

Quantification and Herbicidal Activity of Mimosine from *Leucaena leucocephala* (Lam.) de Wit

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ABSTRACT: Laboratory experiments have been performed to quantify the amount of mimosine (an allelochemical) from *Leucaena leucocephala* and evaluate the herbicidal activity of mimosine on selected invasive weeds in Malaysia. The mimosine amount in an aqueous extract of shoot, mature leaf, and seed parts of *L. leucocephala* were quantified by utilizing high-performance liquid chromatography. The herbicidal activity of mimosine was tested on the growth (i.e., radicle length, shoot length, and fresh weight) of three selected invasive weed species (i.e., *Ageratum conyzoides*, *Emilia sonchifolia*, and *Tridax procumbens*) in five different concentrations (i.e., 0, 10, 25, 50, and 100 ppm) by utilizing the bioassay petri dish method. The mimosine amount was highest in the shoot aqueous extract (1.41×10^4 ppm), followed by the seed aqueous extract (8,463 ppm), and finally, the mature leaf aqueous extract (1,881 ppm). Mimosine inhibited the growth of weed species as the concentration increased. More than 50% inhibition of all the bioassay weed species was observed when they were applied at the mimosine concentrations of 50 and 100 ppm. These results provide benchmark information for controlling weeds in the agriculture field in a sustainable manner and for the future development of bioherbicides.

KEYWORDS: Allelopathy, Herbicidal activity, Mimosine, *Leucaena leucocephala*, Weeds.

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INTRODUCTION

Secondary metabolites have diverse chemical structures; more than 80,000 structures have been identified in plants, 20,000 structures in microorganisms and fungi, and 20,000 chemical structures in amphibians, reptiles, arthropods, and marine organisms (Wink, 2004). Most secondary metabolites can be separated, and their structures can be determined by mass spectrometry nuclear magnetic resonance (e.g., H-NMR and C-NMR), chromatography [e.g., high-performance liquid chromatography (HPLC) and gas chromatography (GC)] and x-ray diffraction (Harborne, 1993). Secondary metabolites are known as allelochemicals in the allelopathic concept, which originate from different species (Whittaker & Feeny, 1971). Putnam (1983) stated that the release of allelochemicals into the environment depends on the process of released allelochemicals (e.g., through exudation or leaching) and the plant organ, such as the root, leaf, fruit, rhizome, and seed. Allelochemical isolation involves several chromatography stages (Inderjit *et al.*, 1999).

Chromatography is one of the methods that can separate several components, which are either static or moving. Static components can be solid or liquid with a solid state, whereas moving components can be in the gas or liquid states (Inderjit *et al.*, 1999). Utilizing GC and

HPLC in allelopathy can detect the presence of allelochemicals in the plant structure and organelles (Inderjit *et al.*, 2005). HPLC is one of the most important chromatography techniques in purification and chemical analysis in the laboratory. The HPLC efficiency can overcome the efficiency of conventional open column LC because HPLC has rapid elution, high efficiency, is suitable in separating unstable chemical compounds, and its column is reusable (Inderjit *et al.*, 1999).

Leucaena leucocephala (Lam.) de Wit, or commonly known as “petai belalang,” is a tropical plant that originated from Central America and South Mexico. This species is categorized under the Family Mimosaceae and can grow from 7 m to 18 m (Shelton & Brewbaker, 1994). This plant is used in South East Asia and Africa to prevent soil erosion and develop soil quality (Hong *et al.*, 2003). Tawata (1990) stated that this plant can be a potential solution to the feeding problem of livestock in developing countries, but it has become limited due to the presence of the chemical compound known as mimosine. Mimosine has been determined as the main allelochemical in *L. leucocephala*, which serves a major function in this plant’s allelopathic activities on other plants and fungi (Tawata, 1990). Xuan *et al.* (2013) also suggested that the mimosine in this plant can potentially serve as a bioherbicide.

Thus, laboratory experiments were performed in the current study to quantify the mimosine amount produced from different plant parts of *L. leucocephala* (i.e., shoot, mature leaf, and seed parts) by utilizing HPLC. The growth inhibitory properties of mimosine at different concentrations were also assayed on the seedling growth of weed species (i.e., *Ageratum conyzoides*, *Tridax procumbens*, and *Emilia sonchifolia*).

METHODOLOGY

Sample collection and preparation

L. leucocephala plant samples (i.e., shoot, mature leaves, and seeds) were collected around Universiti Kebangsaan Malaysia (UKM) from January 2015 to June 2015. These fresh samples were cleaned by distilled water prior to storage at 4°C. The pure mimosine employed was L- mimosine (98%, from the “Koa-hoale” seed, Sigma- Aldrich Company). The tested invasive weed species utilized in the experiments were *A. conyzoides*, *T. procumbens*, and *E. sonchifolia* collected from UKM. The plant sample collection was conducted following the method described by Ishak *et al.* (2016). Each plant sample (10 g) was immersed in 100 mL distilled water for one hour at room temperature. The solution was filtered and injected into the HPLC system in 2 µL (Xuan *et al.*, 2006).

HPLC

The HPLC (Agilent Technologies) system employed was “1220 infinity LC” with a chromatography column (Zorbax Eclipse Plus C₁₈; Agilent Technologies). Quantification with HPLC was performed by utilizing the method of Xuan *et al.* (2006). The mobile phase applied was a mixture solution of 10 mM potassium–dihydrogen phosphate, 10 mM phosphoric acid, and acetonitrile (45:45:10) with a flow rate of 1.2 mL per minute. Approximately 0.1% sodium 1–octanesulfonate was added to the mixture to act as the surface active agent, and the mimosine was detected at a wavelength of 280 nm.

Phytotoxic effects of mimosine on the tested weed species

The phytotoxic test of mimosine on the tested weed species was conducted utilizing the bioassay petri dish technique. This experiment was modified based on the work of Xuan *et al.* (2006). The filter paper in the petri dishes was moistened with different mimosine concentrations (i.e., 10, 25, 50, and 100 ppm). Distilled water served as the control in this experiment. Ten seeds of tested weed species (i.e., *A. conyzoides*, *T. procumbens*, and *E. sonchifolia*) were placed separately in each petri dish. The petri dishes were then incubated at 28°C for 7 days. The germination percentage, radicle length, and shoot length of the tested weed species were then recorded after incubation. This experiment was performed in three replicates and conducted twice.

Statistical analysis

Experiments were conducted utilizing a completely randomized design in three replicates. The data were analyzed by ANOVA with the Duncan Multiple Range Test at 0.05% significance using SPSS software version 22.

RESULT AND DISCUSSION

Mimosine quantification by HPLC

Table 1 shows that the presence of mimosine in the different aqueous extracts of the young leaf, mature leaf, and seed of *L. leucocephala* was detected utilizing HPLC. The aqueous extract of the shoot part of *L. leucocephala* has the highest mimosine amount (1.41×10^4 ppm), followed by the aqueous extract from the seed part (8,462.9 ppm), and finally the mature leaf part (1,881 ppm). Mimosine was detected in all aqueous extracts of the *L. leucocephala* plant parts at a wavelength of 280 nm at 1.212 minutes utilizing HPLC (Figure 1).

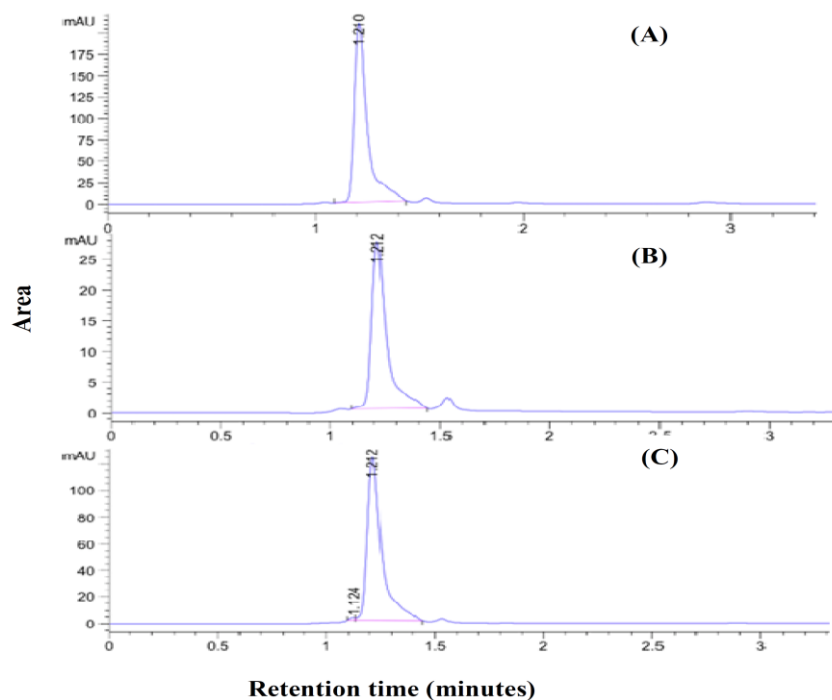


Figure 1. Mimosine determination in the (A) shoot, (B) mature leaf, and (C) seed of *L. leucocephala* by utilizing HPLC.

Table 1. Mimosine content from the aqueous extract of different plant parts of *L. leucocephala*.

Plant parts	Retention time (minutes)	Peak area (mAU × s)	Total/ Area	Total concentration (ppm)
Shoot	1.21	953.8	14.81	1.41×10^4
Mature leaf	1.21	127	14.81	1881
Seed	1.21	571.6	14.81	8462.9

Xuan *et al.* (2006) reported that mimosine has been detected in several *L. leucocephala* plant parts that grow in Japan utilizing HPLC, such as young leaf, mature leaf, seed, and flower. Phenolic compounds, such as phenolic acid, coumaric acid, and benzoic acid, have been detected in four types of rice cultivars (e.g., Gin shun, Kasawala mundara, Philippine 2, and Juma 10); these rice cultivars have allelopathic effects on the growth of the weed *Echinochloa crus-galli* (Chung *et al.*, 2001). Allelochemicals, such as mangiferin, have been detected in *Cyclopia* spp. utilizing HPLC and Thin Layer Chromatography (De Nysschen *et al.*, 1996). Proestos *et al.* (2006) utilized HPLC and gas chromatography mass spectrometry to determine that *Lavandula vera* contains several phenolic acids, such as gallic acid, vanillic acid, caffeic acid, catechin, and naringenin. Several chemical compounds, such as prodelfinidin, flavonol, and flavones, have been detected in *Vicia faba* with HPLC (Baginsky *et al.*, 2013).

The shoot part of *L. leucocephala* contains the highest mimosine amount, followed by the seed, and finally, the mature leaf. These results are consistent with those obtained by Xuan *et al.* (2006), who demonstrated that the shoot of *L. leucocephala* grown in Japan had the highest mimosine amount (2.66%), followed by the seed (2.38%), and finally, the mature leaf part (0.47%). A previous study by Clement *et al.* (1997) determined that the phenethylamine amounts in *Acacia berlandieri* varied depending on the growing season. The phenethylamine amount in *A. berlandieri* was higher during the winter season than the summer season.

Phytotoxic effects of mimosine on the weed species

Figure 2 shows that mimosine significantly inhibits the growth of all three weed bioassay species, namely, *A. conyzoides*, *T. procumbens*, and *E. sonchifolia*. The growths of these weed bioassay species depend on the applied mimosine concentration. The radicle lengths of *A. conyzoides*, *T. procumbens*, and *E. sonchifolia* were inhibited by 98%, 82%, and 69% at a concentration 100 ppm compared with the control, respectively. Mimosine was almost 100% inhibited compared with the control, and the radicle length of *A. conyzoides* at all applied concentrations was the longest compared with those of *T. procumbens* and *E. sonchifolia*.

Figure 2 also shows that the shoot lengths of *A. conyzoides*, *T. procumbens*, and *E. sonchifolia* were inhibited by 94%, 85%, and 81% at a concentration of 100 ppm compared with the control, respectively. Furthermore, the fresh weight of *A. conyzoides*, *T. procumbens*, and *E. sonchifolia* were inhibited by 94%, 88%, and 88% compared with the control when applied at a mimosine concentration of 100 ppm. Overall, the growth of *A. conyzoides*, *T. procumbens*, and *E. sonchifolia* began to be inhibited at the mimosine concentration of 10 ppm.

The inhibited growth of *A. conyzoides* was the highest compared with *T. procumbens* and *E. sonchifolia* (Figure 2).

Xuan *et al.* (2006; 2013) revealed that a mimosine concentration of 100 ppm can completely inhibit 100% of the radicle and shoot growth of *Brassica rapa*. Mimosine began to inhibit the radicle and shoot growth of *Bidens pilosa* and *Lolium multiflorum* at a concentration of 25 ppm, and the inhibition percentage increased as the mimosine concentration increased. Singh *et al.* (2002) reported that the growth of *A. conyzoides* was inhibited by the presence of monoterpenoid compounds, such as cineol and citronellol.

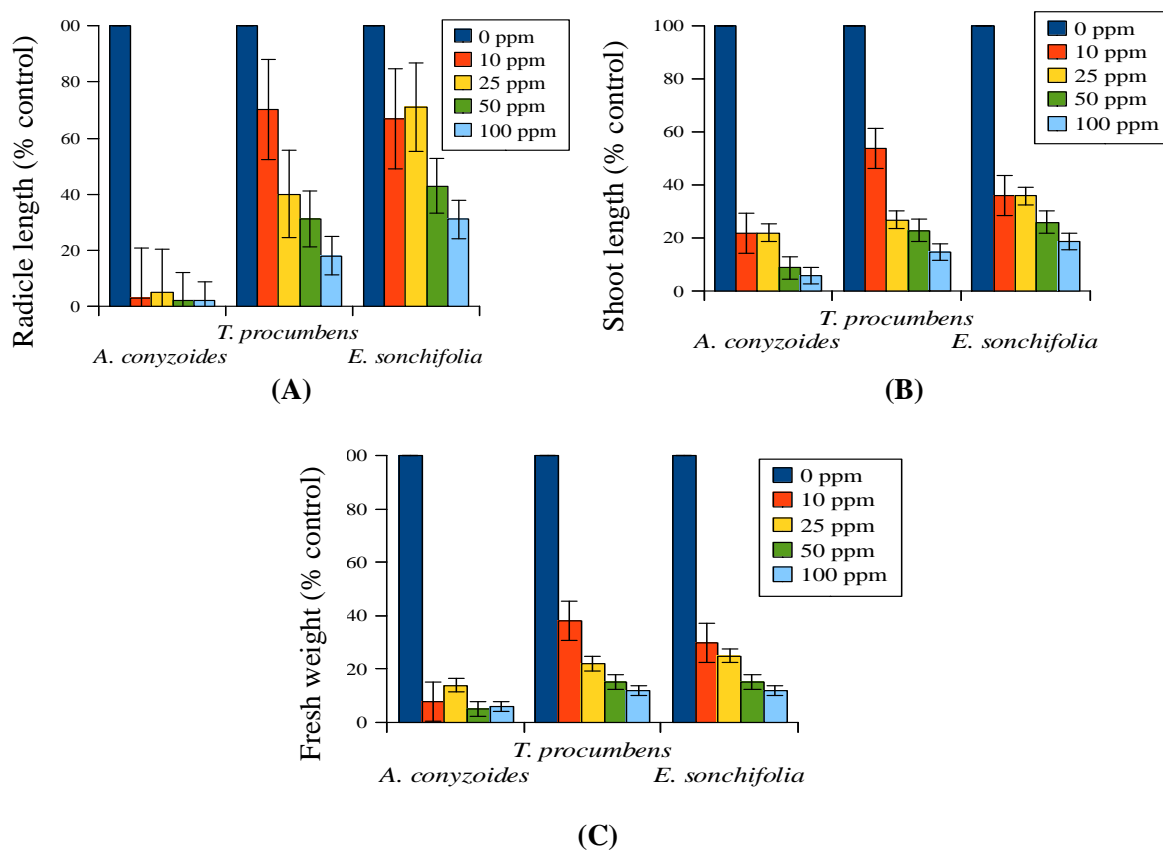


Figure 2. Inhibitory effects of mimosine in the (A) radicle length, (B) shoot length, and (C) fresh weight of *A. conyzoides*, *T. procumbens*, and *E. sonchifolia*.

CONCLUSION

Mimosine is an alkaloid found in *L. leucocephala* parts in Malaysia. Thus, mimosine acts as one of the main allelochemicals in *L. leucocephala*, whereas the shoot and seed of *L. leucocephala* had higher mimosine contents than the mature leaf part. Overall, mimosine inhibits the growth of *A. conyzoides*, *T. procumbens*, and *E. sonchifolia* as the applied mimosine concentration increased. The highest inhibitory effects were observed in the growth of *A. conyzoides*. Thus, mimosine acts as an allelochemical that has inhibitory effects on weed growth. Results suggest that the inhibitory mechanism of mimosine must be investigated, and mimosine can potential serve as a safe bioherbicide.

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