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Research Article

Allelopathic potential of *Acacia pennata* (L.) Willd. leaf extracts against the seedling growth of six test plants

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Abstract

Acacia pennata (L.) Willd (Mimosaceae), a woody climbing plant, is used as a traditional medicinal plant in the South and Southeast Asia regions and has been documented to have various pharmacological effects. However, the allelopathy of this plant still remains unclear. Thus, the allelopathic potential of *A. pennata* leaf extracts was examined against the seedling growth of dicot plants [alfalfa (*Medicago sativa* L.), cress (*Lepidium sativum* L.), and lettuce (*Lactuca sativa* L.)] and monocot plants [barnyard grass (*Echinochloa crus-galli* (L.) Beauv.), Italian ryegrass (*Lolium multiflorum* Lam.), and timothy (*Phleum pratense* L.)] at six different concentrations. The results showed that the *A. pennata* leaf extracts inhibited the seedling growth of all the test plant species at concentrations ≥3 mg dry weight (D.W.) equivalent extract mL^{-1}. The inhibitory activity of the extracts against both shoot and root growth varied with concentration and tested plants. The concentrations required for 50% inhibition of the test plant shoots and roots were 1.5-16.1 and 1.4-8.6 mg D.W. equivalent extract mL^{-1}, respectively. The root growth of all the test plant species was more sensitive to the extracts than their shoot growth, except alfalfa. The results of the present study indicate that the *A. pennata* leaf extracts may have allelopathic potential and may contain allelopathic substances. Therefore, further studies are required for isolation and identification of the growth inhibitory substances which are responsible for the allelopathic effect of *A. pennata.*

Keywords: *Acacia pennata*; allelopathic potential; growth inhibitor; medicinal plant

Introduction

Weeds, among other crop pests, cause the greatest potential yield losses in agricultural production (Oerke, 2006; WSSA, 2019). Accordingly, the application of synthetic herbicides has been the most significant practice in controlling weeds in crop fields (Varshney *et al.*, 2012). However, the widespread and unbalanced application of synthetic herbicides has harmful effects on human health and agriculture (such as the destruction of important beneficial insects and soil microorganisms), and has a toxic residual effect on the environment (Aktar *et al.*, 2009; Chauhan *et al.*, 2018). Furthermore, a number of herbicide-resistant weed biotypes have evolved because of high selection intensity of synthetic herbicides (Caseley *et al.*, 2013). To date, approximately 262 species (152 dicots and 110 monocots) of herbicide-resistant weeds have been reported globally (Heap,

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2020). Hence, searching for natural alternative ways of controlling weeds is needed to reduce the risks of synthetic herbicides (Chai *et al.*, 2015; Islam *et al.*, 2017).

In recent decades, the exploitation of a natural phenomenon called allelopathy has been suggested for weed management and crop productivity in agricultural practices (Li *et al.*, 2010; Bhadoria, 2011). Many allelopathic plants have been used in different ways such as intercrops, cover crops, components of crop rotation, for mulching and residue incorporation in soil, and directly spraying the extract on weeds (Cheema *et al.*, 2000; Rehman *et al.*, 2019). However, the number of successful allelopathic plants is still limited. Therefore, it is important to search for plants with high allelopathic potential to develop effective natural weed control methods.

Many researchers have focused on allelopathic medicinal plants (Li *et al.*, 2009; Poonpaiboonpipat and Jumpathong, 2019) because medicinal plants possibly contain more bioactive compounds than other plants (Islam and Kato-Noguchi, 2014). Several secondary metabolites in medicinal plants can act as allelochemicals to other plants, inhibiting their growth and development (Shurigin *et al.*, 2018). For example, Ladhari *et al.* (2013) reported that three phytotoxic substances isolated from *Capparis spinosa* suppressed the lettuce seedling growth. Furthermore, Kato-Noguchi *et al.* (2019) have been reported that two isolated compounds from *Acmella oleracea* inhibited the growth of cress and barnyard grass. Notably, there are a number of medicinal plants in the Mimosaceae family (Wickens and Pennacchio, 2002; Saha *et al.*, 2018), which comprises many genera and species (Ebinger *et al.*, 2000; Orchard and Wilson, 2001; Maslin *et al.*, 2003; Wiart, 2006). Numerous plants in the Mimosaceae family have been reported to possess a wide array of biologically active constituents as well as allelopathic activity. For instance, aqueous extracts of *Acacia cambagei* foliage suppressed up to 90% of ryegrass seedling growth in Australia (An and Pratley, 2005). In addition, seed extracts of *Acacia cyanophylla* from Tunisia were the strongest inhibitor of the germination and seedling growth of *Lactuca sativa* and *Peganum harmala* (El Ayeb *et al.*, 2013). Similarly, Sahid *et al.* (2017) found that mimosine compounds isolated from *Leucaena leucocephala* inhibited the growth of selected invasive weeds in Malaysia. However, several medicinal plants in the Mimosaceae family have still not been investigated for allelopathic properties.

Acacia pennata (L.) Willd., a large woody prickly climber, has been used for both medicinal and culinary purposes in the South and Southeast Asia regions (Lalchhandama, 2013; Terangpi *et al.*, 2013). The leaf, stem, and roots are valued as herbal medicines for treating body aches, snake venom and fish poisoning, and stomach pain, respectively (Pullaiah, 2006; Khare, 2008; Lalchhandama, 2013). The anti-inflammatory, antioxidant, and anti-parasitic activities of *A. pennata* have been documented (Dongmo *et al.*, 2005; Sowndhararajan *et al.*, 2013). Very recently, Lalnunhluva *et al.* (2019) have been evaluated the effect of aqueous extracts of *Parkia timoriana, A. pennata* and *Trevesia palmata* on four understory crops for selecting suitable crop combinations in agroforestry systems. However, concentrations of aqueous methanol extract of *A. pennata* on common crops and weed species have not been reported. It was therefore of interest to assess the allelopathic potential of this plant species under laboratory condition for weed control purpose.

Materials and Methods

Plant materials

The leaves of *Acacia pennata* were collected from the Yezin area, Nay Pyi Taw Division, Myanmar (19°45′ N and 96°6′ E) during May-June 2019. The collected leaves were dried in the shade, ground into a fine powder, and kept in a refrigerator at 2 °C until extraction. Three dicots [alfalfa (*Medicago sativa* L.), cress (*Lepidium sativum* L.), and lettuce (*Lactuca sativa* L.)] and three monocots [barnyard grass (*Echinochloa crus-galli* (L.) Beauv.), Italian ryegrass (*Lolium multiflorum* Lam.), and timothy (*Phleum pratense* L.)] were selected as test plants. Alfalfa, cress, lettuce, and timothy were chosen because of their known seedling behaviors, whereas barnyard grass and Italian ryegrass were used due to their common distribution in cultivated fields (Islam *et al*., 2017).

Extraction of A. pennata leaves

The leaf powder (50 g) of *A. pennata* was extracted with 500 mL of 70% (v/v) aqueous methanol and kept in a sealed container for 48 h. The extract was then filtered using a sheet of filter paper (No. 2; Toyo Ltd., Tokyo, Japan). The residue was extracted again with an equal amount of methanol for 24 h and filtered. The two filtrates were mixed and evaporated at 40°C with a vacuum rotary evaporator to get the crude extract.

Growth bioassay

The crude extracts of the *A. pennata* leaves were diluted with methanol. An aliquot of leaf extract at final assay concentrations of 1, 3, 10, 30, 100, and 300 mg dry weight equivalent extract mL^{-1} was added to sheets of filter paper (No.2) in 28 mm Petri dishes. The methanol was then evaporated under a laminar flow cabinet. Consequently, 0.6 mL of 0.05% (v/v) aqueous solution of polyoxyethylenesorbitan monolaurate (Tween 20; Nacalai, Kyoto, Japan) was added to all the Petri dishes. Tween 20 was used as a non-toxic surfactant for seedling growth of all the test plants. Ten germinated seeds of barnyard grass, Italian ryegrass, and timothy (germinated in the dark at 25 °C for 48, 60, and 48 h, respectively), and ten seeds of alfalfa, cress, and lettuce were arranged on each Petri dish. As a control treatment, the seeds in the Petri dishes were treated with Tween 20 without leaf extracts. Finally, the shoot and root length of all the test plant species were measured after 48 h incubation in the dark at 25 °C. The seedling length percentage was calculated using the following formula: percentage of seedling length (%) = (length of treatment/length of control) × 100 (Krumsri *et al*., 2019).

Statistical analysis

The bioassay experiments were conducted using a completely randomized design (CRD) with three replications, and the experiment for each test plant was repeated twice (10 seedlings/replication, n=60). Experimental data were analyzed using SPSS version 16.0, and the significant difference between treatments was investigated by carrying out post-hoc Tukey's test at p=0.05. The interaction between the tested plants and concentrations was subjected to two-way analysis of variance (ANOVA), and the paired *t*-test was used to investigate the concentration required for 50% inhibition (I_{50} value) of shoot and root growth. The I_{50} values were analyzed using GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, California, USA).

Results

The crude extracts of the *A. pennata* leaf was obtained in the yield of 9.1 g. The leaf extracts were applied in different concentrations on the test plants. The results showed that the aqueous methanol extracts of the *A. pennata* leaves significantly inhibited the seedling growth of all the test plant species at the concentration of 1 mg D.W. equivalent extract mL^{-1}, except the roots of Italian ryegrass (Figures 1 and 2). At 10 mg D.W. equivalent extract mL^{-1}, the shoot and root growth of alfalfa, lettuce, Italian ryegrass, and timothy were inhibited by more than 50%. At 100 mg D.W. equivalent extract mL^{-1}, the shoot growth of lettuce and cress was completely inhibited, while alfalfa, barnyard grass, Italian ryegrass, and timothy were inhibited to 3.9, 34.2, 3.3, and 0.3% of control growth, respectively. At the same concentration, the root growth of lettuce and timothy were completely inhibited and that of alfalfa, cress, barnyard grass, and Italian ryegrass were inhibited to 5.2, 3.7, 2.1, and 1.7% of control growth, respectively. In addition, the two-way ANOVA showed that concentrations of the extract and growth (shoot and root) of the test plant species had significant interaction (Table 1).

The I_{50} values of the *A. pennata* leaf extracts for the shoot and root growth of the test plant species ranged from 1.5 to 16.1 and from 1.4 to 8.6 mg D.W. equivalent extract mL^{-1}, respectively (Figure 3). The I_{50} values for the cress, barnyard grass, Italian ryegrass, and timothy shoots were 7.3, 16.1, 10.3, and 4.5 mg D.W. equivalent extract mL^{-1}, respectively, which were significantly greater than those for their roots ($p \leq 0.05$) at 4.7, 3.3, 8.6, and 2.5 mg D.W. equivalent extract mL^{-1}, respectively.

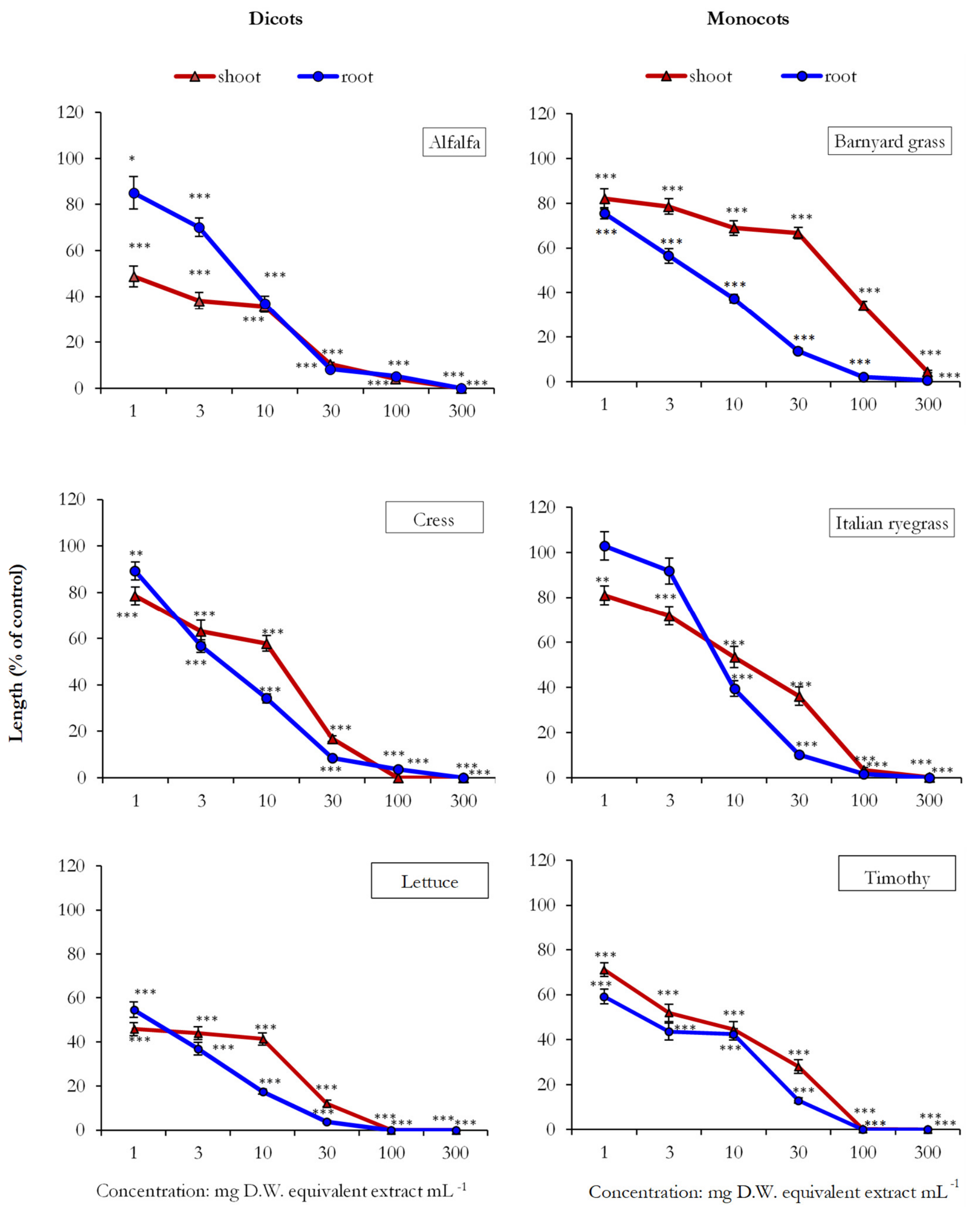

Figure 1. Effect of *Acacia pennata* leaf extracts on the seedling growth of six test plant species at six different concentrations

The vertical bars show standard error of the mean

The significant differences between treatments and control are indicated by asterisks: *$p<0.05$, **$p<0.01$, and ***$p<0.001$

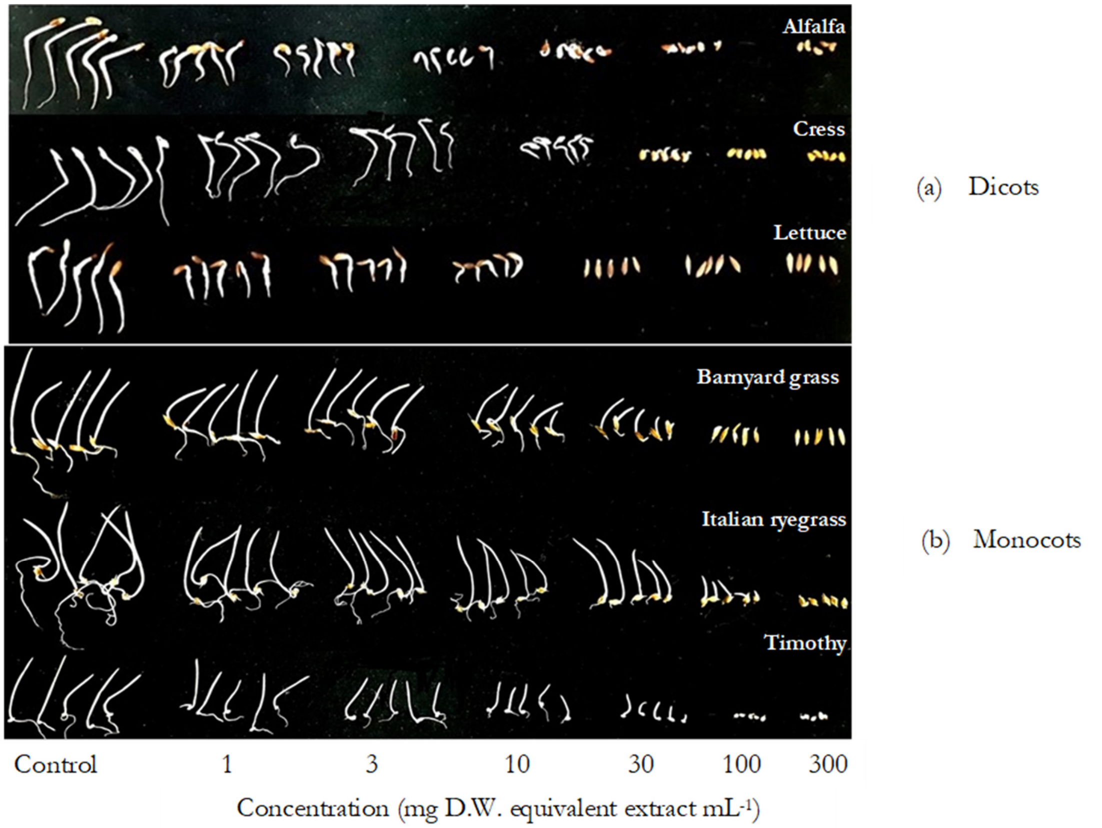

Figure 2. Effect of *A. pennata* leaf extracts on seedling growth of six test plant species at six different concentrations

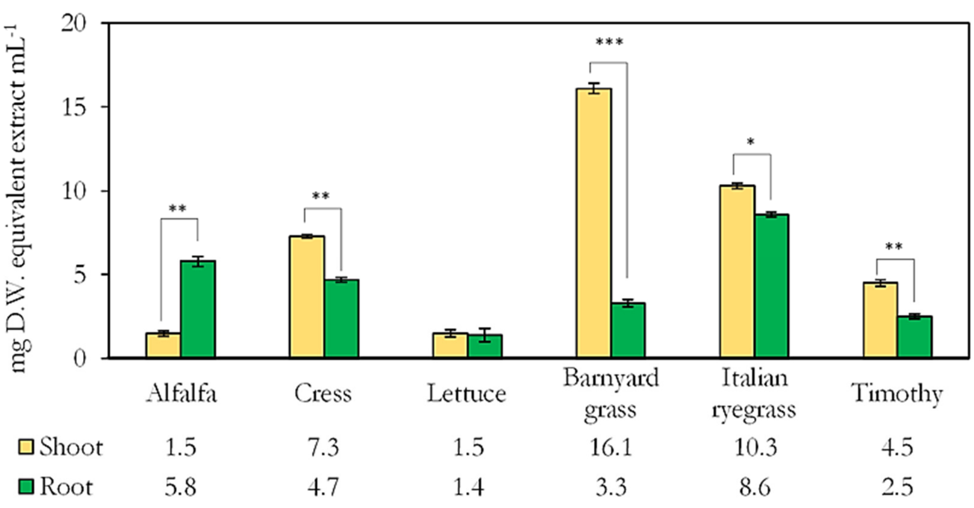

Figure 3. Concentration of *A. pennata* leaf extracts required for 50% inhibition (I_{50} values) of the shoot and root growth of six test plant species

The vertical bars show standard error of the mean. The significant differences between I_{50} values of shoot and root growth are indicated by asterisks: *$p<0.05$, **$p<0.01$, and ***$p<0.001$ (paired *t*-test)

Table 1. Two-way ANOVA for shoot and root growth of test plant species exposed to six different concentrations of *A. pennata* leaf extracts

		Shoot growth		Root growth	
Source of variation	df	Inhibition %			
		F	P	F	P
Test plant species	5	30.98	< 0.0001	44.54	< 0.0001
Concentration	5	120.30	< 0.0001	973.84	< 0.0001
Test plant species × concentration	25	2.31	0.0106	14.45	< 0.0001

df = degree of freedom

Discussion

In this experiment, the aqueous methanol was used as solvent for extraction. It has been found that the aqueous solvent cannot dissolve most of the non-polar bioactive substances (Horky, 2020). Many researchers reported that a wide range of phytochemical substances in medicinal plants has been successfully extracted by the aqueous methanol (Sultana *et al.*, 2009; Boonmee *et al.*, 2018; Kato-Noguchi *et al.*, 2019). Therefore, *A. pennata* leaves were extracted using the aqueous methanol to obtain both polar and non-polar bioactive allelochemicals in the extracts. Our experimental results indicate that the significant inhibitory activity of *A. pennata* leaf extracts against seedling growth was observed in both the dicot plants (alfalfa, cress, and lettuce) and monocot plants (barnyard grass, Italian ryegrass, and timothy) (Figures 1 and 2). Furthermore, the inhibitory activity increased with increasing extract concentration. Such inhibitory activity has also been found in other research, which reported that increasing concentrations of extracts of *Aloe ferox* (Arowosegbe and Afolayan, 2012), *Capparis spinosa* (Ladhari *et al.*, 2013), *Filicium decipiens* (Bari and Kato-Noguchi, 2017), and *Oxalis europea* (Zaman *et al.*, 2018) result in increased growth inhibition against different kinds of crop and weed species.

The I_{50} values for shoot growth showed that alfalfa and lettuce had the highest sensitivity to the leaf extracts, and barnyard grass exhibited the lowest sensitivity (Figure 3). On the other hand, the I_{50} values for root growth showed that lettuce was the most sensitive and Italian ryegrass was the least sensitive. Similar trends have been reported by Suwitchayanon *et al.* (2013) and Krumsri *et al.* (2019), who found that aqueous methanol extracts of *Cymbopogon nardus* and *Dischidia imbricata* showed the highest inhibitory potential against lettuce seedling growth and the lowest inhibition against barnyard grass, respectively. Additionally, El-Mergawi and Al-Humaid (2019) reported different inhibitory activity of *Tamarix mannifera* extracts against lettuce, barnyard grass, canary grass (*Phalaris minor*), and purslane (*Portulaca oleracea*). Moreover, some species of genus *Acacia* such as *Acacia cambagei* (An and Pratley, 2005) and *Acacia cyanophylla* (El Ayeb *et al.*, 2013) extracts showed the inhibitory effect on the germination and the seedling growth of *Lolium perenne*, and *Lactuca sativa* and *Peganum harmala*, respectively. The variation in sensitivities of all the test plants to the extracts indicates that the inhibitory activity was species specific. Different sensitivity to the extracts between plant species may be due to the different biochemical and physiological characteristics of each test plant species (Kobayashi, 2004; Sodaeizadeh *et al.*, 2009). Furthermore, the I_{50} values showed that the roots of the test plant species were more sensitive to the extracts than their shoots, except the roots of alfalfa (Figure 3). El-Mergawi and El-Desoki (2018) and Rob and Kato-Noguchi (2019) also reported that the extracts of *Apium graveolens* and *Garcinia pedunculata* had a greater inhibitory effect against the roots compared with the shoots of several tested plant species. Higher root sensitivity to the extracts could be due to higher root tissue permeability compared with the shoots because the shoot surface of plants is more protected with a well-coated cuticle layer, while that of roots is not covered (Bessire *et al.*, 2007; Gulzar *et al.*, 2016).

Conclusions

The aqueous methanol extracts obtained from *A. pennata* leaves showed inhibitory activity, and the inhibition varied with extract concentration and test plant species. The findings of this study suggest that the leaf extracts of *A. pennata* may possess allelopathic potential and may contain allelopathic substances. Hence, *A. pennata* leaves could be a candidate for isolation and characterization of allelopathic substances.

Authors' Contributions

EHK carried out the experiment, analyzed the data and wrote the manuscript.

HKN designed the experiments, supervised the data analysis and contributed greatly to the writing of the manuscript.

Both authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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