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Effect of Lipophilic Extract from *Lasia spinosa* (L.) Thwaites (Araceae) on Seed Germination and Seedling Growth of the Invasive Plant *Mimosa diplotricha* C. Wright ex Sauvalle

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ABSTRACT

The effect was studied of the lipophilic extract from *Lasia spinosa* (L.) Thwaites rhizome on the seed germination and seedling growth of the invasive plant*, Mimosa diplotricha* C. Wright ex Sauvalle during September–November 2012 using five different extract concentrations (0, 10, 20, 30 and 40 mg.mL^{-1}). The results revealed that 1 wk after transplanting, the extract had an effect on the seed germination and on seedling growth and characteristics. When the concentration was increased, the shoot and root length decreased significantly ($P < 0.05$) Thin layer chromatography screening and preliminary testing and detection using different specific reagents showed the presence of terpenoids, phenolic compounds, coumarin, alkaloids and *C*-glycoside in the extract.

Keywords: lipophilic extract, *Lasia spinosa*, *Mimosa diplotricha*, seed germination, seedling growth

INTRODUCTION

Lasia spinosa (L.) Thwaites is a rhizomatous herb in the family Araceae. It is an edible native plant of Thailand, commonly known as "Phaknam" (Boyce, 2012) and there has been little study of its utilization. *Lasia spinosa* is widely distributed in tropical Asia, as far as New Guinea in the south and to South China in the north, while in Thailand, *L. spinosa* can be found throughout many regions, especially in wet areas, among deciduous and evergreen forest, and along streams and ditches (Boyce, 2012). Botanical characteristics include: prickly stem, sagittate leaf blade when juvenile, deeply pinnatifid, enclosed basal part, linear spathe, purple or brownish spadix and quadrangular dark-green fruits when ripe.

A previous phytochemical investigation reported the isolation of triglochinin from leaves (Nahrstedt, 1975). Dinda *et al*. (2004) isolated β-sitosterol acetate and stigmasterol from the rhizome. Williams *et al*., (1981) isolated flavones (*C*-glycosides and proanthocyanidins), which are chemicals that are characteristics of the subfamily Lasioideae (Dinda *et al*., 2004). Moreover, Van *et al*. (2006) was able to isolate the flavonol 3'-methyl quercetin-3-*o*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside,which can be used as a chemotaxonomic marker of the genus *Lasia*. There are few reports on the biological activities of this plant. the current study investigated the effect of crude extract from *L. spinosa* on the seed germination of one invasive species in Thailand (*Mimosa diplotricha* C. Wright ex

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Sauvalle) which has adapted well to Thailand's environment through its growth, distribution and rapid production of many seeds and seedlings. It is a weed found in crop plantations and tropical pastures (Waterhouse and Norris, 1987). One plant can produce 10,000 seeds annually, and seeds can remain dormant for up to 50 years (Holm *et al*., 1977; Parsons and Cuthbertson, 1992). The plant has the following characteristics: fast growing, erect shrub; scrambling habit; four-angled; forms dense thickets in a short time; bright green, compound leaves; alternate leaf blade; lanceolate; pale pink flowers clustered in a fluffy ball; flowering period from August to February; seed setting from September to February; and profusely branched roots with nodules (Sankaran, 2008). *M. diplotricha* is a large plant in forest ecosystems and on agricultural land and in pasture. It can cause heavy damage to crops like sugar cane and coconut, and thick growth of Mimosa prevents the regeneration, reproduction and growth of indigenous species in all infested areas. *M. diplotricha* scrambles vigorously over other plants, forming dense tangled thickets up to 2 m in height. It also smothers and kills indigenous flora. Therefore the effect of crude extract from *L. spinosa* on the seed germination and growth of *M. diplotricha* should be investigated to help inhibit and control its population. Haig (2004) reported that phenolic compounds and cyanide inhibit seed germination and the growth of plants. Therefore, it would be expected that some chemical compounds from *L. spinosa* may inhibit and delay the seed germination and growth of *M. diplotricha*.

MATERIALS AND METHODS

Plant materials

In September 2012, rhizomes of *Lasia spinosa* (L.) Thwaites were collected from Saiyok National Park, Kanchanaburi province, Thailand. The voucher specimen of *L. spinosa* (KUFF 017) was deposited at the Faculty of Forestry, Kasetsart University, Bangkok, Thailand and the examined specimens (BKF 112365) were deposited at the Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Bangkok, Thailand. The seeds of the invasive plant, *Mimosa diplotricha* C. Wright ex Sauvalle were used for examination of the presence of bioactive compounds.

Preparation of plant extracts

Fresh rhizomes of *L. spinosa* (500 g) were separated, cleaned and chopped into small pieces, and then powdered using an electronic mill. The rhizome powder was macerated with methanol (CH_3OH) for 7 d in the dark at room temperature.

Extractions

After 7 d, the extracts were filtered through filter paper (Whatman No.1), then subsequently concentrated using a rotary evaporator at 37–39 °C, with the crude extract being a dark green, semi-solid. The concentrated crude extract was partitioned into two parts—the hydrophilic extract (in distilled water) and the lipophilic extract (in chloroform). The lipophilic extract was stored at -75 °C for future experiments.

Phytochemical analysis by thin layer chromatography

Thin layer chromatography (TLC) of the *L. spinosa* lipophilic extract was performed on TLC pre-coated silica gel 60 GF_{254} plates (20 × 20 cm; Merck; Darmstadt, Germany) using a solvent system of hexane:ethyl acetate (9:1), and the Rf values were calculated.

Thin layer chromatography screening

The TLC plates were sprayed with detecting reagent for screening major secondary metabolites using different reagents which consisted of: 1) anisaldehyde-sulfuric acid reagent followed by heating on the TLC hotplate at 110 °C for 10 min for terpenoids detection; 2) iodine fuming for organic compounds detection (Merck,

1980); 3) vanillin sulfuric acid reagent followed by heating on the TLC hotplate at 110 °C for 10 min for phenolic compounds detection; 4) 10% NaOH for coumarin detection; 5) Dragendorff's reagent for alkaloids detection; 6) Kedde's reagent and Raymond's reagent for unsaturated lactones ring detection (Farnsworth, 1966); and 7) ultraviolet light at wavelengths of 365 nm and 254 nm for detection of fluorescence compounds.

Biological activities test: seed germination and growth of seedling

The inhibitory effects on the seed germination and growth of *M. diplotricha* were tested using *Lasia spinosa* lipophilic crude extract at different dilutions in methanol (10, 20, 30, and 40 $mg.mL^{-1}$) and then compared with methanol which was used as the control.

Weed seeds (*M. diplotricha*) were soaked in water at 100 °C for 1 min followed by soaking in cool water for 24 hr. Twenty seeds were placed on blotting paper in a Petri dish and all treatments were replicated three times and incubated in the dark at room temperature. Seeds were examined and counted on the second and fourth days after they had been taken from pretreatment and during incubation; the number of seeds germinated for all treatments in the Petri dishes were counted and recorded for their shoot and root length at the fourth day.

Transplanting of the seedlings began on the fifth day of the study. The process of transplanting and observation were undertaken as follows: 1) the seedlings were transplanted from the Petri dishes to seedling trays; 2) the transplanted seedlings were selected from seedlings initiating foliage; 3) the 10 seedling sample was planted in decomposed rainforest leaf soil in appropriate conditions of water-saturated soil; 4) the seedling trays were placed in a chamber; and 5) on the eighth day of the experiment, the seedlings were pulled out from the seedling trays, and the shoot and root lengths were recorded. Seedlings characteristics were compared before transplanting.

Statistical analysis

The germination percentage was calculated using Equation 1:

$$\text{Germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Number of total seeds}} \times 10 \quad (1)$$

The biological activities test was designed as a completely randomized design and the results were presented as means using one-way analysis of variance and Duncan's multiple range test (DMRT). Significance was tested at the 95% significance level. The SPSS (version 16.0; Bangkok, Thailand) statistical software package was used.

RESULTS

Qualitative analyses of thin layer chromatography screening

The phytochemical screening for major secondary metabolites can be investigated by color detection after spraying the TLC plates with different reagents. The results on TLC plates appeared as follows: 1) violet bands had R_f values of 0.3, 0.4 and 0.91 and the grey-green band had an R_f value of 0.1 for detection of terpenoids using anisaldehyde sulfuric acid as the spraying reagent; 2) dark violet bands had R_f values of 0.33 and 0.95 for detection of phenolic compounds using vanillin sulfuric acid as the spraying reagent; 3) the yellow-green fluorescent band under long wavelength (365 nm) ultraviolet light had an R_f value of 0.56 for detection of coumarin after being sprayed with 10% NaOH in ethanol; 4) the orange band at the base line was used for detection of alkaloids after spraying with Dragendorff's reagent; and 5) the red-brown color on the picrate paper was used for detection of *C*-glycoside using Hager's reagent. The results are shown in Table 1.

Biological activities test: Seed germination and growth of seedling

The lipophilic extract from *L. spinosa* rhizome showed a significant difference in seed

germination percentage of *M. diplotricha* between the first count (second day after germination) and the final count (fourth day) indicating that the extract could inhibit most seed germination at the first count but not at the final count. There was a significant difference in seedling growth in the extract inhibited shoot and root lengths compared to the control. The root length was longer than the shoot length for the control treatment when the concentration was at 10 mg.mL^{-1}, the shoot length was nearly equal to the root length at the concentration of 20 mg.mL^{-1} and the shoot length was shorter than the root length at concentrations of 30 and 40 mg.mL^{-1} (Table 2).

One week after transplanting and with an increasing level of extract concentration, seedlings had differences in length and in their characteristics, appearing damaged with abnormalities such as a short primary root and the loss of cotyledons, epicotyls and primary leaves. Seedling vigor was classified into three groups—seedlings were classified as strong in the control group and in some seedlings at a concentration of 10 mg.mL^{-1}; seedlings appeared weak at concentrations of 10 and 20 mg.mL^{-1}; and seedlings appeared abnormal at 20 to 40 mg.mL^{-1} (Figure 1).

After transplanting, the shoot length slightly decreased at the treatment concentrations of 10 and 20 mg.mL^{-1} when compared with the control treatment, but the shoot lengths at the treatment concentrations of 30 and 40 mg.mL^{-1} were nearly equal to the length at the control treatment which showed that the extractions had stimulated shoot growth. The seedling characteristics and leaf photosynthetic process after transplanting from the culture dish were

Table 1 Lipophilic extract from *Lasia spinosa* Thwaites rhizome detected on thin layer chromatography plates.

Chemical compound	Reagent	Result
Terpenoids	Anisaldehyde sulfuric acid	+
Phenolic compounds	Vanillin sulfuric acid	+
Coumarin	10%NaOH in ethanol	+
Alkaloids	Dragendorff's reagent	+
C-glycoside	Hager's reagent	+

+ = Positive test.

Table 2 Percentage of seed germination and length of *M. diplotricha* seedling after treatment with different concentrations of the lipophilic extract from *L. spinosa* rhizome.

Concentration (mg.mL^{-1})	Percentage of germination (%)[1] Mean ± SE		Length of seedling (cm) [1] Mean ± SE	
	Second day	Fourth day	Shoot	Root
0	100.00^{c} ± 0.00	100.00^{a} ± 0.00	1.68^{d} ± 0.10	2.97^{d} ± 0.05
10	98.33^{c} ± 1.67	100.00^{a} ± 0.00	1.17^{c} ± 0.04	1.39^{c} ± 0.04
20	98.33^{c} ± 1.67	100.00^{a} ± 0.00	1.01^{b} ± 0.03	1.02^{b} ± 0.03
30	91.67^{b} ± 1.67	100.00^{a} ± 0.00	0.81^{a} ± 0.01	0.70^{a} ± 0.02
40	86.67^{a} ± 1.67	98.88^{a} ± 1.11	0.80^{a} ± 0.03	0.60^{a} ± 0.03
P value	*	ns	*	*

[1] Average of triplicate experiments.

$^{a-d}$ = Values in a column with the same lowercase superscript letter are not significantly different at the 95% significance level using Duncan's multiple range test.

* = Significant at the 0.05 level.

ns = Not significant.

affected by the extract. Conversely, there was a clear, decreasing trend in the root length when the concentration of the lipophilic extract was increased. In the treatment concentrations of 10 and 20 mg.mL^{-1}, the root length showed a decreasing trend after transplanting. The root length with the treatment concentration of 30 mg.mL^{-1} decreased by a factor of three when compared with the control (Table 3). An extract concentration of 30 mg.mL^{-1} or higher had a significant effect on the seedling growth and especially on the root growth pattern.

DISCUSSION

The results showed that the major secondary metabolites in the extracts were terpenoids, phenolic compounds, coumarins, alkaloids and *C*-glycosides, as have been reported in other studies (Nahrstedt, 1975; Dinda *et al*., 2004; Van *et al*., 2006). The effect of the lipophilic extract from the *Lasia spinosa* rhizome was demonstrated by its inhibition and the delay of the seed germination of the invasive plant species, *Mimosa diplotricha*. This was

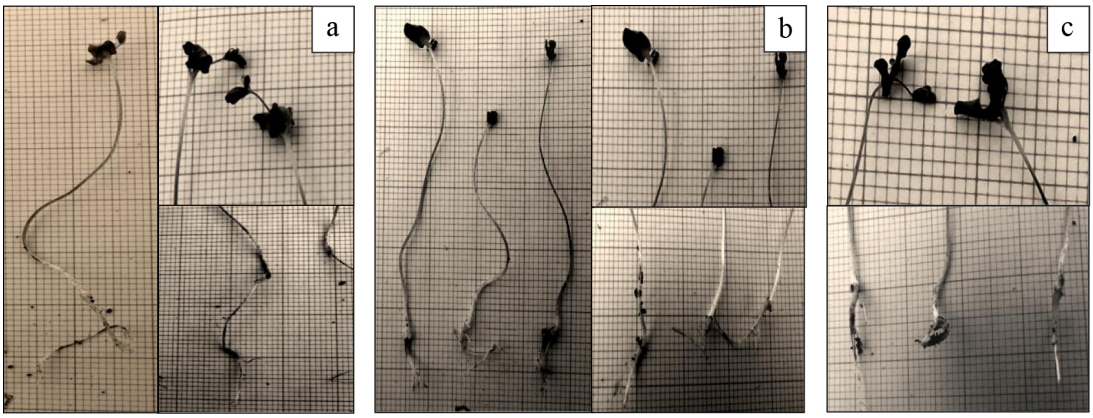

Figure 1 Preliminary classification of seedling, length and characteristics for seedlings appearing damaged and abnormal: (a) Strong seedling; (b). Weak seedling; (c) Abnormal seedling. Large bold line grid = 1 cm in all images.

Table 3 Seedling length differences in (*Mimosa diplotricha*) before transplanting and 1 week after transplanting.

Concentration (mg.mL^{-1})	Shoot length of seedling[1] (cm) Mean ± SE		Root length of seedling[1] (cm) Mean ± SE	
	Before transplanting	1 wk After transplanting	Before transplanting	1 wk After transplanting
0	$1.60^{d} \pm 0.06$	$5.14^{b} \pm 0.10$	$2.97^{c} \pm 0.04$	$3.37^{d} \pm 0.04$
10	$1.15^{c} \pm 0.07$	$4.43^{a} \pm 0.06$	$1.03^{b} \pm 0.07$	$2.52^{c} \pm 0.04$
20	$1.05^{c} \pm 0.03$	$4.54^{a} \pm 0.09$	$0.99^{b} \pm 0.06$	$1.63^{b} \pm 0.07$
30	$0.77^{b} \pm 0.03$	$5.11^{b} \pm 0.07$	$0.82^{a} \pm 0.03$	$1.32^{a} \pm 0.06$
40	$0.61^{a} \pm 0.04$	$5.00^{b} \pm 0.09$	$0.80^{a} \pm 0.03$	$1.23^{a} \pm 0.06$
P value	*	*	*	*

[1] Average of 10 seedlings per treatment.

[a-d] = Values in a column with the same lowercase superscript letter are not significantly different at the 95% significance level using Duncan's multiple range test.

* = Significant at the 0.05 level.

explained by Hoagland and Williams (2004) who described how low concentrations of allelopathic compounds, nonactive compounds, and crude extract may physically or chemically mark the action of allelochemicals, such as phenolic compounds, that can influence nitrogen absorption, membrane permeability, respiration, the balance of plant hormones, water potential and inhibit the seed germination and growth of weeds in a cropping system. In addition, the potential use of allelochemicals as herbicides isolated from many families of terrestrial and aquatic plants has been reported (Putnam, 1988).

The results showed significant differences in the seedling growth with the inhibition of the shoot and root length as follows: there were long shoot and root lengths in the control (1.68 and 2.97 cm, respectively), but the root length was longer than the shoot length in the treatment at a concentration of 10 $mg.mL^{-1}$. The shoot length was nearly equal to the root length at the treatment concentration of 20 $mg.mL^{-1}$, and the shoot length was shorter than the root length at the treatment concentrations of 30 and 40 $mg.mL^{-1}$. Inderjit and Dakshini (1992) and Ahmed *et al*. (2004) reported the inhibitory effects of allelochemicals on the photosynthetic rate, and toxic substances from the extract affected cell division, causing a reduction in the root cell growth and a reduction in the carbohydrate content. This may have led to a decrease in the mineral uptake for growth and development.

After transplanting into a different media, the trend of root length clearly decreased when the concentration of the lipophilic extract was increased, but the shoot length following the treatment at concentrations of 30 and 40 $mg.mL^{-1}$ showed that the extract stimulated the shoot growth. This can be explained by the observations of Fenner and Thompson (2005) who explained that in an epigeal species, the cotyledons are borne aloft on a short stem (hypocotyl), followed by the shoot growing towards the light and generally becoming photosynthetic upon establishment. This observation after transplanting, suggested that the stimulation of shooting was just the result of the elongation of the hypocotyl. However, Creelman *et al*. (1990) found that the water potential in legume seeds decreased from the root to the hypocotyl crook and they showed that the water potential in the elongation zone of the hypocotyl was not uniform and was in fact negative immediately below the hypocotyl crook. Bensen *et al.* (1990) showed that the elongation responses of seedling hypocotyls are not yet clear. Other research found that the hypocotyl elongation rate is reliant on a balance between the concentrations of hormones or chemical compounds in the tissue (Rujin *et al.,* 1999).

The current study found that not only the lipophilic extract from the rhizome of *Lasia spinosa* delayed seed germination and inhibited shoot and root growth but that chemical compounds present in the extract also affected plant activity. Coumarin affected seed dormancy and reduced cell division. Phenolic compounds, such as flavonoids, affected physiological processes and system II photosynthesis. Tannins affected plant hormones that could promote the growth and inhibit the synthesis of enzymes and some proteins. Alkaloids affected plants at the molecular level of seed germination. Terpenoid substances have been reported to inhibit cell division, such as monoterpene (Rizvi and Rizvi, 1991; Reigosa and Pedrol, 2002). Moreover, Duke *et al*. (2000) explained that the chemical compounds from plant extracts possess a structural diversity and complexity, but that some chemical compounds act directly as herbicides. Therefore, it would be expected that some chemical compounds from *L. spinosa* (Table 1) would have an effect on the seed germination and growth of *M. diplotricha*. In the present study, the biological activity tests were undertaken only in the laboratory. In the future, a longer experimentation process should be applied to clarify the effect of the extract on treated seeds with regard to general safety when using the extract from *Lasia spinosa*. This research could be applied

through a weeding control approach, although the current study seemed to imply that the extract from the rhizome could positively stimulate the shoot growth but did not strengthen the seedling of the weed plant *(M. diplotricha*) as evidenced through the appearance of the characteristics of weak and abnormal seedlings.

CONCLUSION

The biological activities of the lipophilic extract at concentrations greater than or equal to 30 $mg.mL^{-1}$ inhibited seed germination and could slow down the growth of the seedling. The seedlings after treatment with 20 to 40 $mg.mL^{-1}$ showed symptoms of weak and abnormal seedlings within 1 wk.

The qualitative analyses using thin layer chromatography screening of the lipophilic extract from the *Lasia spinosa* rhizome showed groups of secondary metabolites such as terpenoids, phenolic compounds, coumarins, alkaloids and *C*-glycoside.

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