Australian Journal of Crop Science

Article | https://doi.org/10.21475/ajcs.26.20.11.pne162
Submitted: 29 October 2025 | Revised: 30 January 2026 | Accepted: 11 May 2026
Pages 922–929

Phenolic profile of *Arachis pintoi* and its potential as a pre-emergence bioherbicide

Isma Muliani Jasadin[1], Muhamad Achmad Chozin[1,2*], and Hafith Furqoni[1]

[1]Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University, Bogor 16680, (West Java) Indonesia

[2]Center for Tropical Horticulture Studies, IPB University, Bogor 16680, (West Java) Indonesia

***Corresponding author: ma_chozin@yahoo.com**

Abstract: Weeds are one of the main factors in decreasing crop productivity, while the intensive use of synthetic herbicides has environmental impacts and leads to weed resistance. This study aims to characterize the phenolic compound profile of *Arachis pintoi* and evaluate its potential as a selective pre-emergence bioherbicide. The methanol extract of *A. pintoi* was analyzed using LC–MS and identified several key phenolic compounds, including salicylic acid, N-feruloylglycine, acetylsalicylate (aspirin), curcumin, zingerol, gentisic acid, and 4-hydroxybenzaldehyde. Pre-emergence germination tests were conducted on maize (*Zea mays* L.) and three weed species, namely *Eleusine indica* L., *Cyperus iria* L., and *Borreria alata* L., at five extract concentrations (0, 50, 100, 150, and 200 g/L). The results indicated that the inhibition of weed germination and early growth increased with higher concentrations, with the maximum inhibition observed at 200 g/L—approximately 66% in *E. indica*, 64% in *C. iria*, and 72% in *B. alata*. Broadleaf weeds were more sensitive than grass and sedge weeds, while the germination and early growth of maize were not significantly impacted. These findings indicate that *A. pintoi* has the potential to be developed as a selective and environmentally friendly natural pre-emergence bioherbicide for sustainable weed control in tropical agricultural systems.

Keywords: Allelopathy; Bioassay; LC-MS; Secondary metabolites; Weed management

Introduction

Global agricultural production faces major challenges due to limited arable land, rising food demand, and the effects of climate change, which decrease crop yields. In recent decades, dependence on synthetic herbicides for weed control has caused various environmental issues, including soil and water pollution and increasing cases of weed resistance to herbicide active ingredients (Parven et al., 2025; Khamare et al., 2022). This situation has driven global efforts to cut back on synthetic herbicide use and develop more eco-friendly and sustainable weed control methods (Thang et al., 2023).

Bioherbicides, which include those based on microorganisms and natural plant compounds, have been developed and marketed in various countries as part of sustainable agriculture systems (Roberts et al., 2022). Several microbial-based bioherbicide products and plant extracts are used in both organic and conventional farming systems (Hasan et al., 2021). However, widespread use of bioherbicides remains limited due to several challenges, such as generally lower field effectiveness compared to synthetic herbicides, variability in response caused by environmental factors like temperature, UV radiation, and soil microbiota, short persistence, relatively high production and formulation costs, and a limited range of weed control (Hasan et al., 2021; Islam et al., 2024; Cai and Gu, 2016). Therefore, research focused on discovering bioactive compounds that are effective, selective, and suitable for tropical agricultural conditions is necessary.

Plant-based bioherbicides derived from plant secondary metabolites, especially phenolic compounds, show significant potential in preventing weed germination and early growth through phytotoxic mechanisms such as enzyme inhibition, disruption of cell membrane integrity, and interference with plant hormone regulation (Mushtaq et al., 2024a; Hussain et al., 2022). *Arachis pintoi*, a tropical legume commonly used as a cover crop, is known to contain various phenolic compounds, flavonoids, and alkaloids that have allelopathic effects on several weed species while also improving soil fertility (Suzuki et al., 2019; Nuryana et al., 2019). Applying bioherbicides during the pre-emergence stage is essential because phenolic compounds typically reach their peak effectiveness before weed germination (Chozin et al., 2018; Sumiahadi et al., 2019).

Although the phytotoxic potential of *A. pintoi* has been reported, most previous studies have relied on analytical techniques such as HPLC or GC–MS, which have limited resolution in fully identifying phenolic compounds (Hoang Anh et al., 2021; Pardo-Muras et al., 2020). Additionally, research on the pre-emergence effects of *A. pintoi* extracts against various weed species and their selectivity for major food crops like maize remains very limited. This gap emphasizes the need for more advanced analytical methods and comprehensive biological testing.

This study aims to identify the main phenolic compounds in *A. pintoi* using high-resolution LC–MS techniques, evaluate its phytotoxic potential as a pre-emergence bioherbicide, and assess its effectiveness against various weed species. The findings are expected to provide a scientific basis for developing selective, safe, and sustainable natural bioherbicides for

tropical agricultural systems. Additionally, detailed metabolite mapping could reveal new allelopathic compounds that might be developed as next-generation bioherbicide candidates to support climate-smart agriculture.

Results and discussion

Profile of phenolic compounds in Arachis pintoi extract based on LC-MS analysis

LC–MS analysis of *Arachis pintoi* extract identified a diverse range of phenolic compounds (Table 1), reflecting the allelopathic role of its secondary metabolites (Ali et al., 2021). The detected compounds included phenolic acids, flavonoids, isoflavonoids, tannins, and lignans, which are known to inhibit germination and suppress weed growth by interfering with cellular respiration mechanisms (Sturm et al., 2018; Hussain et al., 2018).

Table 1. Phenolic compound content in *Arachis pintoi* based on LC-MS analysis.

No.	Compound	Area (%)	Phenolic category	Retention time (min)	Allelopathic mechanism
1	Salicylic acid ($C_7H_6O_3$)	37.47%	Phenolic acid (Hydroxybenzoate)	9.91	Inhibition of weed seedling growth (de Lima et al., 2022)
2	N-feruloylglycine ($C_{12}H_{13}NO_5$)	17.48%	Derivative phenolic acid (conjugated hydroxycinnamate)	7.09	Inhibits nutrient uptake and cell respiration in weeds (Schandry and Becker, 2020).
3	Aspirin ($C_9H_8O_4$)	17.36%	Phenolic ester (salicylate derivative)	6.70	Inhibition of protein synthesis and respiration in weeds (Hoang Anh et al., 2021).
4	Curcumin ($C_{21}H_{20}O_6$)	14.31%	Complex polyphenol (curcuminoid)	16.38	Inhibition of respiratory enzymes and seed germination in weeds (Latif et al., 2017)
5	Zingerol ($C_{11}H_{16}O_3$)	4.05%	Simple phenol (aromatic ketone)	9.07	Disruption of growth hormone regulation during early stage (Latif et al., 2017)
6	Gentisic acid ($C_7H_6O_4$)	5.36%	Phenolic acid (Hydroxybenzoate)	6.68	Inhibition of seed germination and weed growth (Li et al., 2010).
7	4-Hydroxybenzaldehyde ($C_7H_6O_2$)	3.70%	Simple phenol derivative (phenolic aldehyde)	6.92	Induces oxidative stress and inhibits radicle growth in weeds (Li et al., 2010).

The LC–MS chromatogram (Fig. 1) displayed peaks with different intensities over a 0–32-minute retention time, indicating the presence of phenolic compounds with diverse polarities. Based on the ChemSpider and mzCloud databases, the major identified compounds included salicylic acid, N-feruloylglycine, aspirin, curcumin, zingerol, gentisic acid, and 4-hydroxybenzaldehyde. Consistent with the variation in polarity, compounds with shorter retention times (6–10 min), such as salicylic acid and gentisic acid, are more polar and readily diffuse into seed tissues, thereby rapidly inhibiting weed germination through disruption of membrane permeability (Li et al., 2010). In contrast, compounds with longer retention times, such as curcumin and zingerol, are less polar with higher affinity for lipid membranes, resulting in slower but more persistent inhibitory effects. This complex phenolic profile supports the potential of *Arachis pintoi* extract as a pre-emergence bioherbicide through selective allelopathic mechanisms against weeds.

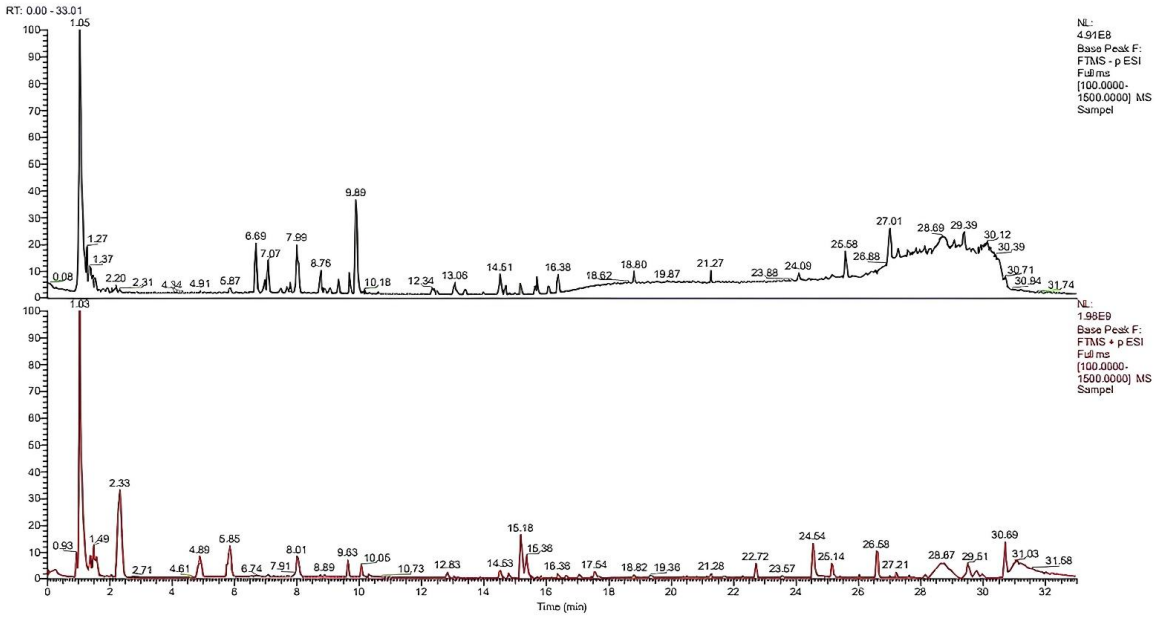


Fig. 1. LC–MS chromatogram of phenolic compounds in *Arachis pintoi* extract. The X-axis represents retention time (minutes), and the Y-axis indicates relative ion intensity (arbitrary units).

Based on chromatogram peak areas, seven dominant phenolic compounds were detected in *A. pintoi* extract, including salicylic acid, gentisic acid, ferulate derivatives, curcumin, zingerol, and aspirin, with varying relative contributions. Salicylic acid and its derivatives are known to enhance defense enzyme activity and phenolic content in plants, thereby suppressing weed growth (Zhang et al., 2025). Curcumin exhibited allelopathic activity against seed germination and root growth of several test plants (Pandey et al., 2025), while gentisic acid and salicylic acid interfered with nutrient uptake and modulated root development (Nutricati et al., 2023). Ferulate derivatives inhibited radicle elongation and affected weed respiration (Hussain et al., 2022), whereas zingerol and aspirin contributed to the inhibition of respiratory enzymes, regulation of growth hormones, and oxidative metabolism (Kong et al., 2024; Lefevere et al., 2020; Zhang et al., 2024).

The presence of both simple phenolics and complex polyphenols in *A. pintoi* is consistent with reports in other tropical legumes, such as *Arachis hypogaea*, where the dominance of hydroxybenzoates and hydroxycinnamates plays a significant role in allelopathic effects (Zhang et al., 2024). This combination of phenolics disrupts photosynthesis, energy metabolism, and increases reactive oxygen species (ROS) in weeds, supporting the potential of *A. pintoi* extract as a pre-emergence bioherbicide (Mushtaq et al., 2024a; Sturm et al., 2018; Shah et al., 2022). The water-soluble and biodegradable nature of these phenolic compounds ensures environmental safety and aligns with integrated weed management principles, making *A. pintoi* a promising candidate for natural bioherbicide applications in tropical agriculture (Thang et al., 2023).

UV–Vis spectral analysis of Arachis pintoi extract

UV–Vis spectrophotometric analysis (Table 2) using the Folin–Ciocalteu method at 765 nm showed an average absorbance of 0.254 with low variation (%RSD ±3.6%), indicating good precision. The total phenolic content of *A. pintoi* extract, quantified using a tannic acid calibration curve, was 22,050.12 mg/kg of extract. The Folin–Ciocalteu reagent reacts with phenolic compounds containing hydroxyl groups (Molole et al., 2022).

Table 2. UV–Vis spectral analysis of *Arachis pintoi* extract.

No.	Wavelength (nm)	Absorbance	Notes
1	311	Over-range	Very high UV absorption
2	445	4.463	Major spectral peak
3	465	3.937	Secondary spectral peak
4	665	2.61	Peak in the red region
5	765	0.094	Absorbance at Folin–Ciocalteu wavelength (scanning)
6	765	0.26	Folin–Ciocalteu assay (Rep-1)
7	765	0.247	Folin–Ciocalteu assay (Rep-2)

The UV–Vis spectrum (Fig. 2) of *A. pintoi* extract showed sharp absorbance at 311 nm and two strong peaks at 445 and 465 nm, indicating conjugated aromatic structures typical of flavonoids and polyphenols (Aniya et al., 2022; Zou et al., 2022). Absorbance decreased toward 665 nm and reached a minimum at 765 nm (0.094), consistent with reports that phenolic acids and flavonoids strongly absorb in the UV range (250–320 nm) due to aromatic conjugation (Ali et al., 2021).

The consistent absorbance at 765 nm confirmed the phenolic character of the extract and served as a relative measure of total phenolic content (Taniguchi et al., 2023). High absorbance in the Folin–Ciocalteu assay indicates strong biological activity, including antioxidant and allelopathic potential influencing weed germination and seedling growth.

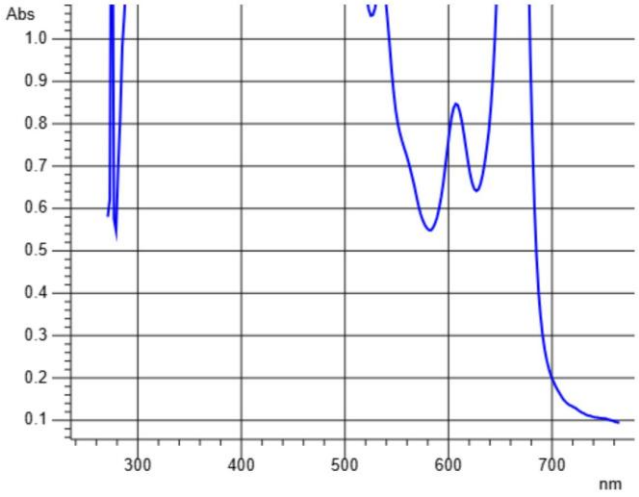


Fig. 2. UV–Vis absorption spectrum of *Arachis pintoi* extract. The X-axis shows wavelength (nm) and the Y-axis shows absorbance (a.u.).

The spectral profile was consistent with the Folin–Ciocalteu results at 765 nm, confirming high phenolic content and supporting the extract's antioxidant and allelopathic activity (Martins et al., 2021). Identified compounds, including hydroxybenzoate derivatives and polyphenols, interfere with respiratory enzymes and affect weed root and shoot development (Jia et al., 2024), reinforcing *A. pintoi*'s potential as an environmentally safe bioherbicide for weed control in crops.

Potential phenolic compounds for pre-emergence bioherbicide

The phenolic profile of *Arachis pintoi* extract was dominated by bioactive compounds with allelopathic potential, indicating strong pre-emergence bioherbicidal activity. LC–MS analysis showed that three major compounds: salicylic acid (37.47%), N-feruloylglycine (17.48%), and aspirin (17.36%), accounted for about 72% of the total chromatogram area. These phenolic acids and derivatives are known to inhibit seed germination and radicle elongation in weeds through multiple physiological pathways (Schandry and Becker, 2020; Li et al., 2010).

The dominance of salicylic acid, comprising over one-third of total phenolics, suggests strong inhibitory potential. Salicylic acid disrupts mitochondrial respiration and induces reactive oxygen species (ROS) formation, limiting energy availability and radicle protrusion (Li et al., 2010). Its high proportion implies that *A. pintoi* may show suppression levels comparable to other phenolic-rich allelopathic plants. This compound also occurs in *Oryza sativa*, *Triticum aestivum*, and *Medicago sativa*, where it inhibits weed growth via oxidative stress, hormonal disruption, and microbial modulation (Mushtaq et al., 2024a; Ali et al., 2021; Li et al., 2010).

N-feruloylglycine, a conjugated form of ferulic acid, represented 17.48% of the chromatogram area. Ferulate derivatives consistently impair radicle elongation and ion uptake during early germination, indicating allelopathic activity (Thang et al., 2023; Li et al., 2010). This intermediate abundance supports its role as a moderate-to-strong inhibitor. Acetylsalicylic acid (aspirin, 17.36%) also exhibits allelopathic and growth-modulating effects during germination and seedling development (Schandry and Becker, 2020; Latif et al., 2017) reinforcing its role as a secondary yet important inhibitor.

The remaining compounds curcumin (14.31%), gentisic acid (5.36%), zingerol (4.05%), and 4-hydroxybenzaldehyde (3.70%) act synergistically to maintain inhibition beyond germination. Curcumin and gentisic acid induce oxidative stress and inhibit respiratory enzymes, prolonging suppression during seedling growth, while zingerol and 4-hydroxybenzaldehyde contribute by modulating ROS balance and hormone regulation (Schandry and Becker, 2020; Latif et al., 2017; Li et al., 2010).

The balanced composition of highly active phenolic acids (salicylic acid, N-feruloylglycine, aspirin) and moderately lipophilic polyphenols (curcumin, zingerol) suggests a synergistic allelopathic mechanism. Polar acids likely cause rapid germination inhibition, whereas less-polar polyphenols ensure sustained metabolic suppression. With over 90% of compounds showing reported allelopathic activity, the LC–MS profile quantitatively supports *A. pintoi* as an effective and environmentally compatible pre-emergence bioherbicide.

Bioassay results of Arachis pintoi extract

Increasing the concentration of *A. pintoi* extract showed a pattern of stronger inhibition of weed germination (Table 3). At a concentration of 50 g/L (A1), germination inhibition reached 41% in *Eleusine indica,* 36% in *Cyperus iria,* and 42% in *Borreria alata.* This inhibition increased with increasing concentration at 200 g/L, which was around 66% in *E. indica,* 64% in *C. iria,* and the greatest inhibition occurred in *B. alata* reaching ±72%, indicating that broadleaf weeds have the highest sensitivity to *A. pintoi* extract. This difference in response levels between species indicates that the allelopathic effect of the extract is specific and influenced by the physiological characteristics of each seed. In contrast, the inhibition of maize germination was relatively low, reaching approximately 8.3% at the highest extract concentration (200 g/L). The higher tolerance of maize seeds indicates more effective physiological defense mechanisms compared to weed seeds, particularly due to their larger seed size and abundant food reserves, allowing maize seeds to maintain metabolic activity during germination despite exposure to allelopathic compounds. Smaller weed seeds with limited reserves are more susceptible to metabolic disturbances and oxidative stress. These results align with a report by Thang et al. (2023), showing that the application of *A. pintoi* extract suppressed weed germination without significantly adversely affecting rice seeds.

The decrease in germination at a concentration of 200 g/L was accompanied by a significant decrease in the percentage of normal germination in the tested weed seeds. The resulting seedlings exhibited abnormalities characterized by curved radicle and plumule growth and brownish tissue discoloration, indicating impaired physiological activity and metabolic processes during the early stages of germination. These disturbances are associated with inhibited germination enzyme activity and increased oxidative stress, which triggers cell damage. This condition aligns with reports by Li et al. (2010) and Mushtaq and Fauconnier (2024b), which state that phenolic compounds can inhibit germination-related enzyme activity and increase oxidative stress, thereby reducing seedling vigor and inhibiting weed seed growth.

The phytotoxic effect of *A. pintoi* extract at the highest concentration was more pronounced in radicle and plumule length parameters. The radicle showed a degree of sensitivity, particularly in *E. indica* and *B. alata* seeds, as evidenced by the inhibition of primary root growth compared to the control. This condition indicates that the early root system is a primary target of allelopathy, as the radicle relies on respiration and cell division processes, which are susceptible to phenolic compounds. Inhibition of the plumule and radicle indicates disruption of cell division and elongation during the early growth. Phenolics suppress hydrolytic enzymes, disturb cell division, and alter membrane integrity and hormonal regulation, reducing early radicle development and seed viability via pre-emergence mechanisms (Khamare et al., 2022; Pardo-Muras et al., 2020).

Table 3. Pre-emergence effects of *Arachis pintoi* extract on germination parameters and seedling growth of weeds and maize.

Species	Treatment	Germination (%)	Germination rate (%/day)	Normal seedlings (%)	Plumule length (mm)	Radicle length (mm)
Eleusine indica L.	A0 (control)	82.5 ± 3.8^{a}	3.9 ± 0.3^{a}	82.5 ± 3.8^{a}	26.0 ± 4.0^{a}	19.0 ± 3.0^{a}
	A1 (50 g/L)	48.5 ± 4.1^{b}	2.3 ± 0.2^{b}	48.5 ± 4.1^{b}	22.0 ± 1.0^{ab}	14.0 ± 1.0^{b}
	A2 (100 g/L)	42.5 ± 5.2^{c}	1.7 ± 0.2^{c}	42.5 ± 5.2^{c}	20.0 ± 3.0^{b}	7.0 ± 1.0^{c}
	A3 (150 g/L)	33.0 ± 3.9^{d}	1.3 ± 0.3^{d}	33.0 ± 3.9^{d}	18.0 ± 3.0^{b}	7.1 ± 1.0^{c}
	A4 (200 g/L)	28.0 ± 3.3^{d}	1.2 ± 0.1^{d}	28.0 ± 3.3^{d}	19.0 ± 3.0^{b}	5.0 ± 1.0^{d}
Pr > F		**	**	**	**	**
Cyperus iria L.	A0 (control)	81.0 ± 2.0^{a}	4.4 ± 0.5^{a}	81.0 ± 2.0^{a}	42.0 ± 3.0^{a}	21.0 ± 3.0^{a}
	A1 (50 g/L)	52.0 ± 5.7^{b}	2.3 ± 0.2^{b}	52.0 ± 5.7^{b}	35.0 ± 3.0^{b}	18.0 ± 3.0^{b}
	A2 (100 g/L)	40.0 ± 5.7^{c}	1.7 ± 0.3^{c}	40.0 ± 5.7^{c}	31.0 ± 2.0^{c}	17.0 ± 2.0^{b}
	A3 (150 g/L)	38.0 ± 4.0^{c}	1.6 ± 0.1^{c}	38.0 ± 4.0^{c}	27.0 ± 1.0^{d}	16.0 ± 2.0^{b}
	A4 (200 g/L)	29.0 ± 2.0^{d}	1.3 ± 0.2^{c}	30.0 ± 2.3^{d}	22.0 ± 2.0^{e}	16.0 ± 1.0^{b}
Pr > F		**	**	**	**	*
Borreria alata L.	A0 (control)	80.0 ± 3.3^{a}	3.8 ± 0.3^{a}	80.0 ± 3.3^{a}	37.0 ± 1.0^{a}	32.0 ± 2.0^{a}
	A1 (50 g/L)	46.5 ± 3.0^{b}	2.2 ± 0.2^{b}	46.5 ± 3.0^{b}	23.0 ± 2.0^{b}	26.0 ± 1.0^{b}
	A2 (100 g/L)	41.0 ± 3.8^{c}	1.6 ± 0.2^{c}	41.0 ± 3.8^{c}	22.0 ± 2.0^{c}	22.0 ± 1.0^{c}
	A3 (150 g/L)	33.0 ± 2.0^{d}	1.3 ± 0.2^{c}	33.0 ± 2.0^{d}	21.0 ± 1.0^{d}	21.0 ± 1.0^{d}
	A4 (200 g/L)	22.0 ± 2.3^{e}	0.9 ± 0.2^{d}	22.0 ± 2.3^{e}	19.0 ± 1.0^{e}	16.0 ± 1.0^{e}
Pr > F		**	**	**	**	**
Maize	A0 (control)	90.0 ± 8.2	2.4 ± 0.2	87.5 ± 5.0	99.0 ± 3.0	86.0 ± 3.0
	A1 (50 g/L)	87.5 ± 5.0	2.1 ± 0.1	87.5 ± 5.0	94.0 ± 8.0	81.0 ± 2.0
	A2 (100 g/L)	85.0 ± 5.8	2.1 ± 0.4	85.0 ± 5.8	93.0 ± 16.0	80.0 ± 3.0
	A3 (150 g/L)	82.5 ± 5.0	2.0 ± 0.2	82.5 ± 5.0	93.0 ± 15.0	80.1 ± 6.0
	A4 (200 g/L)	82.5 ± 5.0	2.0 ± 0.1	82.5 ± 5.0	91.0 ± 15.0	79.1 ± 2.0
Pr > F		ns	ns	ns	ns	ns

Note: The values presented are the mean ± standard deviation. Numbers followed by different letters in the same column indicate significant differences based on the DMRT test at the 5% level.

Arachis pintoi extracts contain phenolics that inhibit α-amylase and α-glucosidase, affecting carbohydrate metabolism in weed seeds (Thang et al., 2023). The extract also modulates hormonal balance and disrupts cell membrane integrity, delaying germination and reducing early seedling growth. These effects make it most effective when applied at the pre-emergence stage before weed seeds emerge at the soil surface (Figs. 3A and B).

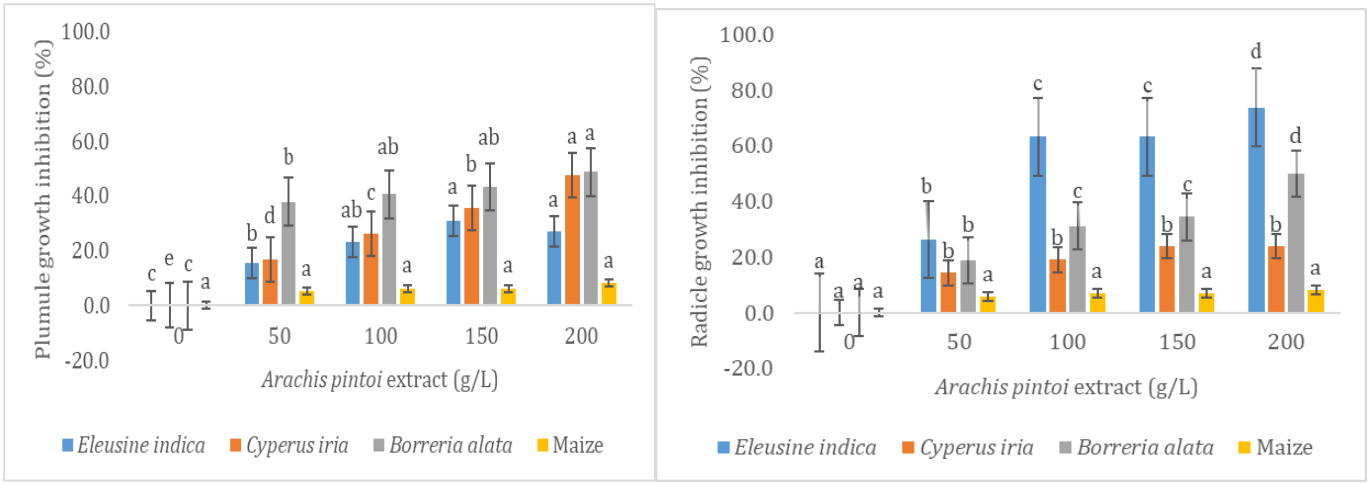


Fig. 3. (A) Plumule growth inhibition 14 days after *Arachis pintoi* extract application (left) (B) Radicle growth inhibition 14 days after *Arachis pintoi* extract application (right).

The reduction in germination rate indicates inhibition of primary metabolism in weeds. *A. pintoi* extract delayed radicle and shoot emergence, reducing early competition for nutrients, water, and light with crops. This aligns with reports on

other legumes, where phenolics suppressed *Amaranthus* spp. and *Digitaria* spp. via pre-emergence mechanisms (Hoang Anh et al., 2021; Pardo-Muras et al., 2020).

The highest inhibition of plumule and radicle growth was observed at a concentration of 200 g/L. The higher rate of radicle growth inhibition compared to plumule growth (Figs. 3A and B) indicates that the radicle is the most sensitive organ to the allelochemical compounds in *A. pintoi* extract. The radicle, as the first organ to interact directly with the growing medium, allows for the uptake of higher amounts of phytotoxic compounds (Khamare et al., 2022). Plumule inhibition reflects overall germination success, so the level of inhibition tends to align with germination rate, while the radicle is more sensitive to allelochemical compounds due to its direct contact with the growing medium. Therefore, in some species, such as *Eleusine indica,* radicle inhibition can be higher even though the reduction in germination rate is not the greatest.

In addition to inhibiting germination, the phenolic compounds in *Arachis pintoi* extract also play a role in inhibiting the growth of germinated weed seeds, as reflected in the high proportion of seedlings that do not develop normally at high concentrations. Successful germination initiation is not always followed by optimal seedling development, indicating that the phenolic compounds in *A. pintoi* extract not only act during the imbibition and dormancy-breaking stages but also continue to influence physiological processes in the early post-germination phase.

Incomplete seedling development in the pre-emergence treatment indicates that the phenolic compounds absorbed during the imbibition phase continue to exert their influence in the early stages of growth. Consequently, although seeds germinate, subsequent development is insufficient to support the formation of functional and complete seedlings. Weed inhibition by *A. pintoi* extract is cumulative, resulting from a combination of seeds that fail to germinate and seeds that successfully germinate but are unable to resume normal early growth. This is consistent with a report by da Silva et al. (2024), which explains that potentially allelopathic plant extracts not only suppress germination but also inhibit the initial growth and vigor of weed seedlings after emergence. This inhibition in the early growth phase results in low weed growth success, thus supporting the use of plant extracts as bioherbicides.

Studies on other weed-controlling plants further support the consistency of this inhibitory effect. Phenolic extracts from legumes such as *Ulex europaeus* and *Cytisus scoparius* effectively inhibited weed germination through cell membrane disruption and hormonal modulation (Pardo-Muras et al., 2020). This reinforces the interpretation that phenolic-induced phytotoxicity is a cross-species phenomenon, providing a scientific basis for the development of allelopathic plant-based bioherbicides in biodiverse tropical environments.

The practical implication of these findings is the potential integration of allelopathic plant extracts into environmentally friendly weed management strategies. Pre-emergence application of aqueous extracts may reduce dependence on synthetic herbicides. This approach not only decreases ecological impact but also supports more sustainable weed management in tropical agricultural systems (Khamare et al., 2022).

Materials and methods

Plant materials

Arachis pintoi plants were collected from Cipanas Village, Cianjur Regency, Indonesia, at an age of approximately 2–3 months. The whole plants, including roots, stems, and leaves, were washed, air-dried, and oven-dried at 45 °C for 50 h (Thang et al., 2023). The dried samples were then ground and sieved through a 0.04 mm (325 mesh) screen to produce a fine powder.

Extraction of phenolic compounds

The fine powder of *Arachis pintoi* (1:3 w/v) was macerated in methanol for 24 h with intermittent agitation. This process was repeated three times. The obtained extract was evaporated using a rotary evaporator and stored at 4 °C until further analysis.

Phenolic compound analysis by LC–MS

Approximately 5 mg of *Arachis pintoi* extract was dissolved in 1 mL of methanol and filtered through a 0.2 µm Nylon membrane prior to analysis. The analysis used UHPLC Vanquish coupled with Q Exactive Plus Orbitrap HRMS (Thermo Scientific) and an Accucore C18 column (100 × 2.1 mm, 1.5 µm, 30 °C). The mobile phase consisted of H_2O + 0.1% formic acid (A) and acetonitrile + 0.1% formic acid (B), with a gradient elution from 5% to 95% B over 25 minutes. Detection was conducted in both positive and negative ionization modes (100–1500 m/z). Identification of phenolic compounds was confirmed using the ChemSpider and mzCloud databases to verify molecular masses and fragmentation patterns.

UV–Vis spectrophotometric analysis

Phenolic compounds were qualitatively examined using a UH5300 spectrophotometer (Serial No. 2375-001) equipped with a six-cell option. The analysis was performed at 765 nm, with a peak threshold of 0.586 and a sensitivity setting of 2, as a qualitative complement to the LC–MS analysis. In addition, the total phenolic content of *A. pintoi* extract was determined quantitatively using the Folin–Ciocalteu method. Briefly, 10 g of the extract was dissolved in acetone to a final volume of 50 mL and homogenized using an ultrasonic homogenizer at 20 °C for 30 min. A 2-mL aliquot of the resulting solution was transferred into a 25-mL volumetric flask, followed by the addition of 10 mL of Folin–Ciocalteu reagent. The solution was then made up to volume with 10.75% Na_2CO_3 and incubated at 37 °C for 30 min. Absorbance was measured at 765 nm using a UV–Vis spectrophotometer. Tannic acid was used as the calibration standard, and the calibration curve was expressed by the linear regression equation $Y = 0.00526x + 0.00346$ ($r = 0.99927$). The phenolic concentration was

calculated from the calibration equation, adjusted for the dilution factor, and expressed as mg/kg of extract based on the initial sample weight.

Pre-emergence bioherbicide assay

The pre-emergence assay was conducted in Petri dishes (90 × 15 mm) using a completely randomized design with five concentrations of *Arachis pintoi* extract (0, 50, 100, 150, and 200 g/L) and four replications. Seeds of maize (*Zea mays*), *Eleusine indica* L., *Cyperus iria* L.and *Borreria alata* L. were sterilized with 1% NaClO and rinsed with distilled water. Twenty-five seeds per dish were placed on Whatman filter paper, treated with 5 mL of the corresponding extract, and incubated in a germinator at 29 °C. Germination and growth of the seeds were monitored daily for 14 days after treatment.

Observations and measurements

Germination percentage (%): Calculated 14 days after sowing using the formula:

$$\text{Germination (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds sown}} \times 100\%$$

Germination rate (%/day): Recorded daily up to 14 days and calculated as:

$$Kct = \sum_{i=1}^{n} \frac{KN_i}{W_i} \times 100\%$$

where KNi is the number of seeds germinated on day i, and Wi is the time in days.

Normal germination (%): Seeds exhibiting primary and secondary roots, hypocotyl, cotyledons, epicotyl, and plumule were recorded daily for 14 days.

Plumule and radicle length (mm): Measured on day 14 using ImageJ software.

Inhibition rate (%): Calculated based on plumule length using the formula:

$$\text{Inhibition (\%)} = \frac{PK - PT}{PK} \times 100\%$$

where PK is the mean plumule length of the control, and PT is the mean plumule length of the treatment.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using an F-test at a 5% significance level, followed by Duncan's multiple range test (DMRT). Statistical analysis was conducted utilizing SAS software (SAS Institute Inc., Cary, NC, USA).

Conclusion

Based on LC-MS analysis, *Arachis pintoi* extract contains major phenolic compounds, including salicylic acid, gentisic acid, N-feruloylglycine, curcumin, zingerol, and aspirin, with a total phenol content of 22,050.12 mg/kg. The extract significantly inhibits the germination and early growth of weeds *Eleusine indica* L., *Cyperus iria* L., and *Borreria alata* L., with the highest effectiveness at a concentration of 200 g/L. However, it has no significant effect on the germination and early growth of maize. The weed inhibition effectiveness in the field may be lower than in the laboratory due to environmental factors and crop cultivation systems. Therefore, *A. pintoi* extract has potential to be developed as a selective pre-emergence bioherbicide, but further field testing is necessary to confirm its stability and consistent effectiveness across various agroecosystems.

Acknowledgements

This study was supported by the Ministry of Research, Technology, and Higher Education of the Republic of Indonesia through the PPS-PTM scheme under contract number 006/C3/DT.05.00/PL/2025, led by Prof. Dr. Ir. Muhamad Achmad Chozin.

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