



Research Article

Application of fern *Davallia denticulata* L. extract using different solvents as biostimulants for Kopay chili (*Capsicum annuum* L.) production

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ABSTRACT

Ferns are a potential source of biostimulant compounds due to their rich content of bioactive secondary metabolites. This study aimed to evaluate different solvents for extracting bioactive compounds from *Davallia denticulata* and to identify the optimal extract formulation for enhancing the growth and yield of Kopay chili. This research was conducted from February to May 2024 at the Plant Physiology Research Laboratory and Greenhouse, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Andalas, Padang. A completely randomized design with 16 treatment combinations involving four solvents (A1 = aquadest, A2 = methanol, A3 = ethanol, A4 = butanol) and four extract concentrations (B0 = 0 mg L⁻¹, B1 = 25 mg L⁻¹, B2 = 50 mg L⁻¹, and B3 = 100 mg L⁻¹) was employed. Plant growth parameters responded differently to various combinations of extract concentration and solvent type. Methanol extract at 100 mg L⁻¹ significantly increased plant height, ethanol at 50 mg L⁻¹ improved shoot dry weight and root biomass, butanol at 100 mg L⁻¹ increased chlorophyll b and total chlorophyll, and methanol at 25 mg L⁻¹ resulted in higher fruit weight. These results indicate that *Davallia denticulata* extract, when properly formulated, holds strong potential as a natural biostimulant to improve growth and productivity in chili cultivation.

Keywords: active compounds; chili growth; plant extract; secondary metabolites; solvents extraction

INTRODUCTION

In recent years, the agricultural industry has faced challenges in boosting food production while efficiently utilizing environmentally friendly resources. Biostimulants have emerged as a promising strategy, particularly in organic farming. They enhance crop productivity, including yield, quality, and production efficiency, while also functioning as biofertilizers and biocontrol agents (Yakhin et al., 2017). Biostimulants improve physiological processes such as root development, nutrient uptake, hormonal regulation, and stress-response mechanisms, plant health, and growth (Martínez-Lorente et al., 2024). Biostimulants are increasingly recognized for their role in sustainable agriculture by enhancing yield and quality while reducing reliance on synthetic inputs (Shanmugam & Seth, 2018). The popularity of plant extracts is also increasing for sustainable weed management (Muntoyib et al., 2024).

Ferns represent a plant group with significant potential as biostimulants. Numerous studies have explored their efficacy in promoting plant growth. Zakiah et al. (2017) reported that *Gleichenia linearis* leaf extract at a concentration of 100 mg L⁻¹ positively

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influenced soybean growth. Similarly, Aulya et al. (2018) demonstrated that fern leaf extract at the same concentration effectively enhanced corn growth. Among the various ferns studied, the *Davallia denticulata* has been identified as a promising biostimulant source due to its rich phytochemical profile, including flavonoids, phenolics, terpenoids, steroids, and saponins (Hendra et al., 2020).

Furthermore, research on several species within the *Davalliaceae* family, including *D. denticulata* from the Mentawai Islands, revealed a terpenoid content of 17.39% in its leaf extract (Mildawati et al., 2020). The efficiency of these bioactive compounds in biostimulant applications is highly dependent on the extraction solvent used, as solvent polarity influences the solubility and stability of specific bioactive components. Despite its promising phytochemical profile, the use of *Davallia denticulata* as a biostimulant, particularly for chili (*Capsicum frutescens*), remains largely unexplored.

The selection of an appropriate solvent is a crucial factor in the extraction process, as it influences the ability to isolate and dissolve active compounds from plant material (Sa'adah & Nurhasnawati, 2017). Commonly used polar solvents for extraction include methanol (CH₃OH), ethanol (C₂H₅OH), butanol (C₄H₉OH), acetic acid (CH₃COOH), and water (H₂O) (Leksono et al., 2018). According to Nawaz et al. (2020), polar solvents facilitate the extraction of various bioactive compounds, including flavonoids, terpenoids, tannins, saponins, polypeptides, steroids, alkaloids, and anthocyanins. Different solvents possess varying polarities, which influence their ability to extract specific classes of bioactive compounds. For instance, polar solvents are more effective in extracting phenolics and flavonoids, while non-polar solvents are better suited for terpenoids and other lipophilic compounds (Lee et al., 2024). Additionally, the concentration of biostimulants applied to plants is critical in determining their effectiveness. Optimal concentrations can enhance plant growth and development, while excessively high or low concentrations may lead to suboptimal or inhibitory effects.

The concentration of biostimulants is pivotal in determining their efficacy, as an optimal dosage can promote plant growth. However, this optimal dosage is not a fixed value, but rather a variable that depends on plant species, environmental conditions, and the specific biostimulant used. This variability adds a layer of complexity to the topic. Excessive application may induce toxicity, and insufficient amounts may yield negligible effects. Higher concentrations may lead to phytotoxic effects, disrupting key physiological processes like photosynthesis and nutrient absorption, while lower concentrations may be insufficient to trigger significant growth responses. Several studies have highlighted the benefits of plant extract-based biostimulants; however, some findings indicate no significant effects on plant development. For instance, research on applying *Gleichenia linearis* extract at varying concentrations showed no impact on the vegetative growth of Kopay chili. Nevertheless, a concentration of 100 mg L⁻¹ was found to improve stem diameter, fresh fruit weight, fruit count per plant, and chlorophyll content (Noli & Labukti, 2022). To date, studies on the optimal concentration of *Davallia denticulata* extract for chili plants are lacking, making this research an initial exploration into its effective dosage range.

The Kopay chili is a high-yielding red chili variety in West Sumatra, characterized by its long fruit, reaching up to 35 cm, with a production of 60.81 tons, an increase of 5.74% compared to the previous year (BPS, 2013). However, this yield remains below its maximum potential of 20 tons per hectare (Atman, 2021). Enhancing Kopay chili productivity and quality can be achieved using natural biostimulants. Based on this background, research on optimizing solvent selection for extracting biostimulants from *Davallia denticulata* is essential to support chili (*Capsicum annum* L.) growth and yield. This study aimed to determine the most effective solvent for extracting bioactive compounds from *Davallia denticulata* and to evaluate their impact on the growth and yield of Kopay chili.

MATERIALS AND METHODS

Research site

This research was conducted from February to May 2024 at the Plant Physiology and Greenhouse Research Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang (Latitude: -0.914518 and Longitude: 100.459526).

The tools used in this study included beakers, measuring cups, filter paper (Whatman No.1), buckets, analytical scales, manual scales, collection plastic, label paper, meters, stationery, cameras, polybags (50 cm x 50 cm), plastic (3 x 20), ovens, centrifuges, sprayers, and spectrophotometers. The materials consisted of *Davallia denticulata* leaves, *Capsicum annuum* L. seeds, distilled water, 70% ethanol, 70% methanol, 80% n-butanol, 80% acetone, organic fertilizer, and plantation soil.

Experimental design and extraction

The experiment used a completely randomized design with a single treatment factor consisting of 16 levels, each representing a unique combination of four solvent types (A1 = aquadest, A2 = methanol, A3 = ethanol, A4 = butanol) and four extract concentrations (B0 = 0 mg L⁻¹, B1 = 25 mg L⁻¹, B2 = 50 mg L⁻¹, and B3 = 100 mg L⁻¹). Each treatment was replicated three times, for a total of 48 experimental units.

The extraction process, utilizing the cold maceration technique with an appropriate solvent, begins by preparing the plant material, which is first thoroughly cleaned, and then macerated for 24 hours at room temperature with occasional agitation. Subsequently, the material was ground to a fine consistency, targeting a particle size of approximately 0.5 mm, using a laboratory grinder, yielding about 10 g of powder.

The ground material was then placed into 100 mL of distilled water (aquadest) and left to soak overnight, following Soares et al. (2020). Similarly, the same procedure was repeated for methanol, ethanol, and butanol solvents.

The following day, the extract was filtered using Whatman No. 1 filter paper to separate the liquid extract from the solid plant residue. Finally, the extract was heated in an oven at 60 °C for aquadest and 40 °C for the methanol, ethanol, and butanol solvents for approximately one hour to evaporate all the solvents, leaving behind the desired solid active compounds. The solid extract obtained after evaporation was considered as 100% concentration.

Stock solutions of 25 mg L⁻¹ were prepared by dissolving 25 mg of the dried extract in distilled water and adjusting the final volume to 1 L. Regardless of the extraction solvent, all solutions were prepared in distilled water.

Application procedure

Kopay chili seeds (a widely cultivated red chili variety in West Sumatra) were obtained from a certified distributor in Payakumbuh City, West Sumatra. The planting media consisted of topsoil, cow manure compost, and charcoal rice husks mixed in a 4:1:1 ratio (m/m). The soil mixture was placed in small plastic pots for seeding and adequately watered, and left for a day before use.

The seeds were maintained in the seedling stage for three weeks. For the subsequent planting phase, the soil mix is transferred into 50 cm x 50 cm plastic bags, which were also watered. In each planting bag, a single seedling was planted. The bags were maintained in a greenhouse.

The plant extract was applied by uniformly spraying 25 mL per plant, targeting all aerial parts. Application began at 14 days after transplanting (DAP) and was conducted weekly starting at 08:00 AM. Treatments continued until the end of the vegetative phase, which occurs approximately 40 DAP. Harvest was conducted until 40 DAP.

Data analysis

The data were collected through observations on various aspects, including plant height, wet and dry weights of the crown, wet and dry weights of the root, chlorophyll a,

chlorophyll b, total chlorophyll, fruit weight, and fruit length. The results of these observations were statistically analyzed using one-way Analysis of Variance (ANOVA). For any significant effects were observed, Duncan's New Multiple Range Test (DNMRT) at a 5% significance level was performed for post hoc comparisons.

RESULTS AND DISCUSSION

Plant height

The statistical analysis revealed a significant effect of *D. denticulata* L. extract on plant height, particularly in relation to its concentration and the type of solvent used (Figure 1). These findings provide valuable insights into the potential of this extract in promoting plant growth through mechanisms likely associated with its phytochemical composition.

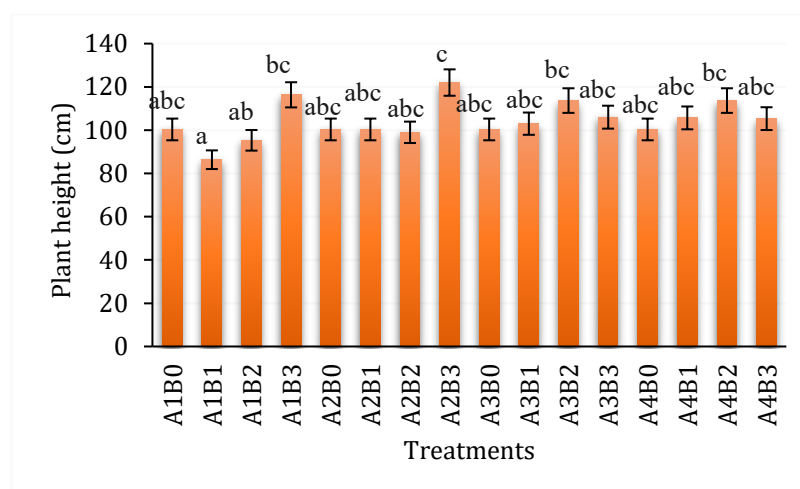


Figure 1. Plant height of Kopay Chili treated with several concentrations of *Davallia denticulata* extracted with several types of solvents. Numbers followed by the same letter in the same treatment and variable indicate no significant difference in the 5% DNMRT test. A1 = aquadest, A2 = methanol, A3 = ethanol, A4 = butanol, B0 = 0 mg L⁻¹, B1 = 25 mg L⁻¹, B2 = 50 mg L⁻¹, B3 = 100 mg L⁻¹

The application of various concentrations of *D. denticulata* L. extract showed a dose-dependent trend in plant height, with the magnitude of the effect also varying across solvent types (Figure 1). Such variation may be attributed to differences in the profile and yield of secondary metabolites extracted, as these compounds can influence vegetative growth either positively or negatively depending on their chemical nature, concentration, and interaction with endogenous plant hormones.

Among these metabolites, terpenoids have been reported to support plant growth. According to Li et al. (2023), terpenoids in plant tissues serve as precursors to gibberellin diterpenoids, which possess bioactivity that stimulates stem elongation and cell expansion or enhances gibberellin function. Gibberellins, derived from terpenoids, play a central role in promoting cell division and elongation, while abscisic acid (ABA), also derived from terpenoids, regulates stress responses and developmental transitions. The balance between these hormones is crucial for optimal growth, and external application of terpenoid-rich extracts may shift this balance toward enhanced elongation growth under favorable conditions.

As shown in Figure 1, the combination of a 100 mg L⁻¹ concentration of *D. denticulata* L. extract with methanol as the extraction solvent resulted in the greatest increase in plant height. This outcome suggests that the effectiveness of the extract was influenced not only by dosage but also by the solvent's capacity to solubilize and preserve bioactive growth-promoting compounds. Methanol, with its intermediate polarity, is known to effectively extract both polar and moderately nonpolar metabolites, including terpenoids, flavonoids,

and certain phenolics. Such compounds may have acted synergistically to enhance hormonal signaling pathways, stimulate photosynthetic efficiency, and promote cell expansion, thereby maximizing the physiological effects on plant growth.

Fresh and dry weight of shoots and roots (g)

The statistical analysis revealed a significant effect of *D. denticulata* L. extract and concentrations on shoot and root weight (Table 1). These findings underscore the importance of extract formulation in influencing biomass accumulation, offering valuable insights for both botanical research and agricultural applications.

Table 1. Fresh and dry weight of shoot dan root biomass of chili treated with different extraction methods and concentratons of *D. denticulata*.

Solvent extract	Concentration (mg L ⁻¹)	Shoot biomass (g)		Root biomass (g)	
		Fresh	Dry	Fresh	Dry
Aquadest	0	140.00abcd	33.16abcd	34.00ab	8.56ab
	25	106.67ab	21.89a	26.01ab	5.82a
	50	95.00a	24.36ab	18.59a	6.14a
	100	135.00abcd	38.25bcd	33.11ab	8.28ab
Methanol	0	140.00abcd	33.16abcd	34.00ab	8.56ab
	25	158.30cd	40.26cd	23.04ab	7.63ab
	50	118.33abc	32.33abcd	27.00ab	6.94a
	100	150.00bcd	38.85bcd	35.12ab	7.03a
Ethanol	0	140.00abcd	33.16abc	34.00ab	8.56ab
	25	126.67abcd	40.88cd	33.51ab	8.94ab
	50	133.33abcd	42.89d	41.45b	12.41b
	100	155.55bcd	40.99cd	33.70ab	7.94ab
Butanol	0	140.00abcd	33.16abcd	34.00ab	8.56ab
	25	168.33d	37.08bcd	29.09ab	7.39ab
	50	168.33d	40.05cd	26.27ab	7.10a
	100	125.00abcd	26.40abc	22.17ab	7.48 ab

Note: Numbers followed by the same letter in the same treatment and variable indicate no significant difference at the 5% DNMRT test.

As presented in Table 1, the application of *D. denticulata* L. extract using ethanol as the solvent at a concentration of 50 mg L⁻¹ produced the most favorable results for shoot dry weight, root fresh weight, and root dry weight. This suggests that the interaction between solvent type and concentration can optimize the extraction and delivery of bioactive compounds that promote biomass development in chili plants. Ethanol, with its ability to extract a broad range of polar and moderately nonpolar metabolites, including terpenoids, flavonoids, and certain phenolic compounds, may have provided an extract composition particularly effective in enhancing both shoot and root growth.

Biostimulants are known to improve plant growth and productivity by modulating physiological processes rather than acting as direct sources of nutrients. The observed increase in biomass may be linked to improved photosynthetic efficiency, enhanced nutrient assimilation, and stimulated hormonal activity, particularly gibberellins and auxins, that regulate cell division and elongation in both shoots and roots. Additionally, phenolic compounds present in ethanol extracts can contribute to root system development by influencing auxin signaling pathways, thereby increasing water and nutrient uptake to support shoot growth.

Previous studies support these findings, e.g., Adisti et al. (2023) reported that *Centella asiatica* extract obtained with methanol and ethanol acted as an effective biostimulant for improving the growth of pagoda mustard. Similarly, Aulya et al. (2018) showed that applying biostimulants at a concentration of 25 mg L⁻¹ enhanced plant height, leaf number, and fresh shoot weight in maize. Tahapary et al. (2020) demonstrated that biostimulant concentration treatments influenced multiple growth parameters in lettuce, including plant height, leaf number, leaf area, fresh weight, root dry weight, root length,

and root number. Collectively, these findings highlight that both the chemical profile of the extract determined by the extraction solvent and the applied concentration play a crucial role in regulating biomass accumulation through established physiological mechanisms.

Chlorophyll levels

The statistical analysis revealed a significant effect of administering *D. denticulata* L. extract, applied at varying concentrations and extracted using different solvents on chlorophyll a, chlorophyll b, and total chlorophyll content in chili plants (Table 2). These findings highlight the potential of *D. denticulata* L. extract as a biostimulant capable of influencing key components of the photosynthetic apparatus, which in turn can have downstream effects on plant growth and productivity.

Table 2. Chlorophyll a, b, and total chlorophyll were treated with different extraction methods and concentrations of *D. denticulata*.

Solvent	Concentration (mg L ⁻¹)	Chlorophyll a (mg g ⁻¹)	Chlorophyll b (mg g ⁻¹)	Total chlorophyll (mg g ⁻¹)
Aquadest	0	1.16ab	2.11ab	3.27a
	25	1.16a	2.17abcd	3.32ab
	50	1.21ab	2.17abcd	3.38abcd
	100	1.27ab	2.10ab	3.38abc
Methanol	0	1.16ab	2.11ab	3.27a
	25	1.30ab	2.28de	3.58def
	50	1.16ab	2.12ab	3.28a
	100	1.27ab	2.27cde	3.53cdef
Ethanol	0	1.16ab	2.11ab	3.27a
	25	1.28ab	2.13abc	3.41abcde
	50	1.36b	2.18abcde	3.54cdef
	100	1.34ab	2.17abcd	3.50bcdef
Butanol	0	1.16ab	2.11ab	3.27a
	25	1.22ab	2.08a	3.29a
	50	1.35ab	2.24bcde	3.60ef
	100	1.30ab	2.31e	3.61f

Note: Numbers followed by the same letter in the same treatment and variable indicate no significant difference in the 5% DMRT test.

Data presented in Table 2 showed that the combination of *D. denticulata* L. extract with butanol as the extraction solvent at a concentration of 100 mg L⁻¹ resulted in a significant increase in chlorophyll b and total chlorophyll content. This effect is likely attributable to the presence of terpenoid compounds in the extract, which are known to play crucial roles in plant growth, development, respiration, and photosynthesis (Zhou et al., 2017). Terpenoids function as precursors for various plant hormones, such as gibberellins, and also contribute to the biosynthesis of photosynthetic pigments, including chlorophyll (Karunanithi & Zerbe, 2019).

Chlorophyll content is a critical determinant of photosynthetic capacity because chlorophyll molecules capture light energy and drive the conversion of carbon dioxide and water into carbohydrates. An increase in chlorophyll b, which expands the range of light wavelengths absorbed by the photosystem, can improve the efficiency of light harvesting and optimize energy transfer within the chloroplast. The observed enhancement in chlorophyll levels under the 100 mg L⁻¹ butanol extract treatment suggests that the bioactive compounds in *D. denticulata*, possibly working in concert with butanol's ability to extract moderately polar and polar metabolites, may upregulate pigment biosynthesis pathways or protect chlorophyll from degradation. These results indicate that *D. denticulata* extract, particularly when prepared with butanol at higher concentrations, holds promise as a natural biostimulant to improve photosynthetic efficiency, which could translate into higher biomass production and overall plant performance under cultivation.

Fruit weight and length (g)

Statistical analysis showed that applying *D. denticulata* L. extract at different concentrations and using various solvents significantly affected the fruit weight and length of Kopay chili plants (Table 3), indicating a positive influence on the plants' generative performance. Fruit development is a complex process regulated by hormonal signaling, carbohydrate allocation, and nutrient partitioning from source tissues (leaves) to sink tissues (fruits). The enhancement observed in this study suggests that the bioactive compounds present in *D. denticulata* extract may have modulated these physiological processes, leading to improved reproductive growth.

Table 3. Fruit weight and length from chili plants treated with different extraction methods and concentrations of *D. denticulata* application.

Treatments	Concentration (mg L ⁻¹)	Fruit weight (g)	Fruit length (cm)
Aquadest	0	218.33abc	17.43a
	25	226.67abc	22.32bc
	50	250.00abc	19.27abc
	100	216.67abc	21.43abc
Methanol	0	218.33abc	17.43a
	25	341.67c	18.21a
	50	270.00bc	20.21abc
	100	243.33abc	20.75abc
Ethanol	0	218.33abc	17.43a
	25	233.33abc	18.32ab
	50	323.33bc	22.55c
	100	266.67bc	20.88abc
Butanol	0	218.33abc	17.43a
	25	255.00abc	21.05abc
	50	196.67ab	20.97abc
	100	123.33a	19.98abc

Note: Numbers followed by the same letter in the same treatment and variable indicate no significant difference in the 5% DMRT test.

The combination of methanol as the extraction solvent and a concentration of 25 mg. L⁻¹ produced the most favorable results, indicating its suitability for enhancing fruit development in chili plants. Methanol, due to its polarity, is effective in extracting a broad spectrum of secondary metabolites, including phenolics, flavonoids, and terpenoids, which can act as hormone mimics or modulators. These compounds may enhance gibberellin- and auxin-mediated fruit set, promote cell division and expansion in developing fruits, and improve vascular transport for nutrient supply during the generative phase.

This finding is consistent with the study by Pohl et al. (2019), which demonstrated that biostimulants derived from seaweed extract improved reproductive effectiveness and increased fruit yield and quality in eggplant. Similarly, Dwitama et al. (2020) reported that biostimulants contributed to greater generative growth in tomatoes, reflected by increased fruit diameter and weight per plant and per plot. Research by Gofar et al. (2022) also confirmed that biostimulant application significantly affected the number and weight of red chili fruits.

In the present study, the use of *D. denticulata* L. extract not only enhanced the generative output of Kopay chili plants but also appeared to improve their overall vigor, possibly through enhanced photosynthetic efficiency, better nutrient assimilation, and hormonal balance. These improvements may have strengthened the plants' physiological resilience and tolerance to environmental stress, allowing them to allocate more resources toward fruit development and maturation.

CONCLUSIONS

Using fern extract extracted with four different solvents produced varied effects on several plant growth parameters, including plant height, shoot wet weight, shoot dry weight, root wet weight, root dry weight, chlorophyll a, chlorophyll b, total chlorophyll, fruit weight, and fruit length. The best results for plant height were observed with a methanol solvent concentration of 100 mg L⁻¹. Meanwhile, an ethanol concentration of 50 mg L⁻¹ significantly increased shoot dry weight and root wet and dry weight. A butanol solvent concentration of 100 mg L⁻¹ enhanced chlorophyll b and total chlorophyll levels. A methanol concentration of 25 mg L⁻¹ improved the weight of Kopay chili fruit.

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