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Evaluating growth and pharmacological potential of *Atherolepis pierrei* Constantin through synergistic fertilization and bioactive compound analysis

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ABSTRACT: This study investigates the impact of various fertilization methods on the growth, yield, and phytochemical composition of Atherolepis pierrei Costantin, a medicinal plant with significant pharmaceutical potential. The experiment examined the effects of organic and chemical fertilizers, including vermicompost, poultry manure, and NPK fertilizers, on plant development and the production of bioactive compounds. Findings reveal that combined fertilization strategies, particularly those involving moderate levels of compost and NPK, optimized plant growth, yield, and biomass accumulation. Phytochemical analysis using Gas Chromatography-Mass Spectrometry (GC-MS) identified several bioactive compounds such as benzaldehyde, 2-hydroxy-4-methoxy, lupeol, and β-amyrin, known for their antioxidant, antimicrobial, anti-inflammatory, and anticancer activities. The study highlights the role of tailored fertilization approaches in enhancing both the physical growth and medicinal potential of Atherolepis pierrei. These results underline the importance of integrated fertilization strategies for sustainable agricultural practices and the production of high-value medicinal plants. Further research is recommended to explore the full pharmacological potential of the identified bioactive compounds and refine fertilization methods for optimizing both crop yield and bioactive compound production.
Keywords: fertilization methods; plant growth parameters; bioactive compounds; sustainable agriculture; medicinal plant utilization.

Avaliação do crescimento e potencial farmacológico de *Atherolepis pierrei* Constantin por meio de fertilização sinérgica e análise de compostos bioativos

RESUMO: Este estudo investiga o impacto de vários métodos de fertilização no crescimento, rendimento e composição fitoquímica de Atherolepis pierrei Constantin, uma planta medicinal com significativo potencial farmacêutico. O experimento analisou os efeitos de fertilizantes orgânicos e químicos, incluindo vermicomposto, esterco de aves e fertilizantes NPK, no desenvolvimento das plantas e na produção de compostos bioativos. Os resultados revelaram que as estratégias de fertilização combinada, particularmente aquelas envolvendo níveis moderados de composto e NPK, otimizaram o crescimento das plantas, o rendimento e a acumulação de biomassa. A análise fitoquímica utilizando Cromatografia Gasosa com Espectrometria de Massa (GC-MS) identificou vários compostos bioativos, como benzaldeído, 2-hidroxi-4-metoxil, lupeol e β-amirina, conhecidos por suas atividades antioxidante, antimicrobiana, anti-inflamatória e anticâncer. O estudo destaca o papel de abordagens de fertilização específicas na melhoria tanto do crescimento físico quanto do potencial medicinal de Atherolepis pierrei. Estes resultados ressaltam a importância de estratégias de fertilização integradas para práticas agrícolas sustentáveis e a produção de plantas medicinais de alto valor. Recomenda-se mais pesquisas para explorar o pleno potencial farmacológico dos compostos bioativos identificados e aprimorar os métodos de fertilização para otimizar tanto o rendimento das culturas quanto a produção de compostos bioativos.
Palavras-chave: métodos de fertilização; parâmetros de crescimento das plantas; compostos bioativos; agricultura sustentável; utilização de plantas medicinais.

1. INTRODUCTION

Medicinal plants continue to gain prominence for their valuable contributions to the pharmaceutical industry, particularly due to their rich reservoir of bioactive compounds. Atherolepis pierrei Costantin, commonly known as cinnamon vine and found along the forest edges of Thailand, is one such underexplored medicinal plant with promising pharmacological potential. Traditionally valued in local medicine, this species offers a compelling subject for research amid the rising global demand for natural pharmaceutical ingredients.

To cultivate medicinal plants sustainably while maximizing their therapeutic value, it is essential to understand the influence of agricultural practices, particularly fertilization, on both growth and phytochemical profiles. Fertilization not only affects biomass production but also modulates the biosynthesis of bioactive compounds, which are central to a plant's pharmacological efficacy. Organic fertilizers, derived from plant and animal sources such as compost, manure, and seaweed extract, play a vital role in sustainable agriculture. These inputs enhance soil structure, improve microbial activity, increase water retention, and release nutrients gradually, thereby maintaining long-term soil fertility (NAGUIB, 2011). Conversely, chemical fertilizers, comprising synthetic nutrients and minerals, are designed for rapid nutrient release and immediate plant uptake. While effective in boosting short-term growth, excessive or unbalanced chemical fertilizer use may contribute to soil degradation, water pollution, and reduced biodiversity (MALGHANI et al., 2010; KHALID; SHEDEED, 2015).

The comparison between organic and chemical fertilizers reveals important distinctions in terms of nutrient content, application methods, environmental impact, and long-term sustainability. Organic fertilizers typically contain lower and variable nutrient levels but promote ecological balance and resilience. In contrast, chemical fertilizers offer standardized and high nutrient content - particularly NPK (Nitrogen, Phosphorus, Potassium) - with ratios reaching up to 60%, making them effective in nutrient-poor soils but potentially detrimental over time if misused (DUBEY et al., 2017; RIVAI et al., 2017; HANDAYATI; SIHOMBING, 2019).

Recognizing the strengths and limitations of both fertilizer types, recent research increasingly explores synergistic or integrated fertilization approaches, combining organic and chemical inputs to optimize plant growth while maintaining soil health and ecological integrity.

This study addresses a critical gap in understanding how fertilization regimes affect the growth and pharmacological attributes of *Atherolepis pierrei* Costantin. Specifically, it evaluates the plant's agronomic performance and bioactive compound profiles under organic, chemical, and combined fertilization treatments. The overarching objectives are: To assess the effects of different fertilization strategies on the growth performance of *A. pierrei*; To analyze the influence of fertilization on the plant's phytochemical composition; To identify optimal fertilization conditions that enhance both biomass yield and pharmacological potential. By integrating agronomic evaluation with bioactive compound analysis, this research aims to contribute to sustainable cultivation practices and inform pharmaceutical development strategies. Ultimately, the findings may support the domestication and commercial utilization of Atherolepis pierrei as a valuable natural resource in herbal medicine and related industries.

2. MATERIAL AND METHODS

2.1. Materials

Atherolepis pierrei Costantin was propagated from plantlets to ensure uniform starting material and cultivated in soil-filled pots to maintain a controlled environment conducive to root development and nutrient availability. The experiment was conducted at the Pauwadee Laboratory, Mae Jo University, from July to December 2023. During this period, plant growth parameters, including height, stem diameter, leaf area, and biomass, were measured to assess the effects of different fertilizer treatments on overall growth and development.

2.2. Methodology

Atherolepis pierrei Costantin was propagated from plantlets and cultivated in soil-filled pots to ensure uniformity and control. The experiment, conducted at the Pauwadee Laboratory, Mae Jo University (July–December 2023), followed a Randomized Complete Block Design (RCBD) with 10 treatment formulas, including a no-fertilizer control. Treatments included varying dosages of NPK fertilizer (T1: 5g, T2: 10g, T3: 15g), compost (T4: 50g, T5: 100g, T6: 150g), and combined applications (T7: 5g NPK + 50g compost, T8: 10g NPK + 100g compost, T9: 15g NPK + 150g compost). Plant height, stem diameter, leaf area, and biomass were recorded to evaluate growth responses across treatments (Table 1; Figure 1).

Table 1. Formulas for treatments.
Tabela 1. Fórmulas para tratamentos.

Compost dosage (g/pot)	NPK dosage (g pot^{-1}) 0	5	10	15
0	Control	T1	T2	T3
50	T4	T7	-	-
100	T5	-	T8	-
150	T6	-	-	T9

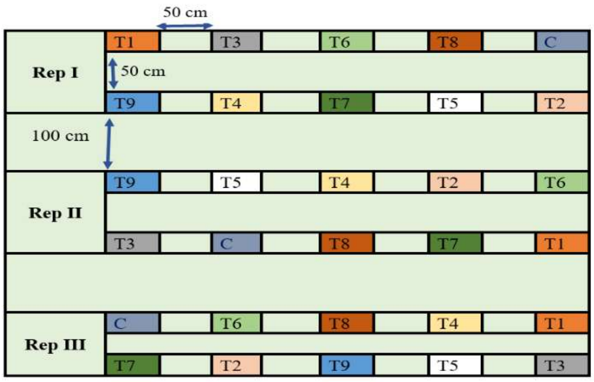

Figure 1. Experimental setup (RCBD).
Figura 1. Configuração experimental (RCBD).

2.3 Growth Monitoring and Phytochemical Analysis

To evaluate the effects of fertilization on plant development, growth parameters were recorded every three days over 93 days. Fertilizer was applied twice: before planting and again on Day 60. Plant height was measured from base to tip using a tape measure, while stem diameter was recorded at specific points using a caliper. Leaf area was assessed with an optical leaf area meter, and the number of leaves per plant was counted to evaluate vegetative growth. Soil moisture and pH were regularly monitored using a Yieryi 4-in-1 pH meter, and light intensity was measured with a light meter under varying conditions. These environmental parameters ensured consistent growing conditions and minimized confounding variables. At harvest, all above-ground biomass was collected, dried, and weighed to quantify yield. Phytochemical analysis was conducted to assess the concentrations of alkaloids, flavonoids, tannins, and phenolics, offering insights into the pharmacological potential of Atherolepis pierrei under different fertilizer treatments.

2.4 Comparison of extraction methods

The extraction was conducted at the Pauwadee Laboratory, Mae Jo University, from November 2–5, 2023, using dried, coarsely ground root and leaf powders of Atherolepis pierrei. For each sample, 5g of dried biomass was placed in a cellulose thimble and extracted using 100 mL of solvent in a pear-shaped flask. The process was carried out at the solvent's specific evaporation temperature for 3 hours to ensure maximum extraction of bioactive compounds (see Table 2). Four different solvents were used to evaluate their efficiency in extracting bioactive compounds from Atherolepis pierrei. This comparative approach aimed to assess the effectiveness of each solvent in isolating target phytochemicals. The results offered valuable insights into solvent-dependent extraction efficiency, highlighting the critical role of solvent selection in optimizing compound recovery from plant materials.

Table 2. Extraction of bioactive compounds with different solvents
Tabela 2. Extração de compostos bioativos com diferentes solventes

Conditions	Water	Chloroform	Hexane	Ethanol
Sample: solvent ratio (g:mL)	5: 100	5: 100	5: 100	5: 100
Temperature (^oC)	100	61-62	68-69	78 -79
Duration (hour)	3	3	3	3

2.5. Analysis of Bioactive Components

2.5.1. DPPH Analysis

The antioxidant activity of the plant extracts was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. Each sample was prepared by dissolving 0.03 g of extract in a 0.004% DPPH ethanol solution. Following incubation, absorbance was measured at 517 nm using a spectrophotometer to determine the reduction in DPPH radical concentration. The scavenging activity was calculated using a standard formula, providing quantitative insights into the antioxidant potential of the extracts.

2.5.2. Total Phenolic Content (TPC) Assay

The Total Phenolic Content (TPC) of the extracts was determined using the Folin-Ciocalteu assay, with gallic acid serving as the standard. A stock solution of 5 g L^{-1} was prepared by dissolving 0.5 g of gallic acid in a mixture of 10 mL ethanol and 90 mL distilled water. From this, five standard solutions were prepared at concentrations of 50, 100, 150, 250, and 500 mg L^{-1} to construct the calibration curve. For the assay, 20 µL of the extract (diluted 1:10 w/w in ethanol) was mixed with 1.58 mL of distilled water and 100 µL of Folin-Ciocalteu reagent. The mixture was incubated at room temperature for 7 minutes. Then, 300 µL of sodium carbonate (Na_2CO_3) solution was added, followed by a second incubation at 40°C for 30 minutes. Absorbance was measured at 765 nm using a spectrophotometer to quantify phenolic content.

2.5.3. GC-MS Analysis

Phytochemical components were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). Essential oils were first isolated from the extracts and prepared for analysis. The samples were injected into the chromatograph, where separation occurred, followed by mass spectrometry to generate spectra for compound identification. Bioactive constituents were identified by comparing the obtained spectra with entries in the NIST 98.1 database. This analytical approach provided detailed insights into the specific bioactive compounds present in Atherolepis pierrei Costantin, contributing to evaluating its medicinal potential.

2.5 Data Analysis

The data is presented as mean ± standard error (SE) based on triplicate observations. Significant differences between means were assessed using SPSS software. A correlation was considered significant when the p-value was less than 0.05.

3. RESULTS

As previously described, the soil was amended with varying concentrations of NPK, compost, and their combinations. These treatments were applied to both plant types, with a planting distance of 50 cm between plants and rows. Each treatment was replicated three times. The experiment was conducted under controlled laboratory conditions, with strict measures taken to prevent pest and insect infestations. Lighting was maintained at a consistent photoperiod of 8 hours per day to ensure uniform growth conditions across all treatments.

3.1. Growth indicators

3.1.1. Leaf Development Dynamics

Following the application of various fertilizer treatments, the number of leaves in Atherolepis pierrei Costantin was monitored every three days over 93 days (Figure 2). The data reveals distinct trends in vegetative growth across the 10 experimental treatments. The control group (C) exhibited a slow and steady increase in leaf number, beginning at an average of 2.67 leaves on Day 0 and peaking at 18.00 by Day 84, followed by a slight decline. T1 showed a progressive increase, reaching a maximum of 18.33 leaves by Day 51, after which the leaf count plateaued. Similarly, T2 demonstrated robust early growth, peaking at 27.00 leaves on Day 60 and maintaining that level through the remainder of the study. T3 initially followed the upward trend but experienced a sharp decline after Day 66, dropping to zero leaves by Day 93, indicating possible stress or over-fertilization at higher NPK levels. T4 and T5, representing moderate compost applications, performed well, with T4 peaking at 20.50 leaves and T5 matching T2's peak of 27.00 around Day 60. T6, the highest compost-only treatment, showed a diminishing trend after initial growth, falling to 17.00 leaves by Day 93, suggesting potential nutrient imbalance or oversaturation. In contrast, integrated treatments T7 and T8 (combinations of NPK and compost) showed stable and consistent growth, peaking at 24.50 and 18.00 leaves, respectively, near Day 84. Treatment T9 also showed a favorable growth curve, reaching a peak of 17.33 leaves by Day 75. Overall, treatments combining moderate levels of NPK and compost (notably T7) appeared to support sustained vegetative development more effectively than high-dose single-source fertilization.

The results of a one-way Analysis of Variance (ANOVA) were conducted to evaluate the effect of different fertilization treatments on the number of leaves in Atherolepis pierrei Costantin. The ANOVA includes three main components: Between Groups, Within Groups, and Total Variation. The

Between Groups component shows a sum of squares of 3734.77 with 9 degrees of freedom, resulting in a mean square of 414.97. This yielded an F-value of 6.158 and a highly significant p-value ($p < 0.001$), indicating a statistically significant difference in the number of leaves among the different treatment groups. The Within Groups component, representing variability within each treatment group, has a sum of squares of 49,398.05 and a mean square of 67.39. The Total variation across all samples amounted to 53,132.82. These findings confirm that the type and concentration of fertilizer treatments significantly influenced leaf development in Atherolepis pierrei, validating the observed growth trends and supporting the importance of fertilizer selection for optimizing vegetative performance.

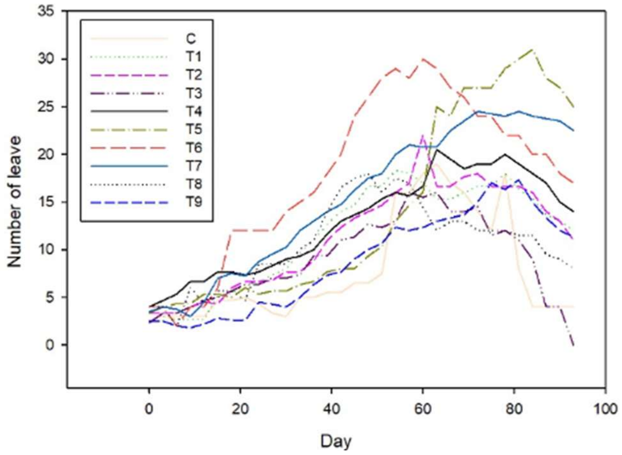

Figure 2. Average number of leaves from *Atherolepis pierrei* Costantin treatments.
Figura 2. Número médio de folhas dos tratamentos com *Atherolepis pierrei* Costantin.

3.1.2. Plant height

Following transplantation into soil pots treated with different fertilizer formulations, the plant height of Atherolepis pierrei Costantin was monitored every three days over a 93-day growth period (Figure 3). The results demonstrate distinct growth trajectories across the treatment groups. The control group (C) showed a steady increase in plant height, reaching a final height of 75.00 cm by Day 93. Treatment T1 displayed consistent growth throughout the period, ending at 84.60 cm. Treatments T2 and T3 followed similar trends, with growth stabilizing around Day 70 and peaking at 72.17 cm and 70.73 cm, respectively. Notably, Treatment T4 exhibited a significant growth response, reaching the highest recorded height of 136.00 cm, indicating a strong positive effect of moderate compost application. Treatment T5 also performed well, achieving a final height of 90.00 cm, showing continuous upward growth. Treatment T6 initially grew gradually but experienced a sharp increase around Day 24, ultimately reaching 135.00 cm - comparable to T4, suggesting a potentially delayed but impactful response to high compost input. Treatment T7, a combined fertilizer treatment, showed steady growth and peaked at 112.88 cm, while T8 reached 77.00 cm, showing moderate but stable growth. Treatment T9 exhibited some fluctuations throughout the growth cycle but still attained a respectable height of 75.73 cm by the end of the trial. An analysis of variance (ANOVA) was conducted to determine whether the differences in plant height across treatments were statistically significant (Table 4). The between-group sum of squares was 73,683.6 with 9 degrees of freedom, resulting in a mean square value of 8,187.07. The within-group variance was 1,088,481.36 with 735 degrees of freedom, yielding a mean square of 1,480.93. The resulting F-value of 5.53 and a significance level of $p < 0.001$ indicate a statistically significant difference in plant height among the treatments. These results confirm that fertilizer type and dosage had a substantial impact on plant growth performance during the experimental period.

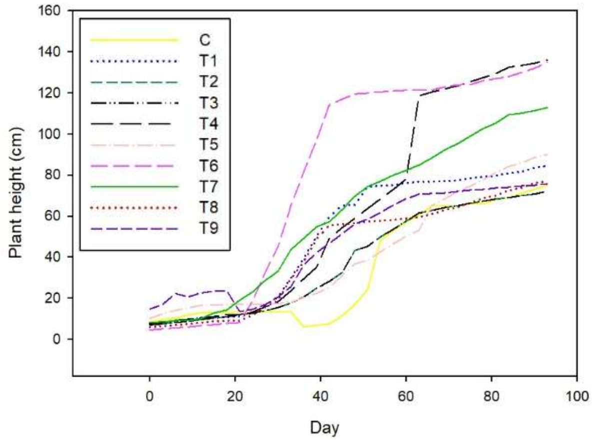

Figure 3. Average plant height of Atherolepis pierrei Costantin under different fertilization treatments over 93 days.
Figura 3. Altura média das plantas de *Atherolepis pierrei* Costantin sob diferentes tratamentos fertilizantes ao longo de 93 dias.

3.1.3 Leave width

After the plants are planted in soil pots, each mixed with a unique formula containing varying fertilizer levels, a monitoring process is initiated. The width of the leaves is recorded every 3 days over a post-planting cycle of 93 days (Figure 4). After transplantation, the leaf width of Atherolepis pierrei Costantin was measured every three days over 93 days, with each treatment group receiving a unique fertilizer formula (Figure 6). The data reveal varied growth trends in response to different fertilization regimes. The control group (C) started with a leaf width of 1.00 cm and gradually increased to 2.30 cm by Day 60, stabilizing thereafter. Treatment T1 displayed consistent growth, reaching 1.93 cm by Day 93. T2 and T3 followed a steady upward trajectory, leveling off at 2.17 cm and 2.00 cm, respectively, after Day 60. Treatment T4 showed a more erratic growth pattern, peaking at 2.55 cm before experiencing slight fluctuations. T5 demonstrated continuous and gradual growth, reaching 2.70 cm by the end of the trial - the highest recorded value among all treatments. T6 maintained a relatively stable width of 2.00 cm, suggesting a limited response to the highest compost application. Treatment T7 achieved a moderate increase, ending at 2.18 cm, while T8 showed variable growth, initially declining but ultimately reaching 2.45 cm. T9 exhibited consistent upward growth, peaking at 2.63 cm by Day 93. These observations suggest that leaf width is influenced not only by the type of fertilizer but also by its dosage and combination. Treatments T5 and T9, in particular, outperformed others, highlighting the potential of moderate to high compost or combined fertilization in promoting broader leaf development. An ANOVA was performed to assess the statistical significance of the differences in leaf width among treatments (Table 5). The between-group sum of squares was 68.38 with 9 degrees of freedom, producing a mean square of 7.60. The within-group variance was 422.79 across 735 degrees of freedom, yielding a mean square of 0.58. The resulting F-value of 13.21 and p-value < 0.001 indicate a statistically significant difference in leaf width across the treatment groups. These results confirm that fertilizer type and concentration significantly influenced leaf development over the 93-day growth period.

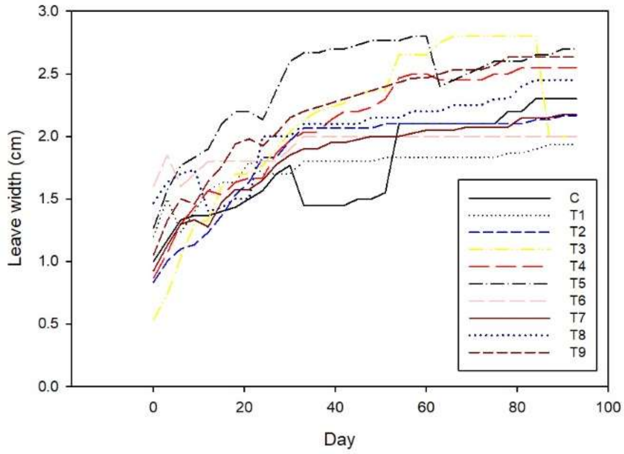

Figure 4. Average leave width of the *Atherolepis pierrei* Costantin treatments.
Figura 4. Largura média das folhas dos tratamentos com *Atherolepis pierrei* Costantin.

3.1.4. Leave length

Leaf length in Atherolepis pierrei Costantin was measured every three days over 93 days following transplantation into soil pots, each treated with a distinct fertilizer formulation (Figure 5). The data reveal considerable variation in leaf elongation across treatments, reflecting differing responses to fertilization strategies. The control group (C) began with an average leaf length of 6.20 cm and gradually increased to 10.70 cm, stabilizing after Day 60. Treatment T1 showed steady growth, reaching 8.67 cm by Day 93. T2 and T3 exhibited moderate growth trends, plateauing at 7.83 cm and 6.70 cm, respectively. Treatment T4 demonstrated a slightly erratic pattern, peaking at 9.45 cm with minor fluctuations afterward. T5 followed a more linear growth trend, culminating at 9.55 cm on Day 93. Interestingly, T6 displayed a unique pattern with a constant leaf length of 12.70 cm throughout the entire period, suggesting a rapid early growth phase followed by stabilization.m Treatment T7 showed a steady increase in leaf length, reaching 9.40 cm, while T8 displayed a decline early on, followed by recovery to a final length of 7.05 cm. T9 exhibited one of the most robust growth patterns, consistently increasing to 11.17 cm by Day 93. These varied growth responses underline the influence of both fertilizer type and dosage on leaf elongation, with treatments T6 and T9 demonstrating superior outcomes. To evaluate statistical differences in leaf length among treatments, an ANOVA test was conducted. The between-group sum of squares was 1282.88 with 9 degrees of freedom, resulting in a mean square of 142.54. The within-group variation was 6715.67 over 708 degrees of freedom, giving a mean square of 9.49. The calculated F-value of 15.03 and p-value < 0.001 indicate highly significant differences in leaf length among the treatment groups. These results confirm that the differences observed in growth patterns are not due to random variation but are significantly influenced by the applied fertilizer treatments.

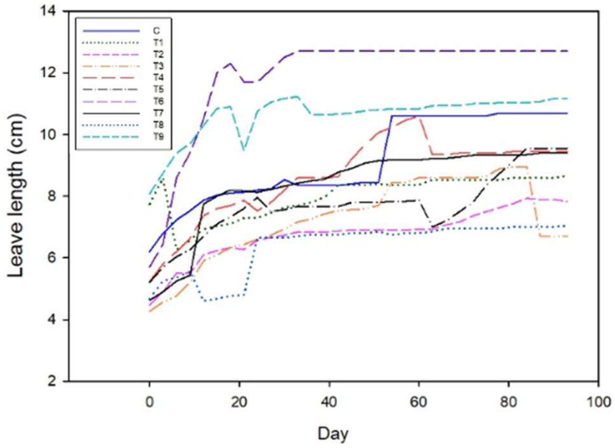

Figure 5. Average leaf length of Atherolepis pierrei Costantin across different fertilizer treatments.
Figura 5. Comprimento médio das folhas de Atherolepis pierrei Costantin sob diferentes tratamentos fertilizantes.

3.1.5. Soil moisture

Following planting, soil moisture in each pot was monitored every three days over 93 days. Each pot was prepared with different fertilizer treatments, including a control (C) and nine variations (T1-T9). The percentage of soil moisture was recorded to evaluate how different fertilization strategies influenced water retention (Figure 8). The control (C) consistently maintained high moisture levels, frequently reaching 100%, with occasional dips to 75%. Treatment T1 followed a similar trend, with soil moisture generally at 100%, except for a few values dropping to 75% and 83.33%. T2 showed variation between 75% and 100%, with most measurements remaining at the higher end. T3 maintained soil moisture around 87.5%, with occasional fluctuations between 75% and 100%. Treatment T4 had a wider range, spanning from 58.33% to 100%, though values frequently returned to the maximum level. T5 was more stable, generally holding between 75% and 100%. T6 consistently sustained 100% soil moisture, with only minor deviations. T7 hovered around 87.5% throughout the period. T8 and T9 displayed moderate variation, mostly maintaining high moisture levels between 75% and 100%, with T9 averaging 91.67%. These results suggest that most treatments were effective at preserving soil moisture over the monitoring period, with some variability depending on the fertilizer type and dosage. An ANOVA test was conducted to assess whether the observed differences in soil moisture across treatments were statistically significant. The between-group sum of squares was 4884.33 with 9 degrees of freedom, yielding a mean square of 542.70. The within-group variation was 118,478.92 across 579 degrees of freedom, resulting in a mean square of 204.63. The calculated F-statistic was 2.65, and the p-value was 0.005, indicating a statistically significant difference in soil moisture among the treatments. This implies that at least two fertilizer treatments led to distinct water retention effects over the 93 days.

3.1.6. Soil temperature

After transplanting, soil temperature was monitored every three days for 93 days. Each treatment group, including the control (C) and nine fertilizer treatments (T1-T9), was assessed to determine how fertilization might influence thermal conditions at the root zone. Under control conditions (C), soil temperature started at 30.0°C and fluctuated between 26.0°C and 30.0°C throughout the observation period. Treatment T1 followed a similar trend, beginning at 30.0°C with minor fluctuations in the same range. This general pattern was consistent across all treatments, with only slight variations, suggesting a stable thermal environment under controlled laboratory conditions. To evaluate whether soil temperature significantly differed among the various treatment groups, a one-way ANOVA was conducted. The test produced an F-value of 1.79 with a p-value of 0.068. Although there is a slight trend toward statistical significance, the p-value exceeds the standard threshold of 0.05. Thus, the analysis indicates that the observed differences in soil temperature between treatments are not statistically significant. This suggests that fertilizer type and concentration did not notably affect soil temperature under the controlled conditions of this study. Further investigation or a larger sample size may be required to confirm any subtle influences of fertilization on soil thermal dynamics.

3.1.7. Soil pH

Following the sowing of Atherolepis pierrei Costantin in soil pots treated with varying fertilizer concentrations, soil pH was monitored systematically every three days over 93 days. The aim was to observe potential shifts in soil acidity or alkalinity under different treatment conditions, labeled as C (control) and T1-T9. Under control conditions (C), soil pH values fluctuated between 6.50 and 7.20, indicating slightly acidic to neutral conditions. Treatment T1 showed a slightly more acidic range, varying between 5.97 and 7.00. Similar patterns of fluctuation were observed across all treatments, with minor variations in pH levels depending on the fertilizer formulation and dosage. These trends suggest that fertilization impacted soil pH to varying extents. To assess whether these pH variations were statistically significant across treatments, an ANOVA test was conducted (Table 9). The between-group sum of squares was 95.951 across 9 degrees of freedom, resulting in a mean square of 10.661. The within-group sum of squares was 942.936 over 579 degrees of freedom, yielding a mean square of 1.629. The test produced an F-value of 6.546 and a p-value < 0.001, indicating that the differences in soil pH between treatment groups were statistically significant. This confirms that the fertilizer type and concentration had a measurable effect on soil acidity or alkalinity during the 93-day growth period.

3.1.8. Biomass

With a planting density of 50 cm × 50 cm, approximately 40,000 plants per hectare can be established. Upon harvesting, both the fresh and dry biomass of Atherolepis pierrei Costantin were measured under different fertilizer treatments (C, T1-T9). The results are summarized in Table 3. Among the treatments, T4 produced the highest fresh biomass yield, recording 194.80 ± 0.5 kg ha^{-1}, while T3 yielded the lowest at 41.73 ± 0.2 kg ha^{-1}. Similarly, T4 also exhibited the highest dry biomass yield at 60.27 ± 0.3 kg ha^{-1}, whereas the control group (C) showed the lowest dry yield, with only 20.13 ± 0.2 kg ha^{-1}. These findings highlight the superior performance of Treatment T4, suggesting that this compost-based formulation significantly enhances biomass accumulation in Atherolepis pierrei Costantin compared to both the control and other fertilization strategies.

Table 3. The average yield of each treatment.
Tabela 3. Rendimento médio de cada tratamento.

Treatment	*Atherolepis pierrei* Costantin yield (kg ha^{-1})	
	Fresh	Dry
C	71.73 ± 0.2	20.13 ± 0.2
T1	178.93 ± 0.5	60.27 ± 0.2
T2	79.20 ± 0.2	28.53 ± 0.2
T3	41.73 ± 0.2	24.93 ± 0.2
T4	194.80 ± 0.5	60.27 ± 0.3
T5	124.13 ± 0.3	34.80 ± 0.2
T6	178.00 ± 0.4	51.00 ± 0.3
T7	143.87 ± 0.4	44.67 ± 0.2
T8	141.60 ± 0.4	42.80 ± 0.2
T9	90.80 ± 0.2	37.73 ± 0.2

Table 4. ANOVA test for fresh and dry yield from treatments.
Tabela 4. Teste Anova para rendimento fresco e seco dos tratamentos.

		Sum of Squares	df	Mean Square	F	Sig.
Fresh yield	Between Groups	42436.741	9	4715.193	31.254	<.001
	Within Groups	1508.654	10	150.865		
	Total	43945.395	19			
Dry yield	Between Groups	3122.057	9	346.895	38.559	<.001
	Within Groups	89.964	10	8.996		
	Total	3212.021	19			

Fresh yield, a key indicator of plant productivity, exhibited significant variation across the different fertilizer treatments. ANOVA results (Table 4) confirmed statistically significant differences in fresh biomass yield ($p < 0.001$), leading to further investigation via post hoc analysis. T4 demonstrated a significantly higher fresh yield than all other treatments, underscoring its effectiveness in promoting above-ground biomass accumulation. In contrast, treatments T3 to T9 recorded significantly lower yields, suggesting either suboptimal nutrient availability or potential adverse interactions associated with specific fertilizer concentrations or combinations. Similarly, analysis of dry biomass yield also revealed significant differences among treatments ($p < 0.001$). Post hoc comparisons identified distinct treatment pairs with statistically different dry matter outputs. These results highlight the variability in how different fertilization strategies influence biomass retention after moisture loss - an important metric for medicinal and industrial applications where dry weight is more relevant. Overall, these findings provide critical insights into the productivity potential of various fertilizer treatments, particularly emphasizing the agronomic superiority of T4. Moreover, the observed differences in both fresh and dry yields underscore the importance of optimizing fertilization practices to ensure both high productivity and sustainability in Atherolepis pierrei Costantin cultivation.

3.2. Extraction methods

The solvent extortion yields are displayed in Table 5. Among the four tested solvents, chloroform yielded very low extract volumes, with all treatments producing less than 1.5 mL. Due to its limited efficiency, chloroform extraction was excluded from further comparisons. The analysis, therefore, focused on ethanol extraction, hexane extraction, and water distillation, which demonstrated higher and more reliable extraction volumes. T3 consistently yielded the highest extract volumes across all three methods, especially under ethanol extraction (11.5 ± 0.5 mL). Treatments T6 and T7 also demonstrated relatively high extract yields. These results suggest that moderate to higher fertilization may improve the extractable content of bioactive compounds.

Table 5. Volume of extracts (mL).
Tabela 5. Volume dos extratos (mL).

Treatment	Ethanol extraction	Hexane extraction	Water distillation
C	6.5 ± 0.3	6 ± 0.2	5 ± 0.2
T1	6.5 ± 0.3	7.5 ± 0.4	6 ± 0.2
T2	7 ± 0.4	7 ± 0.4	5.5 ± 0.2
T3	11.5 ± 0.5	9.5 ± 0.5	8 ± 0.5
T4	7.5 ± 0.4	9 ± 0.5	7 ± 0.4
T5	7.5 ± 0.4	8.5 ± 0.5	7.5 ± 0.4
T6	8.5 ± 0.4	9 ± 0.5	8 ± 0.4
T7	10 ± 0.5	8 ± 0.5	7 ± 0.4
T8	8 ± 0.5	7.5 ± 0.4	6.5 ± 0.3
T9	7 ± 0.4	6.5 ± 0.3	5 ± 0.2

Table 6. Number of peaks of GCMS peaks of treatments.
Tabela 6. Número de picos de GCMS dos tratamentos.

Treatment	Ethanol extraction	Hexane extraction	Water distillation
C	0	0	0
T1	16	15	10
T2	5	4	4
T3	20	16	15
T4	10	10	10
T5	15	11	10
T6	16	16	15
T7	17	15	11
T8	11	10	4
T9	4	4	0

The complexity and abundance of compounds were evaluated using Gas Chromatography-Mass Spectrometry (GC-MS), where the number of peaks corresponds to the number of identifiable phytochemical constituents in the extract as indicated in Table 6. Ethanol extraction consistently yielded the highest number of GC-MS peaks across treatments, indicating it was the most effective solvent for recovering diverse bioactive compounds. Treatment T3 again stood out, producing the highest peak count (20 peaks), suggesting a rich and complex phytochemical profile. Treatments T6 and T7 also demonstrated high chemical diversity. In contrast, hexane extraction and water distillation produced fewer peaks, with the control and T9 showing no detectable peaks in several cases. These results emphasize the importance of both fertilization and extraction strategies in maximizing the detection of pharmacologically relevant compounds.

3.3. Phytochemical components

3.3.1. Total phenol content (TPC)

The Total Phenolic Content (TPC) of Atherolepis pierrei Costantin was analyzed under different fertilization treatments, labeled C (control) and T1 through T9 (Figure 6). Measurements were recorded in absorbance (ABS at 765 nm) and expressed as gallic acid equivalents (mg GAE/g). The analysis provides insights into the influence of treatment conditions on the accumulation of phenolic compounds known for their antioxidant properties. As shown in Figure 16, the control group (C) had a TPC value of 0.236 ± 0.03 mg GAE/g, representing the natural baseline of phenolic content without treatment intervention. In contrast, T9 showed the highest TPC value, reaching 4.902 ± 0.03 mg GAE/g, indicating a more than 20-fold increase relative to the control. This substantial enhancement suggests that the specific fertilization conditions in T9 significantly promote phenolic biosynthesis or extraction efficiency. Treatment T7 also recorded a high TPC value of 3.358 ± 0.03 mg GAE/g, further emphasizing the positive effect of certain fertilization strategies. Other treatments (T1–T8) varied in TPC values between 1.213 and 3.358, all showing higher values than the control. These findings highlight the potential of strategic fertilization in maximizing the recovery of bioactive compounds, which may be beneficial for pharmaceutical, nutraceutical, and functional food applications. To evaluate whether the observed differences in TPC across treatments were statistically significant, an ANOVA test was performed. The results indicate a significant difference between treatments ($F = 54.119$, $p < 0.001$). The between-group sum of squares was 26.908 with 9 degrees of freedom, and the within-group variation was 0.552 across 10 degrees of freedom. These values confirm that the increase in phenolic content across treatments is not due to random variation but is a direct result of the experimental fertilization strategies. Post hoc tests further revealed that treatments such as T9, T7, and T3 had significantly higher TPC compared to others, providing strong evidence of their biochemical enhancement capabilities.

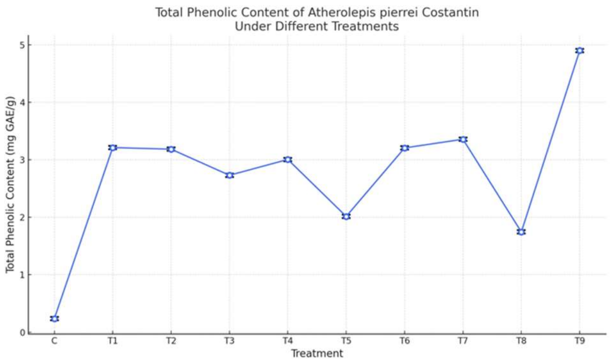

Figure 6. Results of total phenol content (mg g-1).
Figura 6. Resultados do teor total de fenol (mg g-1).

3.4.1. DPPH Radical Scavenging Activity

The DPPH The antioxidant activity of *A. pierrei* Costantin was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, with absorbance measured at 517 nm. The results, expressed as percentage scavenging activity, revealed substantial variation across treatments (Figure 7). The control group (C) exhibited the lowest activity at 11.53%, representing the baseline antioxidant potential of the untreated plant. In contrast, all fertilized treatments (T1–T9) demonstrated significantly higher DPPH values, indicating enhanced free radical scavenging capacity. Among them, Treatment T9 recorded the highest DPPH activity at 29.33%, followed closely by T8 (28.89%) and T3 (24.12%). Treatments T1, T2, and T7 also showed moderately high activity, with values ranging from 21.09% to 22.75%. Meanwhile, Treatments T4, T5, and T6 had relatively lower but still improved values (approximately 14.3–14.6%) compared to the control. These findings indicate that fertilization substantially improves the antioxidant potential of Atherolepis pierrei extracts, particularly under the conditions applied in T9 and T8. Statistical analysis using one-way ANOVA confirmed significant differences among the treatment groups ($F = 27.094$, $p < 0.001$), validating the influence of fertilization on antioxidant activity. The consistency of standard deviation (±0.4) across all treatments suggests uniform measurement precision. Overall, the results highlight the efficacy of specific fertilization strategies in enhancing the bioactive properties of Atherolepis pierrei, with potential applications in the development of antioxidant-rich natural products.

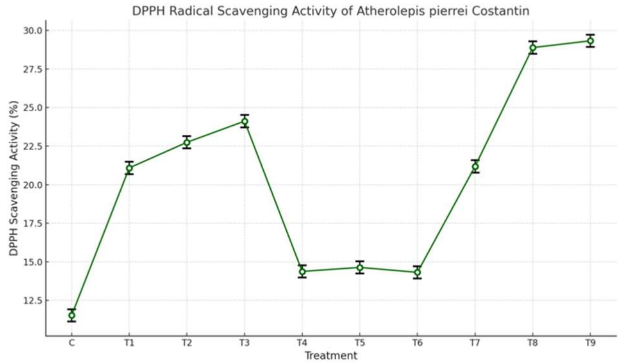

Figure 71. DPPH results.
Figura 7. Resultados do DPPH.

3.4.2. GCMS

Gas Chromatography-Mass Spectrometry (GC-MS) analysis revealed a diverse and complex array of bioactive compounds in Atherolepis pierrei Costantin extracts, with results varying notably by extraction method and treatment (Figure 8). Among the three methods tested ethanol extraction, hexane extraction, and water distillation ethanol consistently yielded the highest number of GC-MS peaks, indicating superior efficiency in extracting a broader spectrum of phytochemicals. Treatment T3 recorded the highest number of peaks (20) under ethanol extraction, suggesting a particularly rich and chemically diverse profile. Similarly, Treatments T7 and T8 also showed high peak counts, reinforcing the effectiveness of certain fertilization conditions combined with ethanol extraction. In contrast, hexane extraction produced fewer peaks overall, and water distillation, particularly in Treatments T1 and T9, failed to detect any GC-MS peaks, reflecting its limitations for this plant material. The compounds identified through GC-MS include a wide range of structurally and functionally diverse molecules, such as benzaldehyde, 2-hydroxy-4-methoxy; phytol; ferruginol; n-hexadecanoic acid; squalene; heneicosane; cyclodecene, 1-methyl-; cyclododecanone; and urs-12-en-24-oic acid, 3-oxo-, methyl ester. These compounds belong to various chemical classes with known or potential pharmacological and therapeutic properties. Notably, long-chain hydrocarbons, esters, terpenoids, and fatty acids were prevalent, hinting at antioxidant, anti-inflammatory, and antimicrobial potential. The analysis also highlighted treatment-specific differences, with Treatment T3 again standing out across multiple extraction types. Conversely, Treatment T8 showed reduced peak counts under water distillation, underscoring the influence of both the extraction method and fertilization strategy on phytochemical yield. These findings emphasize the importance of selecting appropriate extraction techniques to maximize the recovery of target compounds and lay a foundation for future research into the bioactivity and applications of Atherolepis pierrei Costantin in pharmaceutical and biotechnological contexts.

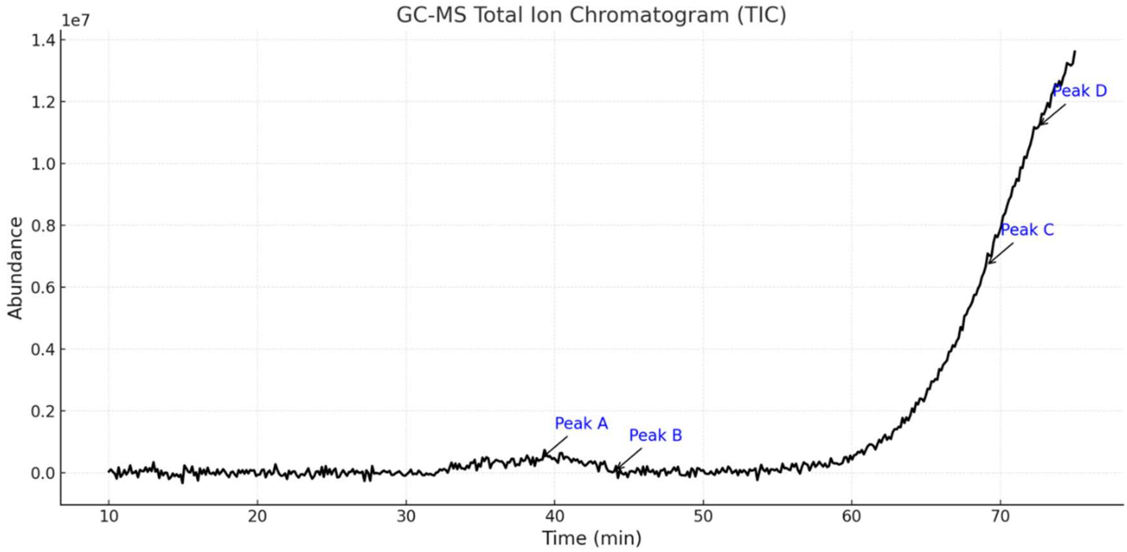

Figure 8. Highest GCMS peaks (T3) observed of *Atherolepis pierrei* Costantin by the best effective extraction method (ethanol extraction).
Figura 8. Maiores picos de GCMS (T3) observados em *Atherolepis pierrei* Costantin pelo melhor método de extração eficaz (extração com etanol).

The phytochemical profiling of *A. pierrei* Costantin revealed the presence of several bioactive compounds with significant therapeutic potential, including benzaldehyde, 2-hydroxy-4-methoxy, lupeol, and β-amyrin. Benzaldehyde derivatives have been widely recognized for their antioxidant and antimicrobial properties, while lupeol is known for its anti-inflammatory and anticancer activities. β-amyrin has demonstrated hepatoprotective and antidiabetic effects, suggesting a broad spectrum of pharmacological relevance. These findings support further investigation into the medicinal applications of these compounds and their role in traditional medicine and natural product development. The effectiveness of different extraction methods, ethanol extraction, hexane extraction, and water distillation, was statistically evaluated using ANOVA, with results presented in Table 18. All three methods showed highly significant differences ($p<0.001$) in extraction efficiency across treatments. Ethanol extraction had the greatest variance between groups ($F=19.389$), followed by hexane ($F=25.956$) and distillation ($F=27.511$). These results underscore the importance of solvent selection in optimizing phytochemical yield and diversity. Notably, Treatment T3 consistently yielded the highest number of GC-MS peaks across all methods, indicating a rich and diverse chemical profile under that specific fertilization regime. While T9 enhanced certain antioxidant and phenolic metrics, it did not maximize compound diversity, highlighting the complexity of phytochemical expression influenced by different treatment conditions. These findings reinforce the need for a tailored approach when selecting fertilization strategies, depending on whether the target outcome is yield, antioxidant capacity, or compound diversity. Organic fertilization, as demonstrated in this study, emerged as a promising strategy for enhancing phytochemical richness. This provides a valuable direction for future research and industrial application, particularly in the development of natural therapeutics and bio-based products. Further studies are recommended to refine treatment combinations for maximizing specific compound groups and to investigate the synergistic effects of bioactive constituents under varying agronomic and extraction conditions.

4. DISCUSSION

This study provides an in-depth evaluation of how different fertilization strategies influence the growth performance and phytochemical profile of Atherolepis pierrei Costantin, a medicinally valuable yet underexplored species. The experimental design, which included varying dosages and combinations of organic (compost) and chemical (NPK) fertilizers, revealed significant differences in both plant growth parameters and biochemical outputs. The results highlight that integrated fertilization, particularly combinations such as vermicompost or poultry manure with NPK, produced optimal outcomes in terms of biomass accumulation, leaf development, and plant height. These findings are consistent with previous studies, which emphasize the synergistic effects of combining organic and inorganic fertilizers to enhance nutrient availability, improve soil structure, and support sustainable crop production (NAGUIB, 2011; HANDAYATI; SIHOMBING, 2019).

Soil parameter monitoring showed that treatments impacted not just plant growth but also soil pH, moisture content, and temperature stability, which are essential for root function and nutrient uptake. The analysis revealed that organic amendments helped maintain soil moisture and slightly more favorable pH conditions, thereby creating a more biologically active rhizosphere, as supported by Khalid; Shedeed (2015). The Randomized Complete Block Design (RCBD) further ensured that observed differences were statistically valid, with ANOVA tests confirming highly significant effects of treatments on variables such as leaf number, plant height, and phenolic content. On the phytochemical front, Total Phenolic Content (TPC) and DPPH antioxidant activity both showed substantial improvement under untreated conditions, particularly with Treatment T9, which yielded the highest TPC (4.902 mg GAE/g) and DPPH (29.33%).

The significant correlation between TPC and antioxidant activity highlights the effectiveness of specific fertilization strategies in boosting the biosynthesis of health-promoting compounds. This aligns with prior research linking phenolic abundance to antioxidant potential in medicinal plants (DUBEY et al., 2017; RIVAI et al., 2017). Furthermore, Gas

Chromatography-Mass Spectrometry (GC-MS) profiling revealed a rich spectrum of secondary metabolites, with Treatment T3 yielding the highest number of chemical peaks across ethanol, hexane, and water distillation methods. Key identified compounds included benzaldehyde, 2-hydroxy-4-methoxy, known for its antimicrobial and antioxidant effects (Lee et al., 2020); lupeol, associated with anti-inflammatory and anticancer properties (Geetha et al., 2012); and β-amyrin, recognized for hepatoprotective and antidiabetic actions (Sultana et al., 2014). These compounds suggest the plant's considerable potential for pharmaceutical and nutraceutical applications.

The diversity of metabolites further underscores the importance of optimizing extraction methods, with ethanol consistently outperforming hexane and water distillation in terms of compound richness. Interestingly, while some treatments maximized specific properties, such as antioxidant capacity or biomass yield, they did not necessarily enhance chemical diversity. For example, Treatment T9, although potent in increasing antioxidant metrics, produced fewer GC-MS peaks compared to Treatment T3. This suggests that optimizing phytochemical expression may require a multi-criteria approach that balances quantity, diversity, and pharmacological relevance. Moreover, the statistical significance of extraction method effects (ANOVA, $p < 0.001$ for all solvents) emphasizes the need for methodological precision in capturing full phytochemical spectra.

The study's findings contribute meaningfully to the discourse on sustainable medicinal plant cultivation, supporting the integration of organic fertilizers not only for soil health but also for maximizing pharmacologically active compound yields. This aligns with global sustainability goals in agriculture, promoting eco-friendly practices while enhancing the economic value of lesser-known medicinal species like Atherolepis pierrei.

5. CONCLUSIONS

In conclusion, this study highlights the significant impact of different fertilization strategies on the growth, yield, and phytochemical composition of Atherolepis pierrei Constantin. The combination of organic and chemical fertilizers, particularly vermicompost or poultry manure with NPK, resulted in the enhanced growth and production of bioactive compounds in the plant. Phytochemical analyses, including GC-MS, identified a range of bioactive compounds, such as benzaldehyde, 2-hydroxy-4-methoxy, lupeol, and β-amyrin, which possess potent antioxidant, antimicrobial, anti-inflammatory, and anticancer properties. These findings demonstrate the pharmaceutical potential of Atherolepis pierrei and underscore the importance of optimizing fertilization practices to maximize both plant productivity and the medicinal value of the plant. Further research is recommended to explore the underlying biochemical pathways and to refine the fertilization strategies for greater yield and enhanced bioactive compound production, contributing to sustainable agricultural practices and the development of novel, natural-based pharmaceutical agents.

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Data availability: Study data can be e-mail from the corresponding author or the second author upon request. It is not available on the website as the research project is still under development.

Conflict of interest: The authors declare no conflict of interest.