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Evaluation of Allelopathic potential of *Amomum krervanh* Pierre ex Gagnep

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Abstract: The allelopathic effect of *Amomum krervanh* Pierre ex Gagnep against 5 test plant species; cress (*Lepidum sativum* L.), lettuce (*Lactuca sativa* L.), alfalfa (*Medicago sativa* L.), timothy (*Phleum pratense* L.) and crabgrass (*Digitaria sanguinalis* L.) was investigated. It was observed that the aqueous methanol extracts of *A. krervanh* inhibited all test plant species with different inhibition values. The shoot and root growth of test plants were inhibited at the concentration greater than 30 mg dry weight equivalent extract/mL and increasing the extract concentration increased inhibition. The concentrations required for 50% growth inhibition of test plants were 2-28 mg dry weight equivalent extract/mL and lettuce seedling was most sensitive to the extract. These results suggested that *A. krervanh* might contain growth inhibitory substances and possess allelopathic activity that could be utilized in the development of bioherbicide for future weed management. Further studies, however, are required to isolate and identify the growth inhibitor causing the allelopathic effect of this plant. It would also be interesting to evaluate the implication on these evaluation results under field conditions.

Key words: Allelopathic activity ・ Medicinal plant ・ Aqueous methanol extract ・ Bioassay plants

INTRODUCTION

The occurrence of weeds in agricultural fields leads to substantial yield reductions causing economic losses all over the world. Modern agriculture relies heavily on the use of synthetic herbicides for managing weeds. These herbicides cause the great concern for agriculture, the environment and human health. These concerns are shifting attention to alternative weed control technologies based on natural products. Herbicide use may be reduced by utilizing allelopathy as an alternate weed management tool in sustainable intensive crop production [1].

Allelopathy is the ability of plant to inhibit the germination of other plants through the production of allelochemicals which may be present in any parts of the plants, i.e. leaves, roots, fruits, stems, rhizomes and seeds, from where they are released to the soil through volatilization, root exudation, leaching and decomposition of plant residues [2]. Allelopathy is also regarded as a bio-chemical warfare as the importance of allelopathy in biological control of weeds and crop productivity has been highly recognized and various methods have been suggested to know the allelopathic effect [3-5]. The role of allelochemicals in agro-ecosystem has attracted the attention of numerous scientists.

Several studies on the screening for the allelopathic potential of medicinal plants have been reported [6-10]. Fujii *et al*. [11] evaluated the allelopathic potentials of 239 medicinal species using the Plant Box Method and 223 species of them suppressed the growth of the plant tested, whereas 17 species enhanced lettuce radicle growth. Gilani *et al.* [12] also surveyed allelopathic potential of 81 Japanese medicinal plants to identify possible candidates as natural herbicides. Nazir *et al*. [13] evaluated allelopathic potential of three herbal species (*Rheum emodi, Saussaurea lappa* and *Potentilla fulgens*) against several traditional crops and the germination of all crops tested 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 [14].

Amomum krervanh Pierre ex. Gagnep, a plant belonging to the family Zingiberaceae, is distributed mainly in Asia including Thailand and Cambodia.

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It is used widely as a folk medicine. The plant possesses pharmacological properties such as antimicrobial, antimalarial and also used in primary healthcare such as constipation, dysentery, digestive disorder [15, 16]. Despite its long usage as testified in traditional folklore, the biological properties of *A. krervanh* and the scientific evidence of its allelopathic effects is scant. Hence, in the present study, the allelopathic effect of *A. krervanh* was investigated against 5 tested plant species and to further characterize the allelopathic substances present in *A. krervanh.*

MATERIALS AND METHODS

Plant Materials: Whole plants (leaves, stem and roots) of *Amomum krervanh* Pierre ex. Gagnep, were collected from Chiang Mai province, Thailand in September 2011. 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. Crabgrass (*Digitaria sanguinalis* L.) was chosen as test plants for bioassay due to their existence of weeds in crop field throughout the world.

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 3, 10, 30 and 100 mg dry weight equivalent extract/mL) 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), which was used for surfactant and did not cause any toxic effects. Timothy and crabgrass were germinated in the darkness at 25°C for 48, 120 and 72 h, respectively. Then, 10 seeds of cress, lettuce, alfalfa, timothy, or crabgrass were arranged on the filter paper in Petri dishes. Treatments with the aqueous solution of Tween 20 without the extract were used as the control. The shoot and root lengths of seedlings was measured at 48 h after incubation in the darkness at 25°C. 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- (sample extracts/ control)] ×100.

The concentrations required for 50% inhibition (defined as I_{50}) of the test plants in the assay was calculated from the regression equation of the concentration-response curves. The values of I_{50} and were summarized in Table 1.

Table 1: I_{50} values of aqueous methanol extracts of *A. krervanh* for shoots and roots of test plants

	I_{50} (mg dry weight equivalent extract/mL)	
Test plant species	Shoot	Root
Cress	5.1	4.7
Lettuce	2.0	3.8
Alfalfa	15.6	7.1
Timothy	27.5	19.3
Crabgrass	21.9	24.1

The values were determined by a logistic regression analysis after bioassays

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 v. 16.0 using one-way ANOVA [17].

RESULTS AND DISCUSSION

Effect of Aqueous Methanol Extracts of *A. krervanh* on Shoot Growth: The inhibitory activity of the extracts on shoot growth of test plant species was summarized in Figure 1. The extracts obtained from 0.03 g dry weight of the *A. krervanh* plants inhibited shoot growth of cress, lettuce, alfalfa, timothy and crabgrass by 95, 83, 69, 7 and 44%, respectively. Exposure to the concentration of 0.1 g mL^{-1}, shoot growth of cress, lettuce, alfalfa, timothy and crabgrass was completely inhibited. Comparing the concentration required for 50% inhibition (defined as I_{50}), lettuce shoots were the most sensitive to the extracts follow by cress, alfalfa and crabgrass. On the other hand, shoot growth of timothy was less sensitive to the extracts (Table 1).

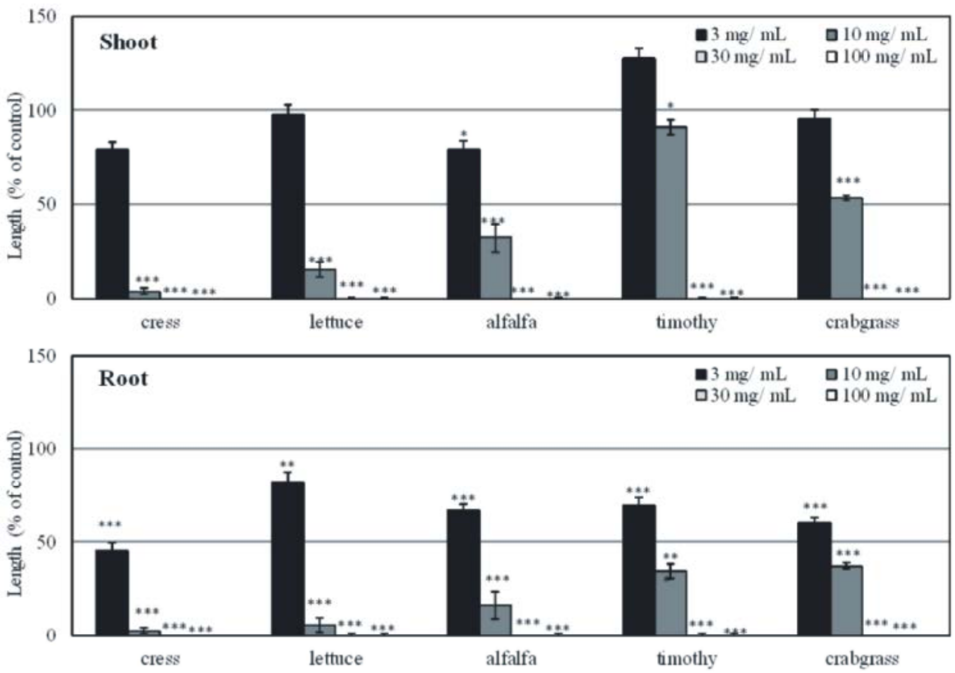

Fig. 1: Effects of aqueous methanol extract of *A. krervanh* on root and shoot growth of cress, lettuce, alfalfa, timothy and crabgrass. Concentrations of tested samples corresponded to the extract obtained from 3, 10, 30 and 100 mg dry weight equivalent extract/mL. Means ± SE from four independent experiments with ten plants for each determination are shown. Asterisk indicates significant difference among treatments in reference to control: *$*P<0.05$, $**P<0.01$, $***P<0.001$*

Effect of Aqueous Methanol Extracts of *A. krervanh* on Root Growth: The extracts obtained from 0.03 g dry weight of the *A. krervanh* plants inhibited root growth of cress, lettuce, alfalfa, timothy and crabgrass by 96, 93, 82, 65 and 61%, respectively (Fig. 1). At the concentration of 0.1 g mL^{-1}, the inhibition of cress, lettuce, alfalfa, timothy and crabgrass was completely inhibited. Comparing the concentration required for 50% inhibition, lettuce roots were the most sensitive to the extract of *A. krervanh* followed by cress, alfalfa and timothy. Crabgrass roots were less sensitive to the extracts (Table 1).

Aqueous methanol extract of *A. krervanh* inhibited shoot and root growth of all test plant species at the concentrations greater than 30 mg dry weight equivalent extract/mL and increasing the extract concentration increased the inhibition. The results are in agreement with earlier studies reporting that degree of inhibition increase with increased extract concentration [18-22].

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 [23]. Thus, inhibition on the growth of test plant species might be due to the presence of allelochemicals in the extract of *A. krervanh* rather than by competitive interference.

Additionally, the aqueous methanol extract of *A. krervanh* had higher growth inhibition on roots than shoots of all the test plant species. Salam and Kato-Noguchi [24] 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 [25].

Results of this study also indicated that inhibitory effects of *A. krervanh* were different on test plant species. Comparing in I_{50} values, root and shoot of dicotyledonous plant (cress, lettuce and alfalfa) were more sensitive to the extract than monocotyledonous plant (timothy, crabgrass) and lettuce seedling was most sensitive to the extract. This unequal susceptibility to the extracts could be due to inherent differences in various biochemicals involved in the process [9].

Therefore, it may be concluded that the aqueous methanol extract of *A. krervanh* may possess allelopathic potential and may contain growth inhibitory substances. The results may have value in enabling weed control based on natural plant extracts and hence this plant could be used for the one of the ingredients for bioherbicide.

However, the isolation and characterization of growth inhibitory substances, which might be responsible for strong allelopathic potential of *A. krervanh* is needed.

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