significantly higher andrographolide concentrations compared to all other treatment combinations, indicating that optimal soil moisture within a well-structured growth medium can enhance the biosynthesis of secondary metabolites. Andrographolide predominantly accumulates in the aerial parts of the plant, with a higher concentration in the leaves. Consequently, interventions that promote shoot biomass are likely to increase andrographolide content. Moreover, the biosynthesis of diterpenoid lactones, such as andrographolide, is entirely dependent on primary metabolic processes, including photosynthesis (Basak *et al*., 2020). Meanwhile, *A. paniculata* plants grown in Mix 1 under low soil water potential resulted in lowest andrographolide content. This reduction is likely due to water stress causing decreased photosynthesis, reduced leaf growth, and impaired enzymatic processes essential for secondary metabolite production, leading to lower andrographolide accumulation (Shil and Dewanjee, 2022; Balamurugan *et al*., 2024). Moreover, drought stress can modulate the accumulation of bioactive compound through several distinct mechanisms. In certain instances, a decline in biomass results in an apparent increase in metabolite concentration when expressed on a dry weight basis, even in the absence of enhanced biosynthetic activity. Alternatively, stress conditions may induce a passive metabolic shift that favors the production of secondary metabolites, or actively upregulate specific enzymes within biosynthetic pathways (Selmar *et al*., 2017).

These observations align with recent studies examining water-stress effects on *A. paniculata* and other medicinal plants. For instance, Misra and Srivastava (2008) reported a similar decrease in essential oil content in Japanese mint subjected to water stress. Moreover, Afshar *et al*. (2014) demonstrated that severe deficit irrigation in milk thistle (*Silybum marianum* L. Gaertn.) significantly reduced oil content by approximately 7.4% relative to fully irrigated controls, underscoring the sensitivity of secondary metabolite synthesis to water availability. In *Rosmarinus officinalis*, severe water stress was found to significantly decrease essential oil yield and the levels of key secondary metabolites, along with declines in antioxidant and antidiabetic activities (Laftouhi *et al*., 2024). However, adverse impact is observed in *Panax quinquefolius*, for which soil moisture stress sharply reduced growth, but induced higher concentrations of certain bioactive ginsenosides per unit dry weight under moderate drought though the total yield still dropped (Zhang *et al*., 2024). Similarly, In *Thymus daenensis*, drought stress also led to increased levels of essential oil components such as thymol under moderate water limitation, but extreme stress reduced overall oil content and altered composition (Nasiri *et al*., 2025). Chen *et al*. (2020) found that drought stress treatments enhanced the concentration of andrographolides in *A. paniculata* leaves, although prolonged and severe stress also depressed overall plant biomass, so the net yield of andrographolide per plant was reduced under the most intense stress conditions. Research done by Punetha *et al*. (2023) on German chamomile (*Chamomilla recutita* L.) also showed that, the proportion of major bioactive compounds was higher under stress, indicating that water stress enhances the concentration of certain secondary metabolites.

Andrographolide content in stem

As illustrated in Figure 3, the interaction between soil water potential and growing media had a significant impact on the amount of andrographolide in the stem ($P < 0.05$). There was a general trend indicating that higher media water status resulted in increased andrographolide accumulation, regardless of the media mix used, with the maximum recorded of 2.284 mg/g. This may be attributed to its improved physical structure, particularly in terms of drainage, porosity, and nutrient-holding capacity, which likely enhanced root development and metabolic efficiency. Under high water availability, these conditions may have promoted optimal physiological processes, including photosynthesis and metabolite transport, leading to enhanced andrographolide synthesis and accumulation in the stem (Chen *et al*., 2020; De Snyders *et al*., 2024; Venkatasai *et al*., 2025).

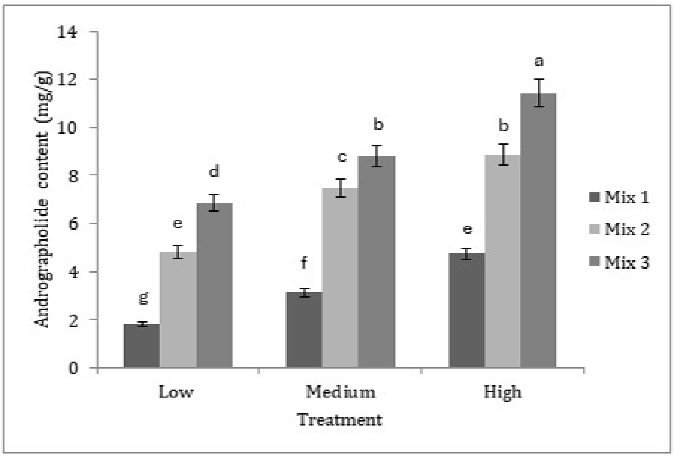

Figure 3: *Andrographolide content in stem influenced by different growing media and media water status (means and standard error bars).*

Mixture 2 showed intermediate andrographolide levels across all treatments, indicating moderate improvement in media conditions compared to Mix 1, but not as favorable as Mix 3. Thus, the highest andrographolide content in the stem was 6.2-fold greater than the lowest recorded content. When water stress and nutrient deficiency occur concurrently, their adverse effects on plant metabolism are often amplified. Water stress induced reductions in photosynthate availability, coupled with diminished enzymatic activity arising from nutrient scarcity, compel plants to reallocate limited resources toward vital survival mechanisms such as osmotic adjustment and reactive oxygen species (ROS) detoxification, rather than secondary metabolic pathways. This metabolic shift ultimately suppresses the biosynthesis and accumulation of bioactive compounds (Copolovici *et al*., 2021; Mulugeta and Radácsi, 2022).

Comparable findings have been documented in relation to the influence of water stress on the biosynthesis of secondary metabolites. For instance, Yadav *et al.* (2014) reported that short-duration water stress altered the concentration of artemisinin in *Artemisia annua* L., highlighting the sensitivity of secondary metabolite pathways to changes in water availability. Additionally, water stress has been associated with significant biochemical and physiological adaptations in plants. Bayat and Moghadam (2019) in a study on *Salvia nemorosa* L., observed that exposure to water stress resulted in marked accumulation of proline, a key osmolyte. This accumulation is a vital component of osmotic adjustment, a mechanism by which plants maintain cell turgor and sustain metabolic activity under conditions of limited water availability. Other study done by Copolovici *et al.* (2021) found that stress treatments caused substantial reductions in total phenolic and flavonoid contents of *Ocimum basilicum*. In *Valeriana jatamansi*, water stress was found to enhance the accumulation of valerenic acid, a sesquiterpenoid of considerable pharmacological importance, with levels increasing 1–6.9-fold across various plant tissues relative to well-watered controls (Pandey *et al*., 2021). In addition, research done by Chungloo *et al.* (2023) demonstrated that exposure of *A. paniculata* to water- stress alone resulted in a substantial reduction in total andrographolide yield, primarily because of a marked decline in overall plant biomass. Under water stress conditions, physiological limitations such as reduced stomatal conductance, impaired photosynthetic efficiency, and restricted aerial parts expansion collectively suppressed vegetative growth. Recent work on *Eleutherococcus senticosus* provides further evidence of stem involvement in secondary metabolism, where water stress upregulated genes in the triterpenoid saponin biosynthetic pathway, leading to elevated saponin accumulation in stems and other tissues. These finding illustrate that while drought often reduces biomass and thereby total metabolite yield, stems can function both as adaptive storage organs and as active sites of stress-induced metabolite biosynthesis, adding a crucial dimension to the understanding of water stress responses in medicinal plants (Wang *et al*., 2024).

Andrographolide content in root

The interaction between soil water potential and growing media significantly affected andrographolide accumulation in roots, as shown in Figure 4. The highest andrographolide concentration was observed in Mixture 3 under high soil water potential, reaching 0.822 mg/g, which was significantly higher ($p < 0.05$)

than all other treatments. This finding reinforces the trend observed in the leaf and stem, where Mix 3 consistently supported greater andrographolide biosynthesis. In contrast, Mix 1 resulted in the lowest andrographolide content under all water treatments, with the lowest value recorded under low soil water potential (0.082 mg/g). Thus, the highest andrographolide content in the root was 10-fold greater than the lowest recorded content.

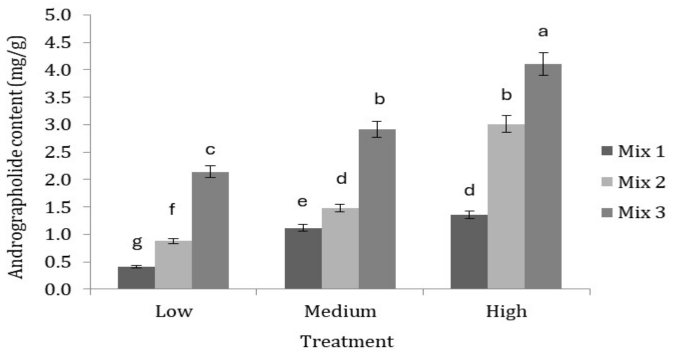

Figure 4: *Andrographolide content in root influenced by different growing media and media water status (means and standard error bars).*

High soil water potential alleviates water stress, thereby sustaining higher metabolic activity, including the enzymatic processes and biochemical pathways involved in andrographolide biosynthesis. This condition may also enhance the uptake or mobilization of precursor substrates necessary for secondary metabolite production. In contrast, low soil water potential imposes physiological stress, which can constrain root development, limit carbon allocation to secondary metabolism, and potentially trigger the downregulation or degradation of andrographolide biosynthetic pathways. The observed 10-fold variation in root andrographolide content emphasizes the critical influence of both the physical properties of the growing medium such as organic matter content, texture, and aeration also soil water status in optimizing the accumulation of root secondary metabolites (Saha *et al*., 2019; Saha and Basak, 2020).

These results agree with recent studies showing that both growing medium composition and water status strongly influence secondary metabolite accumulation in *A. paniculata*. Recent research by Isa *et al*. (2021), found that mixtures with higher organic content (which improve water-holding capacity) combined with higher soil water potential led to greater biomass and often higher secondary metabolite content, implying that water availability is a key driver of both growth and bioactive compound accumulation. Other findings on *Melastoma malabathricum* demonstrated compost markedly improved soil chemical properties, including pH and nutrient availability, which subsequently increased nutrient uptake and promoted the biosynthesis of key bioactive compounds such as total phenolics, flavonoids, and anthocyanins. These results highlight the potential of organic amendments in modulating soil fertility and driving secondary metabolism in medicinal plants, thereby improving both growth and phytochemical quality of *M. malabathricum* (Rusli *et al*., 2022). Rawat *et al*. (2021) reported that growing media significantly influenced the accumulation of the steroidal sapogenin diosgenin of *Costus speciosus*. The study also found that plants grown under favorable growing media condition exhibited substantially higher secondary metabolite content.

Water stress has been shown to influence both the composition and concentration of secondary metabolites in medicinal plants. Zhang *et al*. (2017) observed that moderate water stress initially increased root biomass in *Stellaria dichotoma* L. However, as water stress intensified, root biomass declined. This suggests a biphasic response, where mild stress may stimulate root growth, but excessive stress leads to detrimental effects. Optimal root development occurred under moderate water stress conditions. The concentration of total saponins and flavonoids increased under moderate water stress but decreased under severe stress. This indicates that while certain levels of water stress can enhance the production of bioactive compounds, excessive stress may impair metabolic processes and reduce their accumulation.

Conclusions and Recommendations

The study concluded that variations in growing media and soil water potential had a significant influence on the accumulation of the main active compound, andrographolide, in *A. paniculata* cultivated under greenhouse conditions. A positive interaction effect was observed, where the combination of optimal moisture and improved media properties significantly enhanced bioactive compound synthesis. These findings suggest that optimizing media composition and irrigation management can substantially improve the medicinal quality of *A. paniculata*, offering valuable implications for commercial cultivation and phytopharmaceutical production. Therefore, it is recommended to use the growing media mixture composed of compost: topsoil: sand in a 3:2:1 ratio, combined with adequate irrigation, to optimize the biochemical constituents of *A. paniculata* grown in greenhouse environments. Further research could investigate the underlying physiological, biochemical, and molecular mechanisms that regulate the enhanced synthesis and accumulation of andrographolide under optimized growing media and soil water conditions. Understanding these mechanisms in greater depth would provide a scientific basis for developing more efficient, standardized, and sustainable cultivation strategies, ultimately facilitating the large-scale production of high-quality *A. paniculata* for medicinal and phytopharmaceutical applications.

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Novelty Statement

The study provides novel information on effect of different growing media and media water status on main bioactive compound (andrographolide) of *Andrographis paniculata*.

Author's Contribution

Muslianie Md Isa: Conceptualization, methodology, data curation and analysis, manuscript writing
Mahmad Nor Jaafar: Supervising, project planning and manuscript editing.
Khairul Farihan Kasim: Data analysis, manuscript writing and editing
Muhamad Firdaus Abdul Muttalib: Data analysis, manuscript editing

Generative AI or AI assisted technology statement
The authors declare that no genrative AI was used in the creation of this manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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