Recruitment limitation by the invasive shrub Mimosa pigra across multiple exposure pathways: evidence for a contributory allelopathic role

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Recruitment limitation by the invasive shrub *Mimosa pigra* across multiple exposure pathways: evidence for a contributory allelopathic role

Running title
Recruitment limitation by *Mimosa pigra*

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Abstract

Early recruitment is a critical bottleneck in plant invasion because suppression at this stage can strongly reduce resident regeneration and reinforce invader dominance. *Mimosa pigra* forms dense stands in wetland and riparian habitats, yet the contribution of leaf-derived chemistry to its invasion success remains insufficiently resolved. We tested whether leaf extracts of *M. pigra* suppress recruitment-related traits across three recipient species and whether the resulting pattern is consistent with chemically mediated interference. Using concentration-gradient bioassays combined with physicochemical controls, four-parameter logistic modelling, activated carbon neutralization, litter-leachate assays, invaded-soil comparisons, and phytochemical profiling, we found strong concentration-dependent reductions in germination, seed vigor, root growth, shoot growth, and biomass accumulation. Root growth was consistently the most sensitive endpoint across species. Activated carbon partly alleviated inhibitory effects, while litter leachates and invaded soils also reduced establishment-related traits. These inhibitory responses were associated with a phenolic- and flavonoid-rich extract profile. Together, the findings indicate that *M. pigra* can limit early recruitment through multiple plausible exposure pathways and support a contributory, rather than exclusive, allelopathic role in the persistence of invaded stands.

Keywords: *Mimosa pigra;* allelopathy; recruitment limitation; invasive shrub; litter leachate; plant–soil interactions

Introduction

Biological invasions can fundamentally restructure plant communities by altering the processes that regulate establishment, persistence, and regeneration (Brown and Barney 2021; Drenovsky et al. 2012). Because changes during germination and early seedling development often scale up to influence long-term neighborhood trajectories (Gómez-Aparicio 2008), suppression at the recruitment stage may be more consequential for community reassembly than competition among already established individuals (Bagavathiannan et al. 2020).

Multiple ecological pathways contribute to invader success, including altered litter dynamics, plant–soil feedbacks, and niche pre-emption (Yuan et al. 2021). Beyond resource-based competition, chemically mediated interference has long been proposed as an additional mechanism through which invaders can reduce the establishment of neighboring plants (Goldstein and Suding 2014). In invasion ecology, allelopathy is most meaningful when it affects traits directly linked to recruitment, such as germination timing, seedling vigor, and root development (Gioria et al. 2023; Hierro and Callaway 2021). However, its ecological importance remains debated because many studies rely heavily on crude extract assays without adequately separating biochemical effects from osmotic or physicochemical artefacts, or without linking inhibition to biologically relevant stages of establishment (Doughari 2012; Hollender et al. 2023; Śliwińska and Tomiczak 2025).

The invasive shrub *Mimosa pigra* L. is an especially relevant model for examining recruitment-limiting mechanisms because it is widely recognized as one of the world's most problematic alien plant species and a high-impact invader of wetland and floodplain systems Kato-Noguchi 2023b). More broadly, invasion success in aggressive alien plants is often associated with the combined effects of rapid regeneration, persistent propagule pressure, trait-mediated interference, and altered neighborhood assembly processes (Brown and Barney 2021; Drenovsky et al. 2012; Gioria et al. 2023). In this context, suppression at the germination and early seedling stage is particularly important because recruitment failure can shape later community trajectories and reduce the recovery capacity of resident vegetation (Bagavathiannan et al. 2020; Gómez-Aparicio 2008). In addition to resource competition, chemically mediated interference has been increasingly discussed as one pathway contributing to invasion success, especially where litter inputs, plant residues, and near-surface release of secondary metabolites can influence establishment (Callaway and Ridenour 2004; Hierro and Callaway 2021; Meiners et al. 2012). However, despite growing interest in allelopathy as an invasion mechanism, the extent to which leaf-derived chemistry of *M. pigra* contributes to recruitment limitation across ecologically relevant exposure pathways remains insufficiently resolved (Kato-Noguchi 2023).

Consequently, the main research gap is not whether *M. pigra* tissues can be phytotoxic under controlled conditions, but whether the observed inhibitory responses are consistent with a recruitment-based mechanism relevant to invasion ecology. In this study, we evaluated whether leaf-derived chemistry of *M. pigra* suppresses multiple early establishment traits across three recipient species and whether this suppression remains consistent across several plausible exposure pathways. We combined germination and seedling-growth bioassays with concentration-response modelling, activated carbon neutralization, litter-leachate assays, invaded-soil comparisons, and phytochemical profiling. Specifically, we tested four hypotheses: (H1) leaf extracts cause concentration-dependent suppression of germination and seedling performance; (H2) root-related traits are more sensitive than aboveground traits; (H3) activated carbon partly alleviates inhibitory effects, consistent with a chemical contribution; and (H4) litter leachates and soils from invaded stands are associated with reduced establishment, supporting ecological relevance beyond crude extract assays.

Materials and methods

Study system and donor species

Field material of the donor species was collected in Tram Chim National Park, Dong Thap Province (Nguyen 2020), Vietnam (10.741104, 105.498193), a seasonally inundated wetland landscape in the Mekong Delta where *Mimosa pigra* occurs along canal margins, floodplain edges, and other disturbed habitats (Walden et al. 2002). The park is characterized by a tropical monsoonal climate with distinct wet and dry seasons, and its hydrological regime creates alternating inundation and exposure conditions (Triet 2005) that strongly influence plant establishment and regeneration. Within this context, dense stands of *M. pigra* can accumulate substantial leaf litter and create near-surface conditions that may influence seed germination and early seedling growth of neighboring species.

The donor species used in this study was the invasive shrub *Mimosa pigra* L. Mature, healthy leaves were collected from established populations in Tram Chim National Park. To minimize variation associated with tissue age or visible damage, only fully expanded and undamaged leaves were selected. Collected material was transported to the laboratory, washed with distilled water to remove adhering debris, shade-dried to constant mass, and ground into fine powder for extract preparation. Plant identity was confirmed using standard taxonomic references and diagnostic field characters of *M. pigra*.

Recipient species

Three recipient species were selected to evaluate whether the inhibitory effects of *M. pigra* were consistent across contrasting recipient types: *Eleusine indica* (annual grass), *Paspalum conjugatum* (perennial grass), and *Ludwigia hyssopifolia* (broadleaf wetland herb). These species differ in growth form, regeneration strategy, and likely exposure to invaded conditions, allowing us to assess whether early recruitment responses were general across recipient species rather than restricted to a single taxon (Whitney and Gabler 2008). Seeds of the recipient species were collected from local populations in and around the study region, air-dried, cleaned manually, and stored in paper envelopes at room temperature until experimentation. Initial seed viability metrics and baseline germination performance for the control group are summarized in

TABLE
Table 1.

Experimental overview

To evaluate whether *Mimosa pigra* can limit early recruitment through multiple plausible exposure pathways, we used an integrated experimental design consisting of six linked components: (i) preparation of crude leaf extract, (ii) physicochemical characterization of treatment solutions, (iii) germination bioassays across a concentration gradient, (iv) seedling growth assays, (v) concentration–response modelling, and (vi) complementary validation-oriented assays involving activated carbon, litter leachates, invaded soils, and phytochemical profiling. This progression was designed to move from controlled detection of inhibitory effects toward stronger inference regarding chemical contribution and ecological relevance.

All direct extract bioassays were conducted using five treatment concentrations (0, 20, 40, 80, and 160 mg mL^{-1}). These concentrations were used to evaluate effects on germination percentage, seed vigor, root growth, shoot growth, and biomass accumulation (Beedi et al. 2018). Additional experiments were then conducted to determine whether inhibitory effects were partly alleviated by activated carbon, whether they were also expressed through litter-derived leachates (Stoler and Relyea 2020), and whether soils collected from invaded habitats showed similar recruitment-limiting patterns.

Preparation of leaf extract and treatment solutions

Air-dried leaves of *Mimosa pigra* were ground to a fine powder, and 100 g of the powder was extracted with 1,000 mL of 70% (v/v) aqueous ethanol (1:10, w/v) for 72 h under ambient laboratory conditions. During the experimental in An Giang Province, Vietnam, mean air temperatures for the area were 26.4°C ± 4.3°C; accordingly, extraction was conducted at approximately ambient laboratory temperature. The extract mixture was then filtered through Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 40°C to remove the solvent. The resulting crude extract was weighed to determine extraction yield and re-dissolved in distilled water to prepare the stock solution for subsequent bioassays (Sarker et al. 2006).

From this stock solution, five treatment concentrations were prepared for biological assays: 0, 20, 40, 80, and 160 mg mL^{-1}. Distilled water served as the control (0 mg mL^{-1}). These concentrations were selected to represent a broad inhibitory gradient and to enable subsequent concentration–response modelling, consistent with commonly used approaches in allelopathy bioassays (Inderjit and Callaway 2003). All treatment solutions were freshly

prepared prior to each experiment or stored at 4°C for no longer than 48 h before use. Leaf extract preparation and physicochemical characterization

Germination bioassay

Seeds of each species were placed in sterile 90 mm Petri dishes lined with two layers of Whatman No. 1 filter paper (25 seeds per dish; four replicates per treatment) and moistened with 5 mL of treatment solution. Dishes were incubated under ambient laboratory conditions with a natural photoperiod for 7 d (Ranal and Santana 2006). Germination was recorded daily, and a seed was considered germinated when radicle emergence exceeded 1 mm.

Final germination percentage (GP), mean germination time (MGT), and seed vigor index (SVI) (Zhang et al. 2020) were calculated as follows:

$$\text{GP (\%)} = \frac{n}{N} \times 100$$

$$\text{MGT} = \frac{\sum(n_i \times t_i)}{\sum n_i}$$

$$\text{SVI} = \text{GP} \times \text{mean seedling length}$$

where n is the number of germinated seeds, N is the total number of seeds, n_i is the number of seeds germinated at time t_i, and mean seedling length is the sum of root and shoot length averaged per seedling.

Seedling growth assay

Uniform newly germinated seedlings were transferred to fresh treatment conditions using the same extract concentrations (0, 20, 40, 80, and 160 mg mL^{-1}) to evaluate post-germination responses. Seedlings were maintained under ambient laboratory conditions for 7 d. At harvest, root length and shoot length were measured, and both fresh and dry biomass were determined. Fresh biomass was recorded immediately after harvest, whereas dry biomass was measured after oven-drying seedlings to constant mass. This approach integrates multiple recruitment-related traits commonly used in allelopathy studies (Inderjit and Callaway 2003), with particular emphasis on root responses as sensitive indicators of belowground interference (Wang et al. 2024).

Activated carbon assay

To assess whether inhibitory effects were partly associated with adsorbable compounds, a factorial experiment was conducted using two extract levels (0 and 80 mg mL^{-1}) in the presence or absence of activated carbon.

Activated carbon was incorporated into the substrate at 1% (w/w), and each treatment combination was replicated four times. Seedling emergence and growth-related traits were recorded after 21 d of exposure. AC was used here as a mechanism-oriented adsorption tool rather than as an artifact-free binary test (Inderjit and Callaway 2003).

Litter-leachate assay

To represent a plausible field exposure pathway, air-dried *Mimosa pigra* litter was used to prepare aqueous leachates at concentrations of 0, 10, and 50 g L^{-1}. The leachates were filtered and applied to germination bioassays following the procedure described above. Seeds were incubated for 7 d under ambient laboratory conditions, and germination percentage, root length, shoot length, and seed vigor index were assessed to evaluate early establishment responses. The inhibitory effect was expressed as percentage inhibition, calculated as:

$$\text{Inhibition (\%)} = \frac{C - T}{C} \times 100$$

where C represents the mean value of the control treatment and T represents the corresponding value under extract treatment.

Soil-origin assay

To evaluate whether recruitment limitation was also expressed through invasion-associated soil conditions, surface soils were collected from beneath established *Mimosa pigra* stands (invaded soil) and from nearby non-invaded sites (uninvaded soil) within the same wetland landscape (Del Fabbro et al. 2014). Visible roots and coarse litter fragments were removed manually before use. Seeds were sown in plastic trays containing field-collected soil, with four replicates per soil type. Seedling emergence and growth-related traits were recorded after 21 d.

Phytochemical analysis

Total phenolic content (TPC) was determined using the Folin-Ciocalteu method and expressed as mg gallic acid equivalents (GAE) g^{-1} dry extract (Genwali et al. 2013). Total flavonoid content (TFC) was quantified using the aluminum chloride colorimetric method and expressed as mg quercetin equivalents (QE) g^{-1} dry extract (Shraim et al. 2021). Chromatographic analysis of *Mimosa pigra* leaf extract was conducted using an HPLC system (Gras et al. 2017) (Agilent 1260 Infinity II, Agilent Technologies, Santa Clara, CA, USA) equipped with a diode array detector (DAD) and controlled by Agilent ChemStation software (version B.04.03).

Statistical analysis

Prior to inferential testing, normality and homogeneity of variance were assessed using the Shapiro–Wilk and Levene procedures, respectively, and percentage data were arcsine-square-root transformed when required to better satisfy model assumptions (Levene 1960; Shapiro and Wilk 1965). The effects of extract concentration on germination and seedling-growth variables were analyzed using one-way analysis of variance, followed by Tukey's honestly significant difference test (Tukey 1949) for pairwise mean separation at $\alpha = 0.05$. For the activated-carbon neutralization experiment, a two-way ANOVA was used to test the fixed effects of extract treatment, activated carbon, and their interaction.

To quantify inhibitory potency, concentration–response data were fitted using a four-parameter logistic (4PL) model (Magis 2013) of the form:

$$Y = c + \frac{d - c}{1 + (x/IC_{50})^b}$$

where Y is the response variable, x is extract concentration, $c$and $d$are the lower and upper asymptotes, b is the slope parameter, and IC_{50} is the concentration causing 50% inhibition. Separate models were fitted for germination inhibition, root inhibition, and shoot inhibition for each target species. Model performance was summarized using R^2, and 95% confidence intervals were calculated for each IC_{50} estimate, following standard dose–response procedures (Ritz et al. 2015). Statistical analyses were conducted in R (R Team Core 2020).

Results

Germination responses across concentrations

Germination declined progressively with increasing concentrations of *Mimosa pigra* leaf extract in all three target species, demonstrating a clear concentration-dependent inhibitory effect on seed recruitment. In the control, germination was high in *Eleusine indica* (93.5%), *Paspalum conjugatum* (84.7%), and *Ludwigia hyssopifolia* (78.9%). However, germination dropped sharply at higher concentrations, reaching only 11.6%, 8.4%, and 6.9% at 160 mg mL^{-1}, respectively (Supplementary Tables

Table S1, Fig. 2). The strongest reductions occurred at 80–160 mg mL^{-1}, where germination was suppressed by roughly two-thirds to more than 90% compared with the control

In addition to reducing final germination, the extract also delayed germination and reduced germination performance indices. Mean germination time increased steadily with concentration, for example from 2.2 to 4.0 d

in *E. indica*, while seed vigor index declined dramatically, from 1410 in the control to only 74 at 160 mg mL^{-1} (Supplementary Tables

Table S1). Similar trends were observed in *P. conjugatum* and *L. hyssopifolia*, indicating that the extract impaired not only germination probability but also the speed and vigor of early seedling establishment.

Although all species were negatively affected, *L. hyssopifolia* and *P. conjugatum* showed slightly greater inhibition than *E. indica*, particularly at the highest concentration where germination suppression exceeded 90% (Supplementary Tables

Table S1). Overall, these results support the hypothesis that leaf-derived compounds from *M. pigra* inhibit germination in a concentration-dependent manner and may limit competitor recruitment at the earliest life stage.

Seedling growth inhibition

Seedling growth was strongly suppressed by *Mimosa pigra* leaf extract across all concentrations and species (Fig. 3; Table S2). Inhibition increased progressively with extract concentration, and root growth was consistently more sensitive than shoot growth. For example, in *Eleusine indica*, root inhibition rose from 25.9% at 20 mg mL^{-1} to 93.1% at 160 mg mL^{-1}, whereas shoot inhibition reached 77.6% at the same concentration (Table S2). Similar root-dominant responses were observed in *Paspalum conjugatum* and *Ludwigia hyssopifolia*.

Biomass accumulation showed the same concentration-dependent decline. In *E. indica*, fresh biomass decreased from 86.1 mg $seedling^{-1}$ in the control to 15.3 mg at 160 mg mL^{-1}, while dry biomass fell from 15.5 to 2.4 mg (Table S2). Comparable reductions were recorded in *P. conjugatum* and *L. hyssopifolia*, indicating that extract exposure impaired whole-seedling establishment rather than only elongation traits.

Across species, response trajectories were broadly similar, with only minor interspecific differences in sensitivity. The most consistent pattern was the stronger inhibition of roots relative to shoots, suggesting that early root impairment represents the primary recruitment bottleneck under extract exposure.

Dose response relationships

All major response variables showed clear nonlinear concentration–response relationships, and 4PL models provided a useful summary of inhibitory potency across species and traits (R^2 = 0.96–0.98; Fig. 4). Across species, root inhibition was consistently the most sensitive response, with lower IC_{50} values than both germination and shoot inhibition. In *Eleusine indica*, IC_{50} values were 33.2 mg mL^{-1} for roots, compared with 45.6 and 55.4 mg mL^{-1} for germination and shoots, respectively. A similar pattern was observed in *Paspalum conjugatum* and *Ludwigia hyssopifolia*, indicating that roots responded at lower concentrations than other traits.

Model parameters indicated a relatively sharp transition from weak to strong inhibition, particularly for root responses. Upper asymptotes approached complete suppression across all endpoints, especially for roots (>98%), while confidence intervals around IC_{50} estimates were narrow, suggesting reliable parameter estimates.

Among species, *L. hyssopifolia* was slightly more sensitive than *P. conjugatum*, whereas *E. indica* showed lower sensitivity overall. However, differences among traits were more pronounced than differences among species, with root responses consistently occurring at lower concentrations than germination or shoot inhibition. This identifies root inhibition as the most sensitive endpoint for subsequent ecological interpretation.

Activated carbon neutralization

Activated carbon (AC) partly reduced the inhibitory effects of *Mimosa pigra* leaf extract on seedling emergence and early growth across all three species (

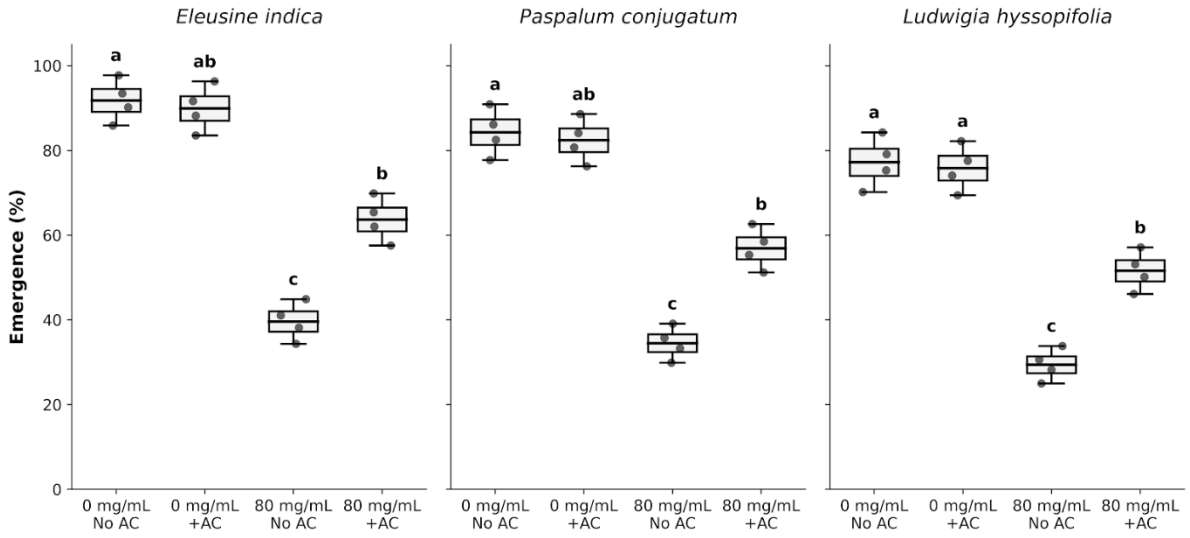

Fig. **5**). At 80 mg mL^{-1}, emergence of *Eleusine indica* increased from 39.6 ± 2.4% without AC to 63.7 ± 2.8% with AC. Similar improvements occurred in *Paspalum conjugatum* (34.5 ± 2.1% to 56.9 ± 2.6%) and *Ludwigia hyssopifolia* (29.4 ± 2.0% to 51.6 ± 2.5%) (

Table 3). In contrast, AC produced only minor changes in the control treatment, with emergence differing by less than 2 percentage points among species.

Growth traits responded similarly. At 80 mg mL^{-1}, root length in *E. indica* increased from 2.1 ± 0.2 to 4.4 ± 0.2 cm with AC, accompanied by an increase in shoot biomass from 0.158 ± 0.014 to 0.244 ± 0.018 g $plant^{-1}$. Comparable increases were observed in *P. conjugatum* (1.8 to 3.8 cm root length; 0.133 to 0.212 g $plant^{-1}$ shoot biomass) and *L. hyssopifolia* (1.3 to 3.0 cm; 0.086 to 0.148 g $plant^{-1}$). Correspondingly, biomass reduction at this concentration declined from 57.5% to 34.4% in *E. indica*, from 59.8% to 36.0% in *P. conjugatum*, and from 62.3% to 35.1% in *L. hyssopifolia* (

Table 3).

Two-way ANOVA supported these responses (

Table **4**). Extract effects on emergence and shoot biomass were highly significant in all species ($P < 0.001$), and the extract × carbon interaction was also significant ($P = 0.003–0.011$). Carbon main effects were comparatively weak, indicating that AC primarily mitigated extract-induced inhibition rather than directly stimulating plant growth.

Invaded versus uninvaded soil effects on seedling establishment

Seedling establishment was consistently lower in soils collected from *Mimosa pigra*-invaded stands than in uninvaded reference soils Across all three species, invaded soils reduced emergence, seedling height, root development, and biomass accumulation after 21 days (Table 5). Emergence declined by approximately 20–23 percentage points across species, while shoot biomass was reduced by about 30–36% relative to uninvaded soils. These reductions were accompanied by parallel decreases in root length and seedling height, indicating coordinated suppression of early establishment traits. The invaded-soil pattern is therefore consistent with soil-mediated recruitment limitation beneath *M. pigra* stands, although this assay does not isolate allelopathy from other invasion-associated soil legacies.

Litter-leachate effects on germination and early seedling growth

Litter-derived aqueous leachates produced a clear concentration-dependent reduction in germination and early seedling growth (Table 6). Even moderate leachate concentrations reduced germination and seed vigor, while stronger effects occurred at the highest concentration tested. Across species, root growth showed greater sensitivity than shoot growth, with root inhibition reaching approximately 60% at 50 g L^{-1}. Seed vigor index declined sharply with increasing leachate concentration, reflecting reduced germination performance and impaired early seedling development (Table 6). These results indicate that aqueous litter leachates can also reduce establishment-related traits, supporting the ecological plausibility of exposure through rainfall leaching and decomposing litter.

Phytochemical composition

The extract was characterized by a phenolic- and flavonoid-rich profile composed of multiple compounds rather than a single dominant constituent. This chemically diverse composition is consistent with the observed inhibitory patterns in the bioassays, where extract exposure reduced germination and suppressed early seedling growth across species. Phytochemical analysis indicated that the leaf extract of *M. pigra* contained substantial amounts of phenolic and flavonoid compounds (Table 7). Total phenolic content (TPC) reached 128.4 ± 6.1 mg GAE g^{-1}, while total flavonoid content (TFC) was 71.3 ± 3.8 mg QE g^{-1}, substantiating that phenolic constituents represented a major fraction of the extract. HPLC–DAD profiling further revealed a chemically diverse mixture of phenolic acids and flavonoids. Among the identified compounds, gallic acid (6.82 min; 280 nm) was detected at 4.62 mg mL^{-1} with a relative peak area of 3.1%. Flavan-3-ols were also present, including epicatechin (14.38 min; 280 nm; 1.84 mg mL^{-1}, 1.6%) and epigallocatechin gallate (EGCG) (17.79 min; 280 nm; 3.05 mg mL^{-1}, 0.8%). Flavonoids constituted another prominent component of the extract. The flavonol glycoside rutin (17.79 min; 360 nm) showed the highest concentration among the quantified compounds (17.21 mg mL^{-1}, 3.0%), followed by the flavonol aglycones quercetin (27.92 min; 360 nm; 9.70 mg mL^{-1}, 1.0%) and kaempferol (29.35 min; 360 nm; 6.46 mg mL^{-1}, 0.9%). All compounds were identified based on retention time and UV spectral matching with external standards.

Associations between phytochemical indices and inhibitory responses

Total phenolic content (TPC) and total flavonoid content (TFC) were strongly associated with germination and seedling growth inhibition across treatments. Root inhibition showed the strongest correlations with both TPC ($r = 0.94$, $P < 0.001$) and TFC ($r = 0.89$, $P < 0.001$), whereas germination and shoot inhibition also increased with higher phytochemical levels ($r = 0.82–0.91$).

Recruitment-related traits were likewise closely linked. Germination inhibition correlated strongly with root inhibition ($r = 0.93$, $P < 0.001$), and root inhibition was tightly associated with biomass reduction ($r = 0.95$, $P < 0.001$) (

Table **8**). These relationships indicate that treatments producing stronger root inhibition also caused greater declines in overall seedling performance. Together, the results suggest that higher phenolic and flavonoid concentrations were associated with coordinated suppression of germination, root growth, and early biomass accumulation (

Table **8**).

Discussion

Across experiments, *Mimosa pigra* consistently reduced germination and early seedling performance, with root growth emerging as the most sensitive response variable. The partial alleviation of inhibition by activated carbon, together with the phenolic- and flavonoid-rich chemistry of the extract, supports the interpretation that these effects were at least partly chemically mediated. More broadly, the results indicate that *M. pigra* can limit early recruitment through multiple plausible exposure pathways, thereby supporting a contributory allelopathic role in invasion.

Recruitment suppression as the dominant response pattern.

One of the main findings of this study was the consistent suppression of recruitment-related traits across all three target species. Increasing extract concentration reduced germination, delayed germination timing, lowered seed vigor, and strongly depressed early seedling growth, indicating that the response was not confined to a single endpoint but extended across successive stages of establishment. This broader recruitment signal is ecologically more informative than final germination alone, because invasion success often depends on whether neighboring species can pass the seed-to-seedling transition and persist beyond initial emergence (Levine et al. 2004; Zhang et al. 2021).

A second prominent feature was the repeated separation between belowground and aboveground responses. Across species, root inhibition occurred at lower extract concentrations than shoot suppression, and root growth remained the most sensitive trait in the dose–response analysis. Similar root-first responses have been reported in other allelopathy studies and are consistent with the idea that belowground tissues are exposed most directly to dissolved allelochemicals during early establishment (Callaway and Ridenour 2004; Uddin et al. 2014).

The activated carbon assay provided additional support for a chemical mechanism (Heidarinejad et al. 2020). Partial recovery after carbon addition suggests that adsorbable organic compounds contributed to the observed inhibition, although activated carbon tests should be interpreted cautiously because carbon can also alter plant performance independently of allelochemical removal (Inderjit and Callaway 2003; Lau et al. 2008)). A comparable interpretation has been reported for *Lantana camara*, where activated charcoal reduced the inhibitory effect of rhizosphere soil on native recruitment, supporting a role for chemically mediated interference in invasion (Kato-Noguchi and Kurniadie 2021). Together with the phenolic- and flavonoid-rich composition of the extract, these findings suggest that the inhibitory effects of *M. pigra* are better explained by a mixture of allelochemicals than by nutrient competition or physicochemical stress alone (Inderjit 1996; Li et al. 2010).

Evidence for chemical mediation

Evidence for chemical mediation in *Mimosa pigra* was strengthened by the activated carbon assay, although the result is best interpreted as supportive rather than definitive. At 80 mg mL^{-1}, activated carbon increased emergence from 39.6% to 63.7% in *Eleusine indica*, from 34.5% to 56.9% in *Paspalum conjugatum*, and from 29.4% to 51.6% in *Ludwigia hyssopifolia*, with parallel improvement in root length and biomass. Recovery of this kind is consistent with the adsorption of bioactive organic compounds, but it does not by itself prove allelopathy because activated carbon can also alter nutrient availability and plant growth independently of allelochemical removal (Inderjit and Callaway 2003; Lau et al. 2008).

The chemical profile of the extract adds an important second line of support. The extract was rich in phenolics and flavonoids and contained several identified polyphenolic constituents, including rutin, quercetin, kaempferol, gallic acid, epicatechin, and epigallocatechin gallate. This is relevant because phenolic compounds are among the secondary metabolites most frequently implicated in plant allelopathy, often acting as mixtures rather than as single dominant toxins (Li et al. 2010). A similar interpretation has been proposed for other invasive species, including *Lantana camara*, where adsorption-based assays and rhizosphere evidence have been used to argue that chemically mediated interference contributes to suppression of neighboring plants (Gentle and Duggin 1997; Kato-Noguchi and Kurniadie 2021).

Taken together, the partial reversal by activated carbon and the polyphenol-rich composition of the extract make a purely physicochemical explanation less likely. This does not demonstrate that any single compound is responsible, nor does it establish field-scale causation, but it does place the inhibitory response of *M. pigra* within a mechanistic framework that is consistent with chemically mediated interference. In this sense, the study goes beyond a simple crude-extract screen by linking recruitment suppression to a plausible chemical basis (Inderjit and Callaway 2003; Kato-Noguchi and Kurniadie 2021).

Ecological relevance for invasion success of *Mimosa pigra*

From an ecological perspective, the main significance of these results lies in the repeated suppression of recruitment-related traits rather than in germination inhibition alone. Across assays, extract exposure reduced emergence, slowed establishment, and strongly constrained early seedling growth, with the strongest effects expressed belowground. Such responses are likely to matter most during the transition from seed arrival to successful establishment, a stage that can strongly influence neighborhood assembly and long-term vegetation

dynamics. In this context, even partial inhibition may be ecologically consequential if it is repeated through litter inputs, leachates, or soil exposure beneath established stands (Levine et al. 2004; Zhang et al. 2021).

This interpretation is consistent with the invasion ecology of *M. pigra*, which is known to form dense, near-monospecific stands in floodplain and wetland habitats and to accumulate abundant litter and propagules. In the Mekong system, the species has been reported to spread across cultivated floodplains and to dominate invaded areas as virtual monocultures, indicating that once established it can strongly reshape local vegetation structure (Rijal and Cochard 2016). The present results suggest that leaf-derived chemical interference may contribute to that dominance by reducing the probability that neighboring species recruit successfully beneath or around invaded stands (Bai et al. 2024). Rather than acting as a stand-alone explanation, this mechanism is more plausibly one component of a broader invasion syndrome that also includes litter build-up, canopy closure, and persistent seed production (Secrett 1996).

The broader relevance of this pattern is that allelopathy becomes most meaningful in invasion biology when it is linked to ecologically realistic pathways and to traits directly tied to establishment (Inderjit et al. 2011). Recent syntheses have emphasized both the widespread occurrence of allelopathic potential among invaders and the need to interpret such effects within realistic ecological settings rather than as isolated extract toxicity (Kalisz et al. 2021). By combining recruitment bioassays with activated carbon, litter-leachate, and invaded-soil evidence, this study evaluates *M. pigra* within a more ecologically grounded framework and supports the view that chemically mediated recruitment limitation may help reinforce persistence of invaded stands (Fig. 6).

Limitations and future directions

Several limitations should be considered when interpreting the present findings. Most assays were conducted under controlled conditions using extract- and substrate-based approaches that are useful for testing mechanism, but they cannot fully reproduce the spatial and temporal complexity of invaded field environments. In particular, exposure intensity in laboratory bioassays may differ from that experienced under natural conditions, where the release, transport, transformation, and persistence of allelochemicals are influenced by litter dynamics, soil properties, hydrology, and microbial activity (Inderjit and Callaway 2003; Wardle et al. 2011; Weidenhamer et al. 2023).

A second limitation is that activated carbon provides supportive rather than definitive evidence for chemical mediation. Although carbon addition partly alleviated inhibition in the present study, activated carbon can also modify nutrient availability and plant growth independently of allelochemical adsorption, which complicates mechanistic inference if interpreted in isolation (Inderjit and Callaway 2003; Lau et al. 2008).

The study focused on early recruitment responses in a limited number of target species, so it does not show whether these effects persist through later survival, competition, or community-level change. Future work should test whether the inhibitory responses observed here are maintained under field conditions, vary across seasons and soil environments, and reduce regeneration beneath established *M. pigra* stands. Further chemical resolution, including fractionation or compound-specific bioassays, would also help clarify the relative contribution of individual metabolites versus mixture effects (Weidenhamer et al. 2023).

Conclusion

This study shows that *Mimosa pigra* can suppress early recruitment across multiple recipient species and multiple plausible exposure pathways. Concentration-dependent inhibition of germination, vigor, root growth, shoot growth, and biomass accumulation, together with partial alleviation by activated carbon and supportive evidence from litter and soil assays, indicates that chemically mediated interference likely contributes to reduced establishment success.

Several lines of evidence suggest that this suppression is at least partly chemically mediated. Partial recovery in the activated carbon assay, together with the phenolic- and flavonoid-rich chemical profile and the inhibitory responses observed in invaded soils and litter leachates, indicates that leaf-derived compounds may contribute to recruitment limitation under ecologically relevant pathways. These findings support allelopathy as a plausible contributory mechanism in the invasion ecology of *M. pigra*, acting primarily through reduced competitor recruitment during early establishment and potentially reinforcing persistence of invaded stands.

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Author contributions

Le Huu Phuoc: Conceptualization, methodology, investigation, formal analysis, data curation, visualization, writing - original draft, writing - review and editing, and project administration.

Vo Thi Huong Duong: Contribution to plant extraction and writing - review and editing.

All authors read and approved the final manuscript.

Data availability

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest

The authors declare no conflict of interest.

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TABLE

Table 1 Study species, functional groups, and seed traits used in recruitment assays

Species	Functional group	Habitat affinity	Mean seed mass (mg)	Seed viability (%)	Baseline germination in control (%)
Eleusine indica	Weed grass	Disturbed open habitats	0.32	96	93.5 ± 1.8
Paspalum conjugatum	Native/perennial grass	Semi-natural understory margins	0.86	91	84.7 ± 2.4
Ludwigia hyssopifolia	Native herb	Riparian/wetland edge	0.14	88	78.9 ± 2.7

Values shown as mean ± SE. Baseline germination values support the use of all three species for dose–response and ecological validation experiments.

Table 2 Extraction yield and physicochemical properties of *Mimosa pigra* leaf extract solutions used in bioassays

Extract concentration (mg mL^{-1})	Extraction yield (%)	pH	Electrical conductivity (mS cm^{-1})	Estimated osmotic potential (MPa)	Matched osmotic control
0	16.8 ± 0.7	6.6	0.12	0.00	Distilled water
20	16.8 ± 0.7	6.4	0.49	-0.02	PEG-20
40	16.8 ± 0.7	6.2	0.93	-0.05	PEG-40
80	16.8 ± 0.7	5.9	1.78	-0.10	PEG-80
160	16.8 ± 0.7	5.6	3.41	-0.21	PEG-160

Table 3 Activated carbon factorial experiment: emergence and growth responses at 21 DAT

Species	Extract (mg mL^{-1})	Activated carbon	Emergence (%)	Root length (cm)	Shoot biomass (g plant^{-1})	Total biomass reduction (%)
Eleusine indica	0	–	91.8 ± 2.7	7.6 ± 0.3	0.372 ± 0.021	0.0
Eleusine indica	0	+	89.9 ± 2.9	7.2 ± 0.3	0.358 ± 0.019	3.8
Eleusine indica	80	–	39.6 ± 2.4	2.1 ± 0.2	0.158 ± 0.014	57.5
Eleusine indica	80	+	63.7 ± 2.8	4.4 ± 0.2	0.244 ± 0.018	34.4
Paspalum conjugatum	0	–	84.3 ± 3.0	6.9 ± 0.3	0.331 ± 0.018	0.0
Paspalum conjugatum	0	+	82.4 ± 2.8	6.5 ± 0.2	0.319 ± 0.017	3.6
Paspalum conjugatum	80	–	34.5 ± 2.1	1.8 ± 0.1	0.133 ± 0.012	59.8
Paspalum conjugatum	80	+	56.9 ± 2.6	3.8 ± 0.2	0.212 ± 0.016	36.0
Ludwigia hyssopifolia	0	–	77.2 ± 3.2	5.8 ± 0.2	0.228 ± 0.015	0.0
Ludwigia hyssopifolia	0	+	75.8 ± 2.9	5.5 ± 0.2	0.221 ± 0.014	3.1
Ludwigia hyssopifolia	80	–	29.4 ± 2.0	1.3 ± 0.1	0.086 ± 0.009	62.3
Ludwigia hyssopifolia	80	+	51.6 ± 2.5	3.0 ± 0.2	0.148 ± 0.012	35.1

Activated carbon partly alleviated inhibitory effects for all species. The modest carbon-only shift in control treatments indicates that adsorption-based mechanism testing should be interpreted together with carbon side effects rather than as an artifact-free binary test.

Table 4 Two-way ANOVA summary for the activated carbon experiment

Species	Response	Extract effect (P)	Carbon effect (P)	Extract × carbon (P)	Partial η^2 for interaction
Eleusine indica	Emergence	<0.001	0.041	0.003	0.29
Eleusine indica	Shoot biomass	<0.001	0.058	0.006	0.25
Paspalum conjugatum	Emergence	<0.001	0.049	0.004	0.27

Paspalum conjugatum	Shoot biomass	<0.001	0.062	0.008	0.23
Ludwigia hyssopifolia	Emergence	<0.001	0.071	0.005	0.26
Ludwigia hyssopifolia	Shoot biomass	<0.001	0.083	0.011	0.21

Significant extract × carbon interactions are consistent with chemically mediated interference, although the low-magnitude main effect of carbon on some responses suggests additional caution in ecological interpretation.

Table 5 Invaded versus uninvaded soil effects on seedling establishment (21 DAT)

Species	Soil origin	Emergence (%)	Seedling height (cm)	Root length (cm)	Shoot biomass (g plant⁻¹)	Biomass reduction vs uninvaded (%)
Eleusine indica	Uninvaded	88.7 ± 2.9	17.8 ± 1.0	8.2 ± 0.4	0.346 ± 0.020	0.0
Eleusine indica	Invaded	69.2 ± 3.1	12.9 ± 0.9	5.6 ± 0.3	0.242 ± 0.018	30.1
Paspalum conjugatum	Uninvaded	81.4 ± 2.7	15.4 ± 0.8	7.1 ± 0.3	0.307 ± 0.017	0.0
Paspalum conjugatum	Invaded	60.5 ± 2.8	11.2 ± 0.8	4.9 ± 0.2	0.213 ± 0.015	30.6
Ludwigia hyssopifolia	Uninvaded	74.6 ± 2.6	11.9 ± 0.7	5.9 ± 0.2	0.204 ± 0.014	0.0
Ludwigia hyssopifolia	Invaded	51.8 ± 2.4	8.1 ± 0.6	3.8 ± 0.2	0.131 ± 0.011	35.8

The invaded-soil pattern supports the hypothesis that *Mimosa pigra* may generate soil-mediated recruitment constraints in addition to direct extract toxicity measured under laboratory conditions.

Table 6 Aqueous litter-leachate effects on germination and early seedling growth (7 DAT)

Species	Leachate concentration (g L⁻¹)	Germination (%)	Root length (cm)	Shoot length (cm)	Seed vigor index	Root inhibition (%)
Eleusine indica	0	92.8 ± 2.0	5.6 ± 0.2	6.4 ± 0.2	1366 ± 60	0.0
Eleusine indica	10	78.6 ± 2.1	4.2 ± 0.2	5.7 ± 0.2	964 ± 45	25.0
Eleusine indica	50	55.3 ± 2.4	2.3 ± 0.1	4.1 ± 0.2	431 ± 29	58.9
Paspalum conjugatum	0	83.5 ± 2.3	5.0 ± 0.2	5.7 ± 0.2	1054 ± 52	0.0

Species	Leachate concentration (g L^{-1})	Germination (%)	Root length (cm)	Shoot length (cm)	Seed vigor index	Root inhibition (%)
Paspalum conjugatum	10	70.8 ± 2.2	3.8 ± 0.1	5.0 ± 0.2	754 ± 38	24.0
Paspalum conjugatum	50	48.7 ± 2.1	2.0 ± 0.1	3.7 ± 0.1	341 ± 24	60.0
Ludwigia hyssopifolia	0	77.1 ± 2.5	4.0 ± 0.2	4.7 ± 0.2	670 ± 33	0.0
Ludwigia hyssopifolia	10	64.9 ± 2.3	3.0 ± 0.1	4.0 ± 0.1	474 ± 27	25.0
Ludwigia hyssopifolia	50	43.2 ± 2.0	1.6 ± 0.1	2.9 ± 0.1	194 ± 18	60.0

Leachate effects are weaker than those of crude ethanolic extracts but remain biologically meaningful, supporting field-relevant release pathways through rainfall leaching and litter decomposition.

Table 7 Phytochemical composition of *Mimosa pigra* leaf extract based on spectrophotometry and HPLC UV DAD profiling

Compound / index	Chemical class	Retention time (min)	Detection wavelength (nm)	Concentration	Relative peak area (%)	Identification confidence
Total phenolic content (TPC)	Bulk index	–	765	128.4 ± 6.1 mg GAE g^{-1}	–	Colorimetric
Total flavonoid content (TFC)	Bulk index	–	415	71.3 ± 3.8 mg QE g^{-1}	–	Colorimetric
Gallic acid	Phenolic acid	6.82	280	4.62 mg mL^{-1}	3.1	Standard match
Epicatechin	Flavan-3-ol	14.38	280	1.84 mg mL^{-1}	1.6	Standard match
Epigallocatechin gallate (EGCG)	Flavan-3-ol	17.79	280	3.05 mg mL^{-1}	0.8	Standard match
Rutin	Flavonol glycoside	17.79	360	17.21 mg mL^{-1}	3.0	Standard match

Quercetin	Flavonol aglycone	27.92	360	9.70 mg mL^{-1}	1.0	Standard match
Kaempferol	Flavonol aglycone	29.35	360	6.46 mg mL^{-1}	0.9	Standard match

Table 8 Pearson correlations between phytochemical indices and inhibitory responses across treatments

Variable 1	Variable 2	Pearson r	P-value	n	Interpretation
TPC	Germination inhibition	0.91	<0.001	15	Strong positive association
TPC	Root inhibition	0.94	<0.001	15	Strong positive association
TPC	Shoot inhibition	0.88	<0.001	15	Strong positive association
TFC	Germination inhibition	0.84	0.001	15	Strong positive association
TFC	Root inhibition	0.89	<0.001	15	Strong positive association
TFC	Shoot inhibition	0.82	0.002	15	Strong positive association
Germination inhibition	Root inhibition	0.93	<0.001	15	Recruitment endpoints covary strongly
Root inhibition	Biomass reduction	0.95	<0.001	15	Root stress closely tracks performance loss

Supplementary Tables

Table S1 Germination responses of competitor species across extract concentrations in Petri-dish assays (7 DAT)

Species	Concentration (mg mL^{-1})	Germination (%)	Germination inhibition (%)	Mean germination time (days)	Germination index	Seed vigor index
Eleusine indica	0	93.5 ± 1.8	0.0	2.2 ± 0.1	18.3 ± 0.7	1410 ± 58
Eleusine indica	20	79.8 ± 2.0	14.7	2.5 ± 0.1	15.1 ± 0.6	1032 ± 51
Eleusine indica	40	60.7 ± 2.3	35.1	3.0 ± 0.2	10.8 ± 0.5	628 ± 37
Eleusine indica	80	33.9 ± 2.1	63.7	3.5 ± 0.2	6.0 ± 0.4	252 ± 21
Eleusine indica	160	11.6 ± 1.5	87.6	4.0 ± 0.3	2.0 ± 0.2	74 ± 10
Paspalum conjugatum	0	84.7 ± 2.4	0.0	2.6 ± 0.1	15.7 ± 0.6	1098 ± 47
Paspalum conjugatum	20	72.1 ± 2.2	14.9	2.9 ± 0.1	13.0 ± 0.5	824 ± 39
Paspalum conjugatum	40	53.6 ± 2.4	36.7	3.3 ± 0.2	9.1 ± 0.4	476 ± 31
Paspalum conjugatum	80	29.1 ± 1.9	65.6	3.9 ± 0.2	5.1 ± 0.3	183 ± 18
Paspalum conjugatum	160	8.4 ± 1.3	90.1	4.4 ± 0.3	1.5 ± 0.2	42 ± 7
Ludwigia hyssopifolia	0	78.9 ± 2.7	0.0	2.8 ± 0.1	14.2 ± 0.5	706 ± 35
Ludwigia hyssopifolia	20	67.5 ± 2.5	14.4	3.0 ± 0.1	11.8 ± 0.4	540 ± 28
Ludwigia hyssopifolia	40	49.3 ± 2.1	37.5	3.4 ± 0.2	8.3 ± 0.3	316 ± 22
Ludwigia hyssopifolia	80	26.8 ± 1.8	66.0	4.0 ± 0.2	4.5 ± 0.3	121 ± 14
Ludwigia hyssopifolia	160	6.9 ± 1.1	91.3	4.6 ± 0.3	1.2 ± 0.1	24 ± 5

DAT, days after treatment. Seed vigor index integrates germination and seedling length, and therefore captures both emergence probability and early establishment quality.

Table S2 Seedling growth responses across extract concentrations (7 DAT)

Species	Concentration (mg mL^{-1})	Root length (cm)	Shoot length (cm)	Root inhibition (%)	Shoot inhibition (%)	Fresh biomass (mg seedling^{-1})	Dry biomass (mg seedling^{-1})
Eleusine indica	0	5.8 ± 0.2	6.7 ± 0.3	0.0	0.0	86.1 ± 3.8	15.5 ± 0.7
Eleusine indica	20	4.3 ± 0.2	5.6 ± 0.2	25.9	16.4	67.4 ± 3.1	12.1 ± 0.5
Eleusine indica	40	2.7 ± 0.1	4.3 ± 0.2	53.4	35.8	48.9 ± 2.7	8.9 ± 0.4
Eleusine indica	80	1.2 ± 0.1	2.8 ± 0.1	79.3	58.2	28.1 ± 2.0	4.8 ± 0.3
Eleusine indica	160	0.4 ± 0.1	1.5 ± 0.1	93.1	77.6	15.3 ± 1.5	2.4 ± 0.2
Paspalum conjugatum	0	5.1 ± 0.2	5.9 ± 0.2	0.0	0.0	73.8 ± 3.2	13.7 ± 0.6
Paspalum conjugatum	20	3.9 ± 0.2	5.0 ± 0.2	23.5	15.3	58.6 ± 2.9	10.9 ± 0.5
Paspalum conjugatum	40	2.4 ± 0.1	3.9 ± 0.2	52.9	33.9	41.7 ± 2.3	7.6 ± 0.4
Paspalum conjugatum	80	1.0 ± 0.1	2.4 ± 0.1	80.4	59.3	22.9 ± 1.8	4.0 ± 0.3
Paspalum conjugatum	160	0.3 ± 0.1	1.1 ± 0.1	94.1	81.4	11.6 ± 1.1	1.8 ± 0.2
Ludwigia hyssopifolia	0	4.2 ± 0.2	4.8 ± 0.2	0.0	0.0	49.2 ± 2.5	8.4 ± 0.4
Ludwigia hyssopifolia	20	3.1 ± 0.1	4.0 ± 0.2	26.2	16.7	38.8 ± 2.1	6.9 ± 0.3
Ludwigia hyssopifolia	40	1.9 ± 0.1	3.0 ± 0.1	54.8	37.5	27.4 ± 1.7	5.0 ± 0.3
Ludwigia hyssopifolia	80	0.8 ± 0.1	1.9 ± 0.1	81.0	60.4	15.1 ± 1.2	2.8 ± 0.2
Ludwigia hyssopifolia	160	0.2 ± 0.0	0.8 ± 0.1	95.2	83.3	7.3 ± 0.8	1.1 ± 0.1

Across all three species, root growth remained the most sensitive endpoint, a pattern consistent with direct exposure of root tissues to dissolved phytotoxic compounds in the germination substrate.

FIGURE

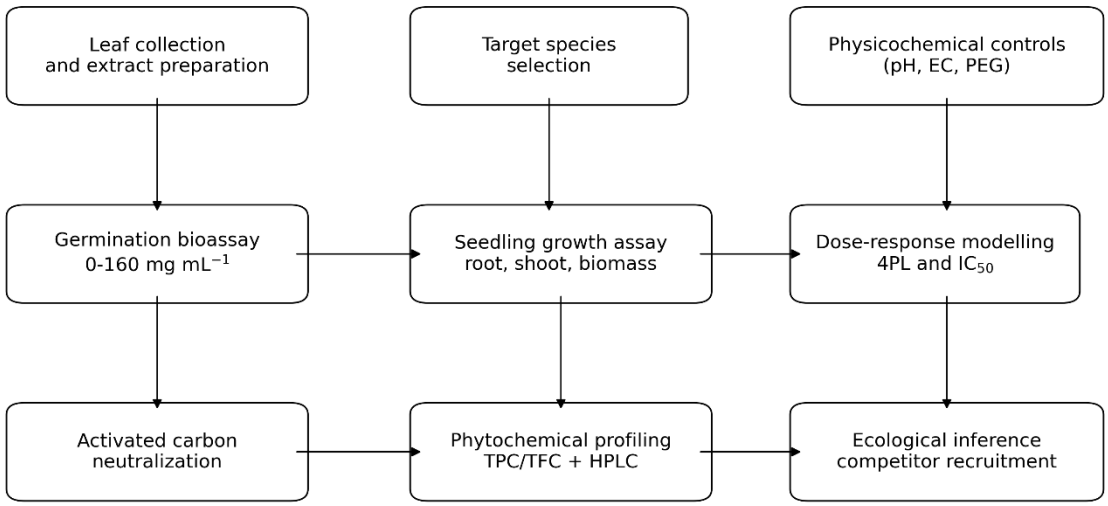


Fig. 1 Schematic overview of the experimental workflow used to test whether leaf-derived chemistry from *Mimosa pigra* suppresses competitor recruitment across different plant functional groups

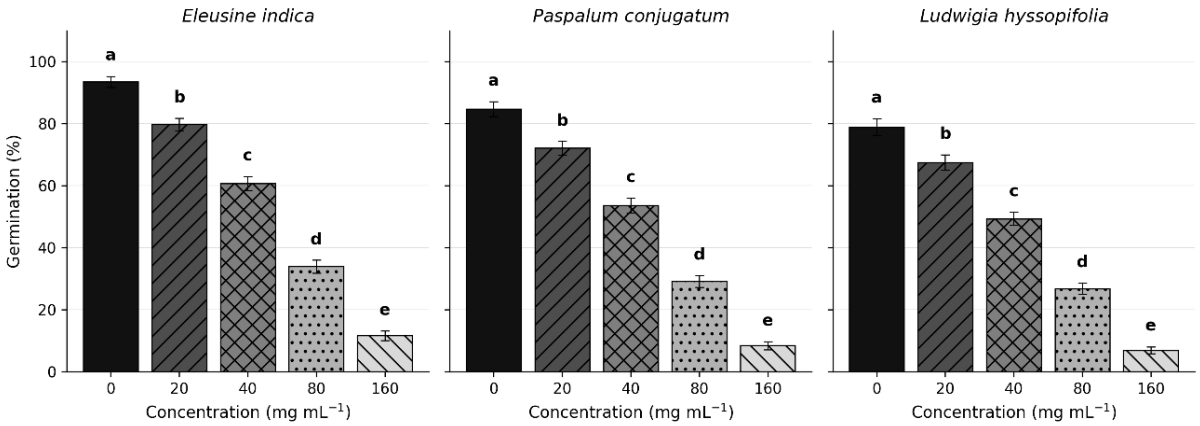


Fig. 2 Germination responses of three target species (*Eleusine indica, Paspalum conjugatum, and Ludwigia hyssopifolia*) to increasing concentrations of Mimosa pigra leaf extract. Bars represent mean ± SE. Different lowercase letters indicate significant differences among concentrations within each species according to Tukey's HSD test ($P < 0.05$).

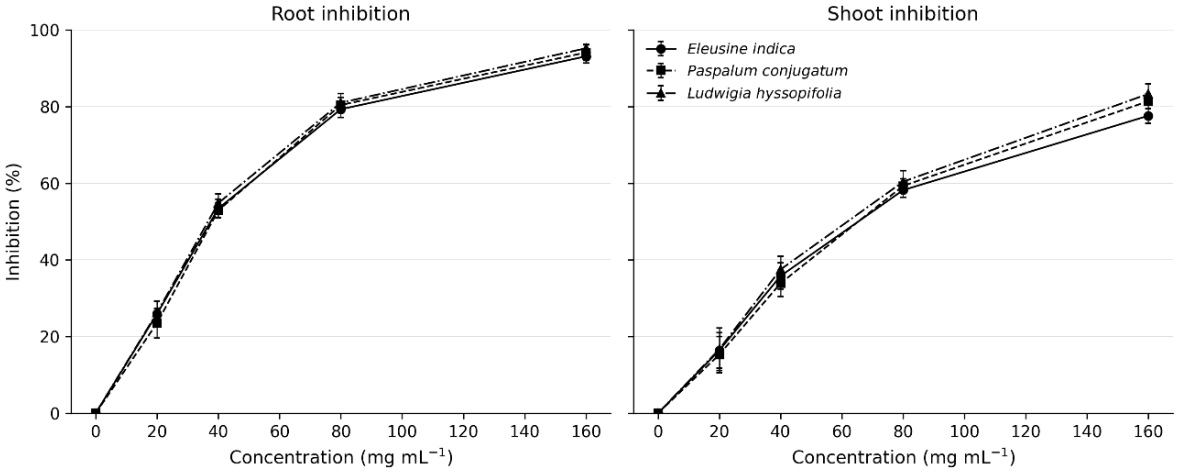
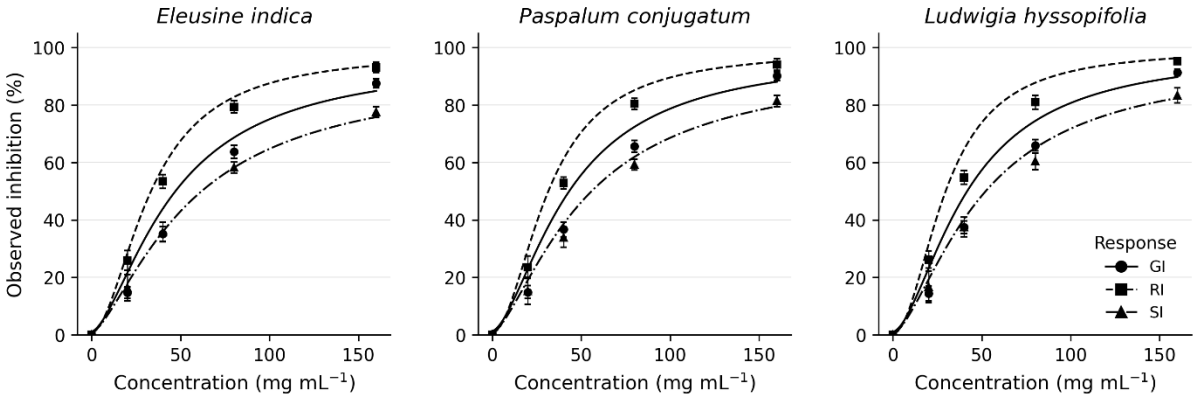


Fig. 3 Concentration-dependent inhibition of seedling growth by *Mimosa pigra* leaf extract, shown separately for root and shoot traits in three target species (*Eleusine indica*, *Paspalum conjugatum*, and *Ludwigia hyssopifolia*). Error bars represent mean ± SE



Species	Response	IC_{50} (mg mL^{-1})	95% CI	Slope	Lower asym.	Upper asym.	R^2
Eleusine indica	GI	45.6	40.1-51.8	1.58	1.2	96.4	0.97
Eleusine indica	RI	33.2	29.0-38.0	1.86	0.9	98.7	0.98
Eleusine indica	SI	55.4	48.6-63.9	1.44	0.7	92.1	0.96
Paspalum conjugatum	GI	43.1	37.9-49.3	1.63	1.4	98.3	0.97
Paspalum conjugatum	RI	31.5	27.6-36.1	1.93	0.8	99.1	0.98
Paspalum conjugatum	SI	52.2	45.7-59.9	1.47	0.6	94.7	0.96
Ludwigia hyssopifolia	GI	41.8	36.4-47.7	1.69	1.1	98.9	0.97
Ludwigia hyssopifolia	RI	29.7	25.8-34.2	2.02	0.5	99.5	0.98
Ludwigia hyssopifolia	SI	49.6	43.0-57.2	1.55	0.4	95.8	0.96

Fig. 4 Four-parameter logistic (4PL) dose–response curves for germination inhibition (GI), root inhibition (RI), and shoot inhibition (SI) of three target species (*Eleusine indica*, *Paspalum conjugatum*, and *Ludwigia*

hyssopifolia) exposed to increasing concentrations of *Mimosa pigra* leaf extract. Points represent observed values with error bars showing mean ± SE, and fitted curves indicate model-predicted responses. The table summarizes the estimated 4PL parameters, including IC_{50}, 95% confidence interval (CI), slope, lower and upper asymptotes, and R^2

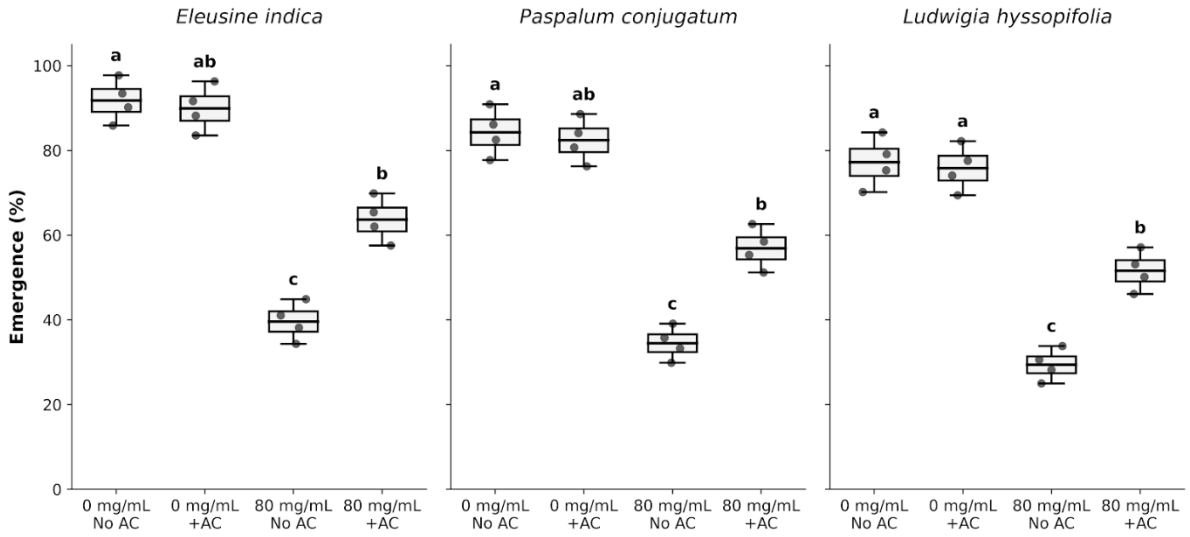


Fig. 5 Partial alleviation of Mimosa pigra-induced inhibition by activated carbon. Seedling emergence of three target species (*Eleusine indica, Paspalu m conjugatum, and Ludwigia hyssopifolia*) was assessed at two leaf extract concentrations (0 and 80 mg mL^{-1}) in the presence (+AC) or absence (−AC) of activated carbon. Each boxplot represents four replicates (N = 4), and error bars represent mean ± SE. Different lowercase letters indicate significant differences among treatment combinations within each species according to Tukey's HSD test ($P < 0.05$).

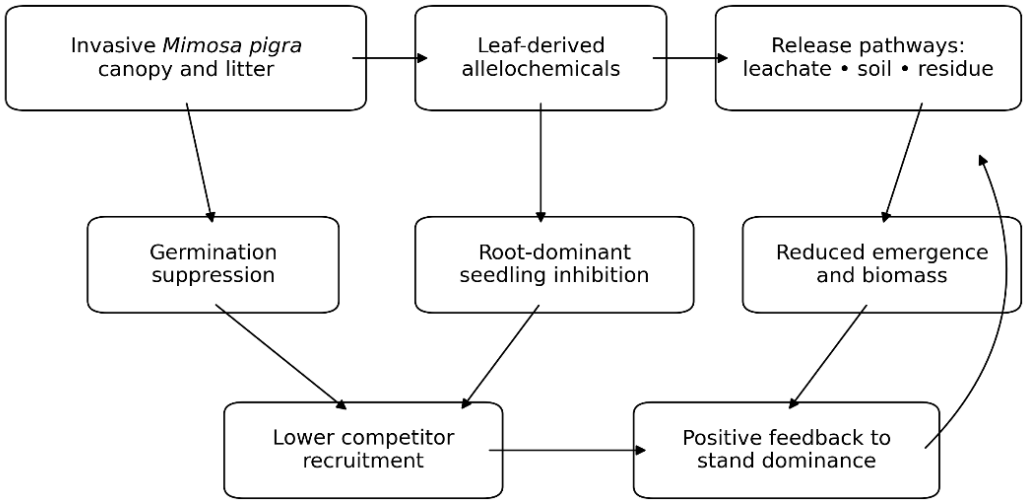


Fig. 6 Conceptual model showing how leaf-derived chemistry from *Mimosa pigra* may suppress competitor recruitment and contribute to invasion success. Allelochemicals released through litter leachates, soil, and plant residues are hypothesized to reduce germination and inhibit seedling growth, thereby lowering competitor recruitment and reinforcing stand dominance. In this framework, allelopathy is interpreted as one component of invasion interacting with litter accumulation and stand closure.

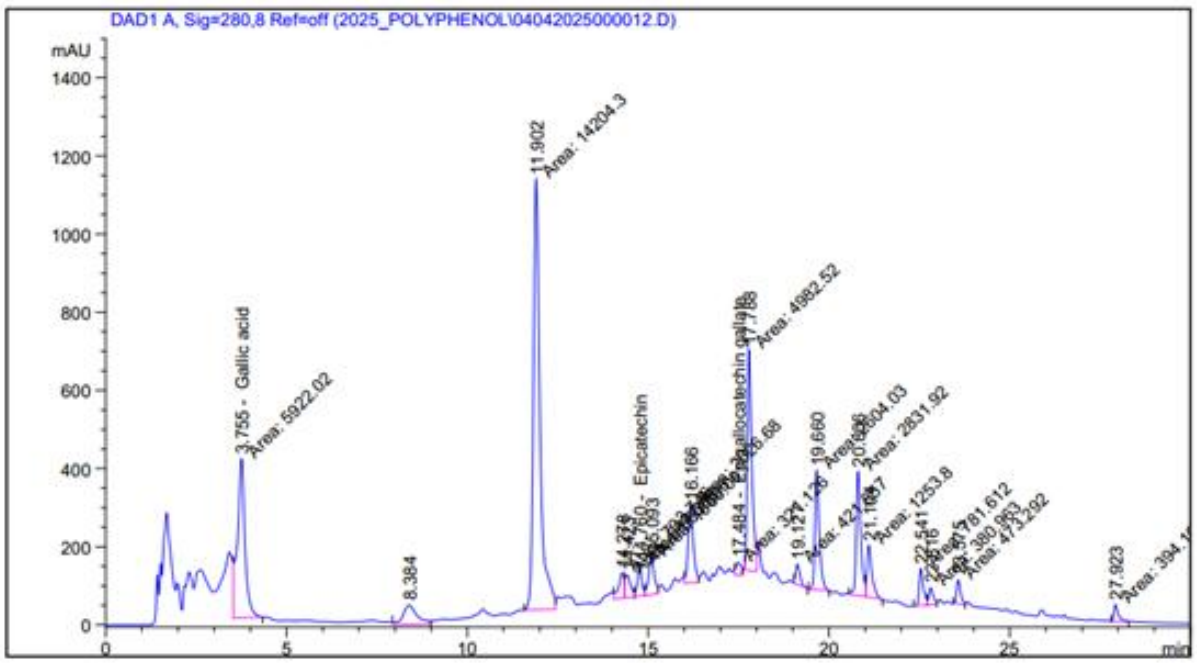


Fig S1 HPLC chromatogram of polyphenolic compounds extracted from *Mimosa pigra* leaves detected at 280 nm. The chromatographic profile shows the relative abundance and distribution of phenolic constituents across the elution time.

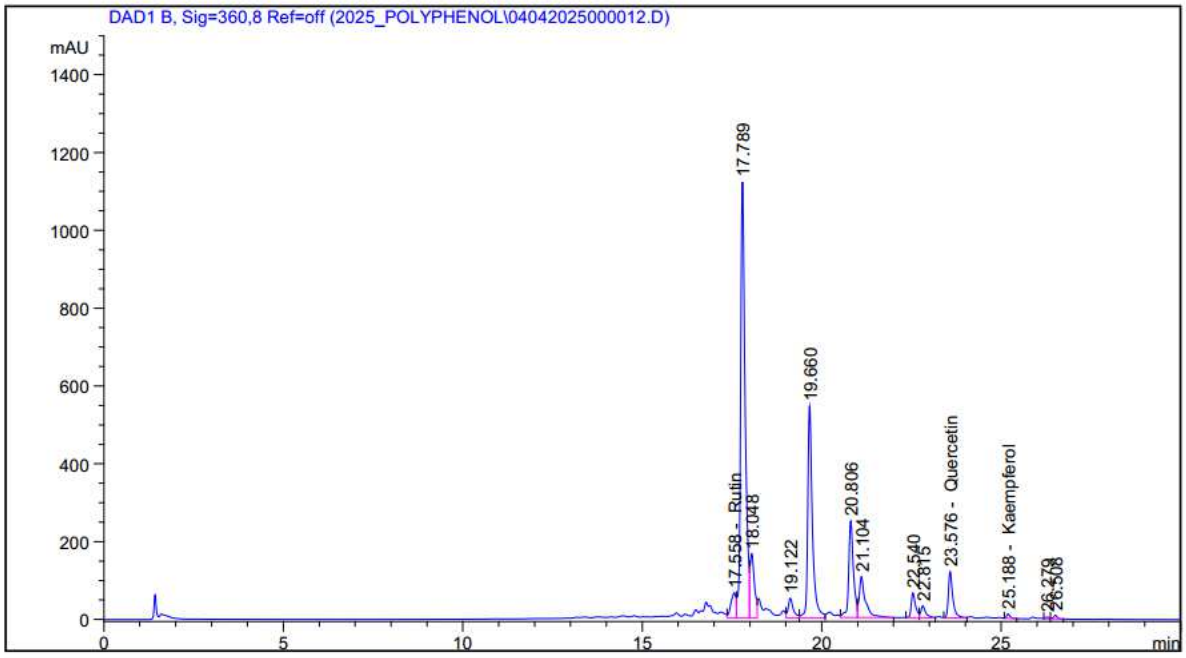


Fig S2 HPLC chromatogram of flavonoid compounds from *Mimosa pigra* leaf extract detected at 360 nm. Major peaks correspond to rutin, quercetin, and kaempferol, with retention times indicated. The chromatogram illustrates the distribution and relative abundance of flavonoid constituents across the elution profile.

Supplementary Files

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- SupplementaryFigureTable.docx