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# Allelopathic Activity of Piper sarmentosum Roxb.

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**Abstract:** The allelopathic effect of medicinal plant, *Piper sarmentosum* Roxb., against 12 test plant species was evaluated. Four different concentrations of 0.01, 0.03, 0.1 and 0.3 g dry weight equivalent extract mL<sup>-1</sup> were used in the study. The hypocotyl and root length were measured compared with control treatments. It was observed that the aqueous methanol extracts of *P. sarmentosum* plants inhibited all test plant species with different inhibition values. The variation may result, in part, from the different test plant species with different sensitivity to allelochemicals. The shoot and root growth of test plants were inhibited at the concentration greater than 0.03 g dry weight equivalent extract mL<sup>-1</sup> and increasing the extract concentration increased inhibition. These results suggesting that *P. sarmentosum* may contain growth inhibition of test plants were 0.001-0.210 g dry weight equivalent extract mL<sup>-1</sup> and alfalfa seedling were most sensitive to the extract. *P. sarmentosum* may be good candidate for isolation and identification of allelochemicals. The information obtained could be utilized in the development of bioherbicide for future weed management.

**Key words:** Allelopathy, *Piper sarmentosum* Roxb. allelopathic activity, medicinal plant, aqueous methanol extract, test plant species

### INTRODUCTION

Weeds cause serious yield reduction problems for crop production. Conventional weed management has been significantly depend on synthetic herbicides. Increasing public concern on human health and on the environmental issues requires alternative weed management systems whichh are less synthetic herbicide dependant or based on naturally occurring compounds (Singh et al., 2003; Sujatha et al., 2010). Allelopathy offers a challenge for practical weed management options (Travlos et al., 2007). Numerous plants are reported to possess allelopathic potential and efforts have been made to apply them for weed control.

Recently, use of allelopathic medicinal plants has been suggested as a viable option for alternative weed management under sustainable agriculture (Fujii, 2001; Hong et al., 2003; Singh et al., 2003; Yang et al., 2007). Several studies on the screening for the allelopathic potential of medicinal plants have been reported. Fujii et al. (2003) evaluated the allelopathic potentials of 239 medicinal species using the Plant Box Method and 223 species of them were found to suppress tested plant growth, whereas 17 species were enhancing lettuce radicle growth. Gilani et al. (2009) also surveyed allelopathic potential of 81 Japanese medicinal plants to find out

possible candidates as natural herbicides. Nazir et al. (2006) evaluated allelopathic potential of three herbal species (Rheum emodi, Saussaurea lappa and Potentilla fulgens) against several traditional crops and the germination of all crops was reduced significantly by aqueous extracts of S. lappa and P. fulgens. Medicinal plants are also useful for pest control and soil improvement besides their pharmaceutical properties (Khanh et al., 2005). To support of these findings, Southeast Asia is rich in various medicinal plants and indigenous farmers traditionally incorporate them into paddy soils which results in the increasing rice yield and decreasing pest damage.

Piper sarmentosum Roxb. (Piperaceae) is a stoloniferous herb or shrublet that is cultivated and found in Southeast Asia. The plant is well known due to its culinary and medicinal properties. As a traditional medicine, the extracts of different parts of the plant has been used to cure many diseases (Saralamp et al., 1996). The plant has also been reported to possess pharmacological properties such as anti-tuberculosis (Hussain et al., 2008), anti cancer (Ariffin et al., 2009), hypoglycaemic (Peungvicha et al., 1998), antimalarial (Rahman et al., 1999), antioxidant (Subramaniam et al., 2003), neuromuscular blocker (Ridtitid et al., 1998) and antiamebic (Sawangjiaroen et al., 2004). Due to these

properties, the plant has a great potential of commercialization as medicinal plant in Southeast Asia. The present study was conducted to investigate the allelopathic activity of *P. sarmentosum* against 12 tested plant species and to further characterize the allelopathic substances present in *P. sarmentosum*.

#### MATERIALS AND METHODS

Plant materials: Whole plants (leaves, stem and roots) of Piper sarmentosum Roxb. were collected from Chiang Mai province, Thailand in August 2010. The plants were washed several times by tap water, dried under the sunlight until the materials dried and then ground into powder. Cress (Lepidum sativum L.), lettuce (Lactuca sativa L.), alfalfa (Medicago sativa L.), timothy (Phleum pratense L.), were chosen as test plants for bioassay because of their known seedling growth behavior. Italian ryegrass (Lolium multiflorum Lam.), ryegrass (Lolium rigidum Gaud.), crabgrass (Digitaria sanguinalis L.), buckwheat (Eriogonum compositum Douglas ex Benth.), Chinese sprangletop (Leptochloa chinensis [L.] Nees.), jungle rice (Echinochloa colona [L.] Link.), barnyard grass (Echinochloa crus-galli [L.] Beauv) and sand fescue (Festuca myuros L.) were chosen as test plants for bioassay because there are common weeds and uniformly distributed throughout the cultivated fields.

**Extraction:** Plant powder (100 g) was extracted with 1 L of 80% (v/v) aqueous methanol for two days. After filtration using one layer of filter paper (No. 2; Toyo Ltd., Tokyo, Japan), the residue was extracted again with 1 L of cold methanol for one day and filtered. The two filtrates were combined and evaporated with a rotary evaporator at 40°C.

Bioassay: An aliquot of the extract (final assay concentration was 0.01, 0.03, 0.1 and 0.3 g dry weight equivalent extract mL<sup>-1</sup>) was evaporated to dryness at 40°C in vacuo by rotary evaporator, dissolved in 3 mL of methanol and added to a sheet of filter paper (No. 2) in a 2.8 cm Petri dish. The methanol was evaporated in a draft chamber then the filter paper was moistened with 0.6 mL of 0.05% (v/v) aqueous solution of polyoxyethylenesorbitan monolaurate (Tween 20; Nacalai, Kyoto, Japan) whichh was used for surfactant and did not cause any toxic effects. Ten seeds of cress, lettuce, alfalfa, or 10 germinated seeds of timothy, sand fescue, buckwheat, crabgrass, barnyard grass, jungle rice, Italian ryegrass, ryegrass or Chinese sprangletop were arranged on the filter paper in Petri dishes. Timothy, sand fescue and buckwheat were germinated in the darkness at 25°C

for 48 h, rabgrass, barnyard grass and jungle rice were germinated in the darkness at 25°C for 120 h and Italian ryegrass, ryegrass and Chinese sprangletop were germinated in the darkness at 25°C for 72 h. The shoot and root lengths of seedlings was measured at 48 h after incubation in darkness at 25°C. Control seeds were sown on the filter paper moistened with the aqueous solution of Tween 20 without the extract. The percentage length of seedlings was then determined by reference to the length of control seedlings. The bioassay was repeated three times with 10 plants for each determination. The inhibition percentage was calculated as follows:

Inhibition (%) =  $[1 - (\text{sample extracts control}^{-1})] \times 100$ 

Concentration-response curves: The concentrations required for 50% inhibition (defined as) of the test plants were determined by concentration-response curves. Filter paper (No.2) was placed into Petri dish and different amounts of the extract were added on it. Final concentrations of the extract were 0.003, 0.01, 0.03 and 0.1 g dry weight equivalent extract mL<sup>-1</sup>. After the methanol evaporated, 10 seeds of cress, lettuce, alfalfa or 10 germinated seeds of timothy, crabgrass, Italian ryegrass, ryegrass, buckwheat, Chinese sprangletop, jungle rice, barnyard grass or sand fescue were arranged on the filter paper in Petri dishes. Control seeds were sown on the filter paper moistened with the aqueous solution of Tween 20 as described above. The shoots and roots lengths of the seedlings were measured at 48 h after incubation in darkness at 25°C. The concentrations required for 50% inhibition (defined as) of the test plants in the assay was calculated from the regression equation of the concentration-response curves. The values of  $I_{50}$  and coefficient of correlation were summarized in Table 2.

**Statistical analysis:** Each treatment of this experiment was carried out with three replications and repeated twice. Treatments were prepared in a completely randomized design. Data were analyzed by SPSS version 11.5 using One-way ANOVA.

# RESULTS

Allelopathic activity of *P. sarmentosum* extract: Figure 1 shows the effects of aqueous methanol extracts of *P. sarmentosum* on shoot and root growth of test plants. The inhibitory effect was increased with increasing concentrations of the extracts. Significantly inhibited shoot and root growth of all test plant species was observed at the concentration greater than 0.03 g dry weight equivalent extract mL<sup>-1</sup> (p<0.05).

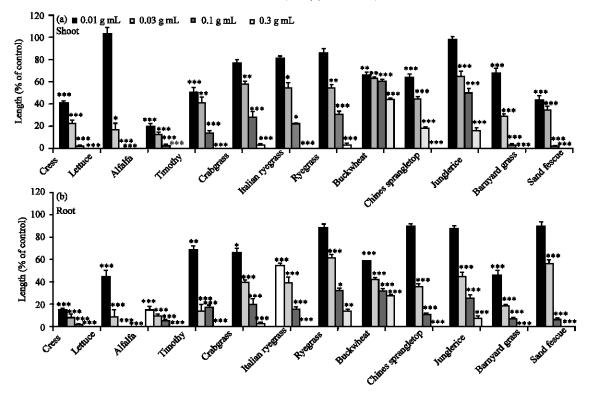


Fig. 1: Effects of aqueous methanol extracts on shoot and root growth of cress, lettuce, alfalfa, timothy, crabgrass, Italian ryegrass, ryegrass, buckwheat, Chinese sprangletop, jungle rice, barnyard grass and sand fescue seedlings. Concentrations of tested samples corresponded to the extract obtained from 0.01, 0.03, 0.1 and 0.3 g dry weight of *P. sarmentosum*. Root and shoot lengths of these seedlings were determined after 48 h of incubation in the dark at 25°C. Means ± SE from three independent experiments with 10 seedlings for each determination are shown. Asterisks indicate a significant difference between control and treatment: \*p<0.05, \*\*p<0.01, \*\*\*p<0.001

Effect of aqueous methanol extracts of P. sarmentosum on shoot growth: The inhibitory activity of the extracts on shoot growth of test plant species was summarized in Table 1. The extracts obtained from 0.1 g dry weight of the P. sarmentosum plants completely inhibited shoot growth of lettuce seedlings (100%) and shoot growth of cress, sand fescue, alfalfa and barnyard grass were inhibited by 98.67, 98.20, 97.64 and 97.20%, respectively. The extracts also inhibited the other seven test plants, timothy, Italian ryegrass, ryegrass, crabgrass, buckwheat, jungle rice and Chinese sprangletop but their magnitudes were less than 90% inhibition. Exposure to the concentration of 0.3 g mL<sup>-1</sup>, shoot growth of crabgrass, ryegrass, buckwheat and jungle rice inhibited by 96.98, 97.50, 53.42 and 84.10%, respectively and shoot growth of cress, alfalfa, timothy, Italian ryegrass, barnyard grass, Chinese sprangletop and sand fescue seedlings was completely inhibited. Comparing the concentration required for 50% inhibition, alfalfa shoots were the most sensitive to the extracts follow by cress. On the other

hand, shoot growth of buckwheat was less sensitive to the extracts (Table 2).

Effect of aqueous methanol extracts of P. sarmentosum on root growth: The extracts obtained from 0.1 g dry weight of the P. sarmentosum plants completely inhibited root growth of lettuce seedlings (Table 1). The extracts inhibited root growth of cress, sand fescue, alfalfa and barnyard grass by 99.51, 93.35, 94.60 and 93.10%, respectively. At the concentration of 0.3 g mL<sup>-1</sup>, the inhibition of crabgrass, ryegrass, buckwheat and jungle rice and was 97.35, 86.50, 71.17 and 93.12%, respectively and the root growth of cress, alfalfa, timothy, Italian ryegrass, barnyard grass, Chinese sprangletop and sand fescue were completely inhibited. Comparing the concentration required inhibition, alfalfa roots were the most sensitive to the extract of P. sarmentosum follow by cress, lettuce, timothy and ryegrass roots were less sensitive to the extracts (Table 2).

Table 1: Inhibition percentage of aqueous methanol extracts of P. sarmentosum on the growth of test plant seedlings

Test plant species	Inhibition (%)							
	Shoot				Root			
	0.01	0.03	0.1	0.3	0.01	0.03	0.1	0.3
Cress	50.65a	$71.35^{b}$	98.67⁰	100.00°	84.34ª	91.31 <sup>b</sup>	99.51°	$100.00^{\circ}$
Lettuce	-5.67ª	84.33 <sup>b</sup>	100.00 <sup>b</sup>	$100.00^{b}$	54.15a	92.81 <sup>b</sup>	$100.00^{b}$	$100.00^{b}$
Alfalfa	78.99⁴	87.14ª	97.64 <sup>b</sup>	$100.00^{b}$	84.74ª	$89.10^{ab}$	$94.60^{bc}$	$100.00^{\circ}$
Timothy	45.32ª	59.63 <sup>b</sup>	86.37°	$100.00^{\circ}$	28.58°	85.48 <sup>6</sup>	$83.70^{b}$	$100.00^{b}$
Crabgrass	18.21ª	40.46°	69.53°	$96.98^{d}$	28.63ª	57.03 <sup>b</sup>	78.77°	97.35⁰
Italian ry egrass	-0.01ª	$34.41^{b}$	75.17°	$100.00^{\circ}$	42.53ª	60.56°	83.96°	$100.00^{d}$
Ryegrass	10.58°	39.27 <sup>b</sup>	63.86°	$97.50^{d}$	-18.02ª	$17.19^{ab}$	$61.00^{bc}$	86.50°
Buckwheat	28.54ª	$31.20^{a}$	36.74ab	53.42 <sup>b</sup>	37.27ª	53.73 <sup>b</sup>	$67.27^{bc}$	71.17°
Chinese sprangletop	36.07ª	54.38 <sup>6</sup>	81.24°	$100.00^{d}$	9.09ª	62.94 <sup>b</sup>	89.05°	$100.00^{\circ}$
Jungle rice	-1.77ª	35.14 <sup>b</sup>	49.14⁰	$84.10^{d}$	2.38a	56.46 <sup>b</sup>	$73.01^{bc}$	93.12°
Barny ard grass	27.02ª	69.55 <sup>b</sup>	97.20°	$100.00^{\circ}$	54.44ª	81.50 <sup>b</sup>	$93.10^{bc}$	$100.00^{\circ}$
Sand fescue	41.37ª	52.31a	98.20°	$100.00^{b}$	9.41ª	$41.44^{b}$	93.35°	$100.00^{\circ}$

Mean with same letters in row is not significantly different at p<0.05

Table 2:  $I_{50}$  values of aqueous methanol extracts of *P. sarmentosum* for shoots and roots of test plants

	I <sub>50</sub> (g dry weight equivalent extract mL <sup>-1</sup> )			
Test plant species	Shoot	Root		
Cress	0.008	0.001		
Lettuce	0.027	0.009		
Alfalfa	0.002	0.001		
Timothy	0.013	0.014		
Crabgrass	0.037	0.020		
Italian ry egrass	0.034	0.014		
Ryegrass	0.040	0.050		
Buckwheat	0.210	0.018		
Chinese sprangletop	0.021	0.024		
Jungle rice	0.080	0.032		
Barny ard grass	0.016	0.009		
Sand fescue	0.009	0.033		

The values were determined by a logistic regression analysis after bioassays

# DISCUSSION

Aqueous methanol extract of P. sarmentosum inhibited shoot and root growth of all test plant species at the concentrations greater than 0.03 g dry weight equivalent extract mL<sup>-1</sup> and increasing the extract concentration increased the inhibition. Such inhibition on the growth of test plant species might be due to the presence of allelochemicals in P. sarmentosum. Similar results have been reported from other studies. Randhawa et al. (2002) found that the germination of Trianthema portulacastrum was suppressed by higher concentration of the sorghum water extract. The results are in agreement with earlier studies reporting that effectiveness of receiver plants to allelochemicals was concentration dependent of inhibitory substances with a response threshold (Caussanel, 1979; Lovett et al., 1989; Hoque et al., 2003; An et al., 2005; Batlang and Shushu, 2007; Ashrafi et al., 2009). The aqueous methanol extract therefore had an inhibitory effect on a wide range of plant species, both of the monocotyledonous plant (timothy, crabgrass, Italian ryegrass, ryegrass, Chinese

sprangletop, jungle rice, barnyard grass and sand fescue) and the dicotyledonous plant (cress, lettuce, alfalfa and buckwheat). In addition to that, aqueous methanol extract of P. sarmentosum had higher root growth inhibition than that of shoot growth of the test plant species except of ryegrass, Chinese sprangletop and sand fescue. Salam and Noguchi (2010) reported that the extracts of allelopathic plants had more inhibitory effect on root growth than on hypocotyl growth because root is the first organ to absorb allelochemical from the environment. Furthermore, the permeability of allelochemicals to root tissue was reported to be greater than that to shoot tissue (Nishida et al., 2005). Results of this study also identified that inhibitory effects of P. sarmentosum were different on test plant species. This unequal susceptibility to different extracts could be due to inherent differences in various biochemicals involved in the process. The species specificity of phytotoxins has also been demonstrated for other allelopathic plants species (Javaid and Anjum, 2006).

The seedlings of each test species used in these experiments were grown in a single Petri dish without intra-species competition for resources, as young seedlings withdraw nutrients from the seeds and light is unnecessary in the developmental stage (Ashrafi et al., 2008). Thus, growth inhibitions of the test plant species are likely to have been caused by the allelopathic reaction rather than by competitive interference. It is important to note that P. sarmentosum had strong inhibitory effect on the growth of noxious paddy weeds barnyard grass and jungle rice. From the present study, it could therefore be concluded that the aqueous methanol extract of P. sarmentosum may possess allelopathic potential and may contain growth inhibitory substances. These results might have value in enabling weed control based on natural plant extracts and hence this plant could be used for the development of bioherbicide in weed management.

However, further research is needed to isolate and characterize allelopathic compounds from the aqueous methanol extract of *P. sarmentosum*.

### CONCLUSION

An aqueous methanol extract of *P. sarmentosum* plants showed an allelopathic effect on all test plant species at the concentration greater than 0.03 g dry weight equivalent extract mL<sup>-1</sup>and increasing the extract concentration increased the inhibition. Therefore, *P. sarmentosum* may contain growth inhibitory substances and possess allelopathic potential (Fig. 1). Further evaluation of allelochemicals in the plants under field conditions is required. The effective natural products could be used as environmentally friendly herbicides to control weeds.

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