Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

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Asian Journal of Plant Sciences 9 (8): 486-491, 2010
ISSN 1682-3974

Screening of Allelopathic Activity of Eleven Thai Medicinal Plants on Seedling Growth of Five Test Plant Species

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Abstract: Eleven species of Thai medicinal plants, namely *Rhinacanthus nasutus* (L.) Kurz, *Clitoria ternatea* L., *Mammea siamensis* Kosterm., *Centella asiatica* (L.) Urban, *Thunbergia lauriflolia* Linn., *Piper sarmentosum* Roxb., *Hibiscus sabdariffa* L., *Moringa oleifera* Lam., *Tinospora tuberculata* Beume, *Tiliacora triandra* (Colebr.) Diels. and *Amomum krervanh* Pierre ex. Gagnep. were evaluated their allelopathic potentials against cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), alfalfa (*Medicago sativa* L.), timothy (*Phleum pratense* L.) and crabgrass (*Digitaria sanguinalis* L.). The aqueous methanol extracts of these medicinal plants had inhibitory activity on all test plant species with different inhibition values. The aqueous methanol extracts of *H. sabdariffa* showed the highest inhibitory effect on cress and alfalfa seedlings. The extract obtained from *P. sarmentosum*, *R. nasutus* and *T. tuberculata* possessed the highest allelopathic potential on lettuce, timothy and crabgrass seedlings, respectively. Inhibitory effects of these medicinal plants were dependent on test plant species. The variation may result, in part, from the different test plant species with different sensitivity to allelochemicals. However, four medicinal plants *H. sabdariffa*, *P. sarmentosum*, *R. nasutus* and *T. tuberculata* possessed high allelopathic potential and may be good candidates for isolation and identification of allelochemicals. It would also be interesting to evaluate the implication on these evaluation results under field conditions.

Key words: Allelopathic activity, medicinal plants, aqueous extracts, methanol extracts, test plant species, inhibitory effect

INTRODUCTION

Due to increasing numbers of herbicide-resistant weed biotypes and environmental concerns about the safety of synthetic herbicides, much attention has recently been focused on the reduction of the synthetic herbicide dependency and finding alternative strategies for weed management which are less herbicide dependant or based on naturally occurring compounds (Sujatha *et al*., 2010). The use of plants with strong allelopathic properties for weed control has shown promising results and allelopathy holds great prospect for meeting some of these demands (An *et al*., 1996; Travlos *et al*., 2007). Many crop and weed species have been observed to have allelopathic properties (Batish *et al*., 2001). Allelopathic effect of medicinal species is of special interest in recent year (Han *et al*., 2008; Li *et al*., 2009). Nazir *et al*. (2006) evaluated allelopathic potential of 3 herbal species (*Rheum emodi*, *Saussaurea lappa* and *Potentilla fulgens*) against some traditional crops. Germination of all crops was reduced significantly by aqueous extracts of *S. lappa* and *P. fulgens*. Khan *et al*. (2009) found the allelopathic effects of 4 medicinal plants by using various methods. Aziz and Fujii (2005) examined allelopathic activities of 14 medicinal plant species grown in plain areas of Pakistan with semi-arid conditions on growth of lettuce (*Lactuca sativa*). Fujii *et al*. (2003) and Gilani *et al*. (2009) also surveyed allelopathic potential of 387 Japanese medicinal plants to find out possible candidates as natural herbicides. Thailand is endowed with a great diversity of indigenous medicinal plants. The local communities of Thailand have been using the medicinal plant species for curing various diseases for a long time (Mahidol *et al*., 2002). The beneficial medicinal effects of these plants typically result from the secondary compounds in the plants which are specific in certain taxa, such as family, genus and species (Parekh *et al*., 2005). The purpose of this study is to carry out an evaluation on allelopathic activity of Thai medicinal plants for future chemical analyses. Thus, 11 species of medicinal plants were collected from the preliminary survey and assessed for the

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effects of their extracts on the growth of 5 tested plant species, cress (*Lepidium sativum* L.), lettuce (*Lactuca sativa* L.), alfalfa (*Medicago sativa* L.), timothy (*Phleum pratense* L.) and crabgrass (*Digitaria sanguginalis* L.).

MATERIALS AND METHODS

Plant materials: Whole plants (leaves, stem and roots) of *Rhinacanthus nasutus* (L.) Kurz, *Clitoria ternatea* L., *Mammea siamensis* Kosterm., *Centella asiatica* (L.) Urban, *Thunbergia lauriflolia* Linn., *Piper sarmentosum* Roxb., *Hibiscus sabdariffa* L., *Moringa oleifera* Lam., *Tinospora tuberculata* Beume, *Tiliacora triandra* (Colebr.) Diels. and *Amomum krervanh* Pierre ex Gagnep. were collected from Chiang Mai province, Thailand in June 2009. 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.) and crabgrass (*Digitaria sanguinalis* L.) were chosen as test plants for bioassay because of their known seedling growth behavior.

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

Bioassay: An aliquot of the extract (final assay concentration was 0.003, 0.01, 0.03 and 0.1 g dry weight of each plant extract equivalent extract/mL) was evaporated to dryness at 40°C *in vacuo* by rotary evaporator, dissolved in 1 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), which was used for surfactant and did not cause any toxic effects. Ten seeds of cress, lettuce, alfalfa, or 10 germinated seeds of crabgrass (germinated in the darkness at 25°C for 120 h) or timothy (germinated in the darkness at 25°C for 48 h) were arranged on the filter paper in Petri dishes. 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:

$$\text{Inhibition (\%)} = \left[1 - \left(\frac{\text{Sample extracts}}{\text{Control}}\right)\right] \times 100$$

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 was analyzed by SPSS version 11.5 using One-way ANOVA.

RESULTS

Effects of aqueous methanol extracts of 11 medicinal plants on the growth of cress seedlings: Table 1 shows the effects of aqueous methanol extracts of 11 medicinal plants on hypocotyl and root growth of cress. The inhibitory effect was increased with increasing concentrations of the extracts and inhibition of the roots was greater than that of the hypocotyls. At concentration of 0.01 g mL^{-1}, the extract of *H. sabdariffa* showed the highest inhibitory effect on root and hypocotyl growth of cress seedlings, followed by *A. krervanh* and *M. siamensis*. At concentration of 0.03 g mL^{-1}, the extracts of *H. sabdariffa* and *A. krervanh* completely inhibited the seedling growth. At concentration of 0.1 g mL^{-1}, the extracts of *T. lauriflolia*, *P. sarmentosum* and *M. oleifera* also completely inhibited the seedling growth.

Effects of aqueous methanol extracts of 11 medicinal plants on the growth of lettuce seedlings: The effects of aqueous methanol extracts of 11 medicinal plants on hypocotyl and root growth of lettuce are shown in Table 2. At concentrations of 0.01 g mL^{-1}, the extract of *P. sarmentosum* had high inhibitory effect, while extracts from the other 10 species had low inhibitory effects. At the concentrations greater than 0.03 g mL^{-1}, *C. ternatea*, *M. siamensis*, *C. asiatica*, *T. lauriflolia*, *P. sarmentosum*, *H. sabdariffa* and *A. krervanh* completely inhibit root and hypocotyl growth of lettuce seedlings.

Effects of aqueous methanol extracts of 11 medicinal plants on the growth of alfalfa seedlings: Table 3 shows the effects of aqueous methanol extracts of 11 medicinal plants on hypocotyl and root growth of alfalfa. At concentration of 0.01 g mL^{-1}, the extract of *H. sabdariffa* exhibited the highest inhibition on hypocotyl and root growth of alfalfa, followed by the extracts of *P. sarmentosum*, *T. lauriflolia* and *A. krervanh*. The extracts of other species, *R. nasutus*, *C. ternatea*,

Table 1: Effects of aqueous methanol extracts on the shoot and root growth of cress seedlings

	Inhibition (%)							
	Shoot				Root			
Plant species	0.003	0.01	0.03	0.1	0.003	0.01	0.03	0.1
Rhinacanthus nasutus	8.8c	13.78c	52.32b	98.18a	8.62c	14.04c	87.89a	99.09a
Clitoria ternatea	6.74cd	24.84c	61.25b	92.43a	-10.96d	19.25c	85.44ab	91.19a
Mammia siamensis	17.48c	74.09a	75.51a	79.98a	36.58b	86.18a	88.69a	88.21a
Centella asiatica	26.88c	62.81b	72.65ab	89.22ab	10.34c	81.02ab	93.29a	94.95a
Thunbergia laurifloria	43.15e	61.41d	77.71c	100.00a	83.73bc	85.63b	89.87b	100.00a
Piper sarmentosum	50.65e	71.35d	98.67ab	100.00a	84.34c	91.31bc	99.51a	100.00a
Hibiscus sabdariffa L.	24.84b	96.33a	100.00a	100.00a	37.29b	97.43a	100.00a	100.00a
Moringa oleifera Lam.	7.56d	71.26b	77.53b	100.00a	33.68c	77.04b	89.01a	100.00a
Tinospora tuberculata Beume	-50.44d	-16.27bcd	-0.42bcd	82.09a	-25.87cd	38.41abc	47.49ab	91.03a
Tiliacora triandra (Colebr.) Diels	-5.85bc	8.75bc	35.10b	83.19a	-26.56c	14.84bc	38.68b	90.06a
Amomum krervanh Pierre	-5.75c	94.87a	100.00a	100.00a	42.91b	96.27a	100.00a	100.00a

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

Table 2: Effects of aqueous methanol extracts on the shoot and root growth of lettuce seedlings

	Inhibition (%)							
	Shoot				Root			
Plant species	0.003	0.01	0.03	0.1	0.003	0.01	0.03	0.1
Rhinacanthus nasutus	2.72d	8.44d	70.31b	95.11a	13.36cd	24.09c	89.18a	99.38a
Clitoria ternatea	36.43d	55.34c	100.00a	100.00a	56.40c	75.33b	100.00a	100.00a
Mammia siamensis	-6.70d	42.98b	100.00a	100.00a	21.71c	57.92b	100.00a	100.00a
Centella asiatica	50.13c	70.74b	100.00a	100.00a	49.26c	70.50b	100.00a	100.00a
Thunbergia laurifloria	32.33d	65.67c	100.00a	100.00a	59.34c	83.17b	100.00a	100.00a
Piper sarmentosum	-5.67d	84.33b	100.00a	100.00a	54.15c	93.44ab	100.00a	100.00a
Hibiscus sabdariffa L.	4.42d	78.13b	100.00a	100.00a	29.66c	84.57b	100.00a	100.00a
Moringa oleifera Lam.	1.34f	18.25e	61.13cd	100.00a	52.19d	73.21bc	87.74ab	100.00a
Tinospora tuberculata Beume	-16.41b	1.13b	0.45b	58.47a	-12.58b	1.13b	10.24b	70.67a
Tiliacora triandra (Colebr.) Diels	0.29c	15.87bc	26.16b	64.65a	14.76bc	26.61b	28.9b	64.05a
Amomum krervanh Pierre	2.69d	83.39b	100.00a	100.00a	16.43c	92.81ab	100.00a	100.00a

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

Table 3: Effects of aqueous methanol extracts on the shoot and root growth of alfalfa seedlings

	Inhibition (%)							
	Shoot				Root			
Plant species	0.003	0.01	0.03	0.1	0.003	0.01	0.03	0.1
Rhinacanthus nasutus	-12.08d	11.83c	77.15a	97.22a	3.90cd	34.71b	87.10a	95.24a
Clitoria ternatea	63.30c	64.62c	84.93ab	92.07a	78.62b	79.41b	90.27a	91.94a
Mammia siamensis	44.21c	62.56abc	82.54a	86.69a	51.43bc	74.36ab	86.70a	90.03a
Centella asiatica	24.38b	29.60b	92.84a	95.83a	17.45b	8.23b	85.00a	91.48a
Thunbergia laurifloria	83.47c	82.32c	91.00bc	100.00a	84.30c	88.21bc	95.75ab	100.00a
Piper sarmentosum	78.99e	87.14cde	97.64ab	100.00a	84.74de	89.10bcd	94.60ab	100.00a
Hibiscus sabdariffa L.	36.35b	96.89a	100.00a	100.00a	46.12b	95.00a	100.00a	100.00a
Moringa oleifera Lam.	13.27f	54.93d	81.82bc	98.57a	37.85e	68.69cd	87.33ab	99.55a
Tinospora tuberculata Beume	2.73c	4.75c	16.14bc	62.86a	16.49bc	18.48bc	26.99b	74.52a
Tiliacora triandra (Colebr.) Diels	-0.81e	6.60de	14.17de	56.74ab	9.18de	26.08cd	43.09bc	77.34a
Amomum krervanh Pierre	18.78c	69.04b	100.00a	100.00a	31.89c	81.77b	100.00a	100.00a

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

M. siamensis, *C. asiatica*, *M. oleifera*, *T. tuberculata* and *T. triandra* against the root and hypocotyl growth of alfalfa seedlings also exhibited inhibitory activity with different percent inhibition.

Effects of aqueous methanol extracts of 11 medicinal plants on the growth of timothy seedlings: Table 4 shows the effects of aqueous methanol extracts of 11 medicinal plants on hypocotyl and root growth of timothy. At concentration of 0.003 g mL^{-1}, aqueous extract of all medicinal plants slightly inhibited root and hypocotyl of timothy. When the concentration was increased to 0.01 g mL^{-1}, the inhibitory effects were increased. The aqueous extract of *R. nasutus* exhibited the greatest inhibition. Exposure to the concentration of 0.1 g mL^{-1}, the extracts of *R. nasutus*, *H. sabdariffa*, *T. triandra* and *T. tuberculata*, completely inhibited the hypocotyl and root growth of timothy.

Table 4: Effects of aqueous methanol extracts on the shoot and root growth of timothy seedlings

	Inhibition (%)							
	Shoot				Root			
Plant species	0.003	0.01	0.03	0.1	0.003	0.01	0.03	0.1
Rhinacanthus nasutus	32.82d	70.76b	100.00a	100.00a	36.05cd	65.14b	100.00a	100.00a
Clitoria ternatea	2.04d	31.52cd	49.37c	78.28ab	14.92d	31.51cd	51.21bc	89.37a
Mammia siamensis	-5.92d	49.39c	79.22b	100.00a	0.50d	34.58c	47.69c	98.29a
Centella asiatica	9.28cd	13.89cd	47.37b	86.10a	-9.70d	20.02c	56.06b	85.76a
Thunbergia laurifloria	0.94d	10.57c	60.16b	95.15a	5.54d	23.64c	60.60b	95.03a
Piper sarmentosum	-4.16d	9.31d	47.01bc	75.43ab	-0.22d	26.43cd	56.06abc	81.78a
Hibiscus sabdariffa L.	3.59d	29.31c	56.31b	100.00a	5.70d	33.28c	66.56b	100.00a
Moringa oleifera Lam.	-32.16e	5.62d	40.71bc	84.27a	10.30d	24.85cd	52.54b	85.83a
Tinospora tuberculata Beume	-16.08e	9.72d	37.71bc	100.00a	-12.08e	27.47cd	51.54b	100.00a
Tiliacora triandra (Colebr.) Diels	-13.30f	20.48e	51.87cd	100.00a	40.68d	63.70bc	73.04b	100.00a
Amomum krervanh Pierre	-32.46e	6.81d	77.40ab	96.24a	26.17c	56.97bc	70.49b	98.07a

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

Table 5: Effects of aqueous methanol extracts on the shoot and root growth of crabgrass seedlings

	Inhibition (%)							
	Shoot				Root			
Plant species	0.003	0.01	0.03	0.1	0.003	0.01	0.03	0.1
Rhinacanthus nasutus	-42.24c	-15.36bc	35.07abc	63.69ab	-40.22c	12.87abc	70.78a	92.09a
Clitoria ternatea	-30.19c	6.93bc	24.31ab	54.86a	-6.81bc	7.50bc	23.73ab	58.76a
Mammia siamensis	-10.10e	44.26cd	71.75ab	91.82a	30.88d	57.24bc	79.03ab	100.00a
Centella asiatica	-20.04d	2.13cd	36.09bc	50.23abc	25.45cd	34.90bc	82.56ab	93.31a
Thunbergia laurifloria	-2.69d	36.44c	52.69bc	97.05a	-3.34d	39.62c	66.98b	97.50a
Piper sarmentosum	11.78c	20.19c	60.02b	96.81a	27.64c	61.64b	79.49ab	97.26a
Hibiscus sabdariffa L.	-16.13c	21.63b	73.85a	100.00a	-20.82c	28.10b	73.69a	100.00a
Moringa oleifera Lam.	-3.70c	64.69b	97.27a	100.00a	5.54c	67.36b	95.65a	100.00a
Tinospora tuberculata Beume	10.62d	77.04b	100.00a	100.00a	36.47c	79.88b	100.00a	100.00a
Tiliacora triandra (Colebr.) Diels	-14.01d	44.98b	100.00a	100.00a	24.43c	51.93b	100.00a	100.00a
Amomum krervanh Pierre	-3.26d	44.34c	100.00a	100.00a	36.84c	60.46b	100.00a	100.00a

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

Effects of aqueous methanol extracts of 11 medicinal plants on the growth of crabgrass seedlings: At concentration of 0.01 g mL^{-1}, the extracts of *T. tuberculata* showed the highest inhibitory activity on hypocotyl and root growth of crabgrass by 77.04 and 79.88%, respectively. When the concentration was increased, the inhibitory effects were increased. At concentration of 0.01 g mL^{-1}, the extracts of *T. tuberculata*, *T. triandra*, *A. krervanh*, *M. oleifera* and *H. sabdariffa* completely inhibited hypocotyl and root growth of crabgrass.

DISCUSSION

Allelopathy in agricultural practices has become more important with the main objectives of using this phenomenon in biological control of weeds (Rice, 1984). As a possible approach, this fact shall be further evaluated and utilized for screening allelopathic plant species (Kebede, 1994; Leather, 1982). The growth inhibitory effects on 11 Thai medicinal plants were confirmed by five test plant species in the present research. The inhibitory effect augmented with increasing concentrations of methanol extracts (Table 1-5). It was also reported that effectiveness of receiver plants to allelochemicals was concentration dependent of inhibitory substances with a response threshold (Lovett *et al*., 1989; Caussanel, 1979; An *et al*., 2005; Ashrafi *et al*., 2009; Batlang and Shushu, 2007). Inhibitory effects of these medicinal plants were different on test plant species. The variation might be attributed to the differences in kind, total amount as well as properties of allelochemicals produced by different species used in this study. Chon *et al*. (2005) reported that the extracts from lettuce plant had potent allelopathic activity and the activity differed depending on cultivar, extract or fraction. However, the extracts of *H. safdarifa*, *P. sarmetosum*, *R. nasutus* and *T. turberculata*, respectively, showed the highest inhibitory effects on cress and alfalfa, lettuce, timothy and crabgrass seedlings.

In addition, the inhibition of the methanol extract of 11 Thai medicinal plants on the root growth of five test plant species was greater than that on hypocotyls growth. These results are in agreement with the results of Stachon and Zimdahl (1980), which 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.

Similar kinds of results were reported from the studies of Chon *et al*. (2000), root length was the best indicator of allelopathic effects of plant extracts because root growth has been reported to be more sensitive to phytotoxic compounds than hypocotyl growth in alfalfa. Furthermore, the permeability of allelochemicals to root tissue was reported to be greater than that to shoot tissue (Nishida *et al*., 2005). The present research suggests that the extracts of *H. safdarifa*, *P. sarmetosum*, *R. nasutus* and *T. turberculata* have showed higher allelopathic effects among all Thai medicinal plants. These four plants, therefore, may be the candidates for isolation and identification of allelochemicals. Further studies, however, should be conducted under greenhouse and field conditions to help evaluate the implications of these potential species.

ACKNOWLEDGMENTS

Authors would like to thankfully acknowledge Government of Japan for the supporting scholarship for Piyatida P.

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