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Effects of *Citrus aurantifolia* (Christm.), *Citrus hystrix* and *Alpinia galanga* on Plant Growth Inhibition

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Abstract

Peels of two species of Citrus, *Citrus aurantifolia* (Christm.) and *Citrus hystrix* and rhizome of *Alpinia galanga* were selected for various extractions. Three extraction solvents: methanol, aqueous and dichloromethane were used in this study. The test methods were seed germination bioassay which focused on percent inhibition of seed germination, seedling length bioassay and shooting length bioassay that reflected the inhibitory effects on root elongation and shoot length of seedling plants of lettuce seeds (*Lactuca sativa* L.) by_suitable germination and growth rate as the representative for weeds in the test such as sensitivity, simplicity, low cost and suitability. The result showed that the 10 mg/ml of methanol extracts of *Citrus hystrix* had significant inhibitory effects on germination of lettuce seeds and inhibition of root length and 10 mg/ml of dichloromethane extracts of *Alpinia galanga* had significant inhibitory effects on inhibition of root and shoot length which could be utilized as bioherbicide for future weed control.

Keywords: *Citrus aurantifolia* (Christm.), *Citrus hystrix*, *Alpinia galanga*, seed germination bioassay, seedling length bioassay, bioherbicide

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1. Introduction

Natural products are sources of a number of pesticides, used directly as crude extracts or pure compounds. Numerous examples exist of such natural products used as fungicides, insecticides and even herbicides. Over the last few decades, interest had growth concerning compounds which could lead to the discovery of natural herbicides that act directly on the weeds and do not cause environmental damage (Tigre *et al*., 2012). However, some serious flaws are associated with the use of these synthetic pesticides including pest resistance and negative impact on natural enemies in addition to enviro nmental and health related concerns. These problems have resulted in the renewed interest in the development and use of botanical pest icides, which could be an appropriate and

non-hazardous alternative to the currently used synthetic agrochemicals as the natural products, they are generally effective, biodegredable and thus pose less threat to the environment (Khan *et al*., 2008)

Citrus is an important crop, the main uses of citrus in food industries include fresh juice or citrus-based drinks. Very large amounts of byproduct wastes, such as peels are formed every year. Recently, a number of studies have proposed that some fruit or vegetable byproducts could be a source of natural antioxidants in order to valorize these wastes. Citrus fruits are princi pally consumed by humans as fresh fruit or processed juice, either fresh chilled or concentrated. After juice is extracted from the fruit, the fruit pulp is mostly dumped as waste at large expense. The manipulation of food processing waste is now becoming a very serious environmental issue. It seemed, therefore, worthwhile seeking how to make use of the waste of citrus fruits (Li *et al*., 2006; Kato-Noguchi *et al*., 2004; Tripoli *et al*., 2007; Nawanopparatsakul *et al*., 2012). The peel of *Citrus junos* fruit was found to possess potent allelopathic activity and a methanol extract of the peel inhibited the growth of several weed species (Kato-Noguchi *et al*., 2004). In Thailand, there are many Citrus species that have more agriculture production and widely use in agriculture and manufacturer. The ethanolic extracts of *Alpinia galanga* exhibited excellent phytotoxic activity against *Lemna minor*. In this research, we screen the inhibitory effects of two citrus species *Citrus aurantifolia* (Christm.) (CA), *Citrus hystrix* (CH) and *Alpinia galangal* (AG)

Phytotoxicity tests, especially the seed germination and seedling length bioassay have obvious advantages over those toxicity using animals and algae (Wang *et al*., 2001). The commonest bioassay model used for weeds is *Lactuca sativa* L., due to its fast germination, uniformity and sensibility (Tigre *et al*., 2012). Thus, the germination rate is also a variable to measure when seed viability and germination tests are conducted. (Valerio *et al*., 2007) Germination rate and root elongation, as a rapid phytotoxicity test method, possess several advantage, such as sensitivity, simplicity, low cost and suitability for unstable chemicals or samples. Seed germination and root elongation tests do not need plant nutrients and adjunctants in the water control. (Daohui *et al*., 2007; Wang *et al*., 2001; Lin *et al*., 2007). To find some new natural source of pesticides from botanical origin, we screened the crude extracts. The objective of these study is to find substances for weeds control, reduce the use of traditional herbicides, diminishing the costs of agricultural production and the resulting environmental impact of their use.

2. Material and methods

2.1 Plant material and preparation of extracs

Peels of *Citrus aurantifolia* (Christm.), *Citrus hystrix* and rhizome of *Alpinia galanga* were gathered from Nakhon Pathom, Thailand. There were shade dried and then cut and extract with methanol, aqueous and dichloromethane. Each three extracts were concentrated wirh rotary

evaporator at 40˚C , under vacuum. Each concentrated crude extract was kept at -20°C until use.

2.2 Species and pretreatment

The study was conducted using lettuce seeds (*Lactuca sativa* L*)* that commonly used for the seed germination and elongation root test, recommended by the US Environmental Protection Agency, the US Food and Drug Administration and the Organization for Economic Cooperation and Development (EPA, 1996; OECD, 2003). Seeds were purchased from a local seed market. The seeds were sterilized in 10% Na hypochlorite solution for 20 minutes to prevent fungal growth, washed with distilled water for several changes.

2.3 Seed germination assay and growth inhibition assay

Seed germination on filter paper was carried out in glass Petri dishes with filter paper in the bottom as the support, ten seeds of lettuce were sowed and 4 mL aliquot of crude extracts was added. Distilled water was used as control. All dishes were stored in growth chamber at 25±2 ˚C in the dark for 120 hours. Seed germination was measured.

Seeds of lettuce that germinated for 24 h on filter paper wetted with distilled water were selected. Five selected seeds were sowed in test tube on agar that on which aliquot of crude extracts was added. All treatments were stored in growth chamber maintained at 12/ 12 h light/ dark period at 25±2°C. After 7 day, the root length and shoot length of lettuce plants were measured.

2.4 Statistical analysis

Each treatment of this study is carried out with three replications, and the experiments were repeated three times. All treatments were conducted in a completely randomized design. Mean values and standard deviation (S.D.) were calculated from the result. One way analysis of variance (ANOVA) was applied for comparison of the mean value. p value<0.05 was regarded as significant.

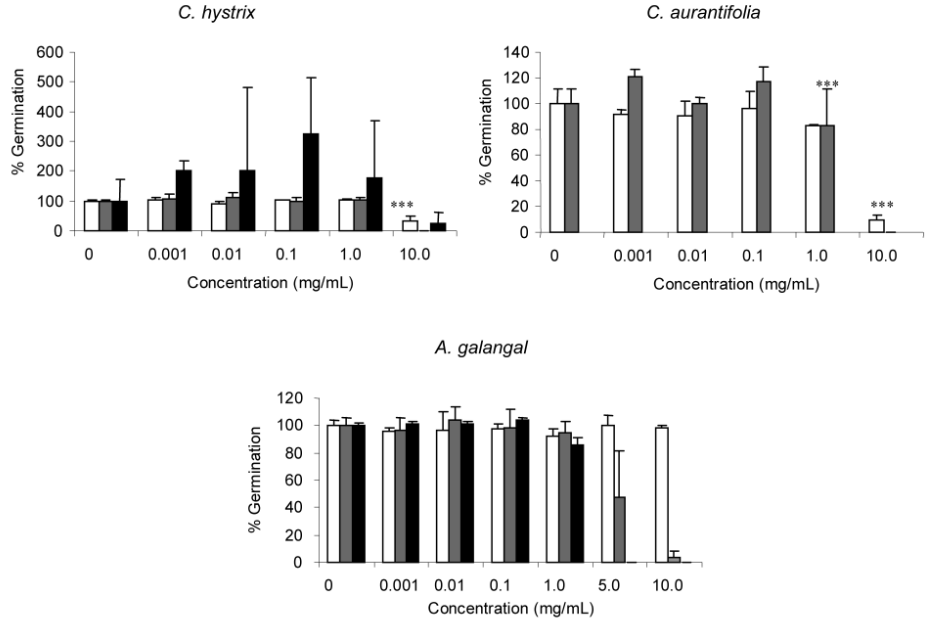

Figure 1. Effect of *Citrus aurantifolia (Christm.)* (CA), *Citrus hystrix* (CH) and *Alpinia galanga* (AG) extracts on lettuce seed germination. Value represents the mean ± S.D. *** p<0.001. ☐ aqueous extract, ▒ methanol extract and ■ dichloromethane extract

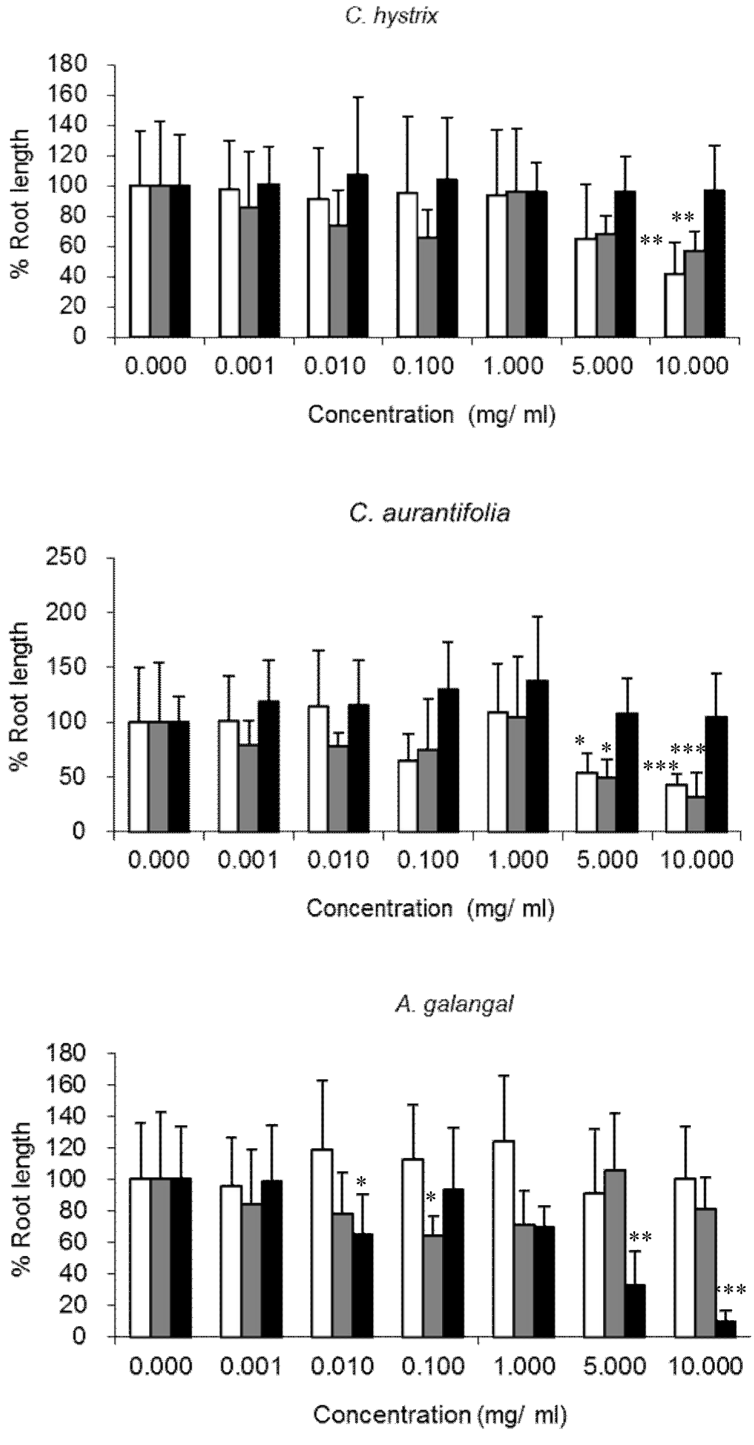

Figure 2. Effect of Citrus aurantifolia (Christm.) (CA), Citrus hystrix (CH) and Alpinia galanga (AG) extracts on lettuce root length. Value represents the mean ± S.D. * $p<0.05$, ** $p<0.01$, *** $p<0.001$. □ aqueous extract, ▒ methanol extract and ■ dichloromethane extract.

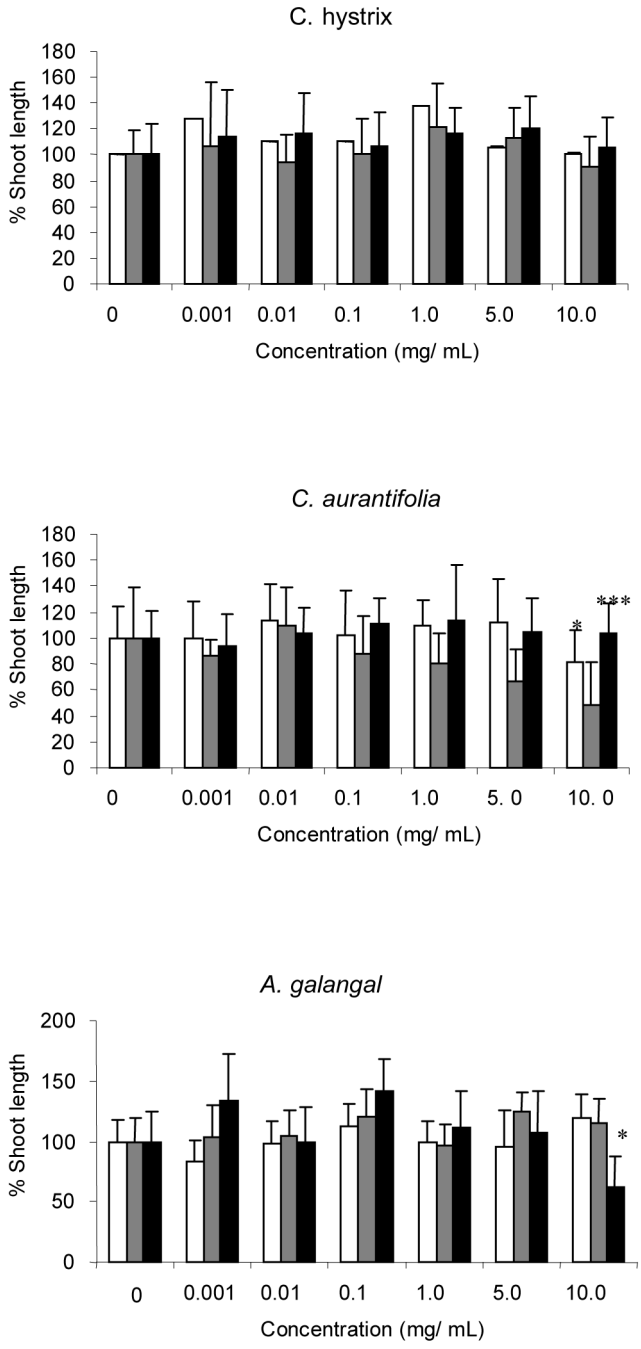

Figure 3. Effect of *Citrus aurantifolia* (Christm.) (CA), *Citrus hystrix* (CH) and *Alpinia galanga* (AG) extracts on lettuce shoot length. Value represents the mean ± S.D. * $p<0.05$, *** $p<0.001$. □ aqueous extract, ▨ methanol extract and ■ dichloromethane extract

3. Result and discussion

In this study, three extracts of CA, CH and AG were subjected to seed germination assay and weed growth inhibition assay. Seed germination assay and weed growth inhibitory activity of compounds was determined at 0.001, 0.01, 0.1, 1.0, 5.0 and 10.0 mg/ml against dicotyledonous. The suitability of seed germination rate, root elongation and shoot length as phytotoxicity test endpoints were evaluated and the effects of CA, CH and AG on seed germination, root length and shoot length are shown in figure 1, 2 and 3 respectively. The results obtained indicated that some of the tested extracts possess tremendous herbicidal activity.

The effects of extractions of CH, CA and AG on the evaluated endpoints of seed germination were presented in figure 1. It was reported that only 10 mg/ml of aqueous extracts of CA, CH and 1mg/ml of methanol extract of CA had significant inhibitory effects on germination of lettuce seeds (p<0.05), the germination was significantly reduced compared to control. Other extracts of CA and CH and all extracts of AG did not have any effect to reduce germination of lettuce. The seed germination bioassay could be relatively low sensitive to many toxic substances, because many chemicals may not be absorbed by seeds and the embryonic plants draws its nutritional requirements internally from seed stored materials and is effectively isolated from the environment (Sunmonu *et al*., 2014)

For the seedling length bioassay, 5 mg/ml of aqueous and methanol extracts of CA and 10 mg/ml of aqueous extracts and methanol extracts of CA and CH and 0.01, 5.0 and 10.0 mg/mL of dichloromethane extracts of AG and 0.1 mg/mL of methanol extracts of AG had significant inhibitory effects on root length of lettuce seeds (p<0.05) (Figure 2). For shoot length, 10.0 mg/mL of aqueous and dichloromethane of CA extracts and 10.0 mg/ml of dichloromethane of AG had significant inhibitory effects on shoot length of lettuce seeds (p<0.05) (Figure 3). In shoot length bioassay showed that all extracts of CH did not have any effect to reduce shoot length of lettuce seeds bioassay.

Seed germination and plant growth bioassay are the most common techniques used to evaluate compost phytotoxicity. There are large variations among bioassays and plant species. The observed inhibitory effect of some extracts on germination and early growth of seeds indicate that extracts contain some inhibitory principles, which upon release into the aqueous medium, inhibited germination, and reduced growth. Germination is normally known as a physiological process beginning with water imbibition by seeds and culminating in the emergence of the rootlet, seeds showing emergence of radical or cotyledon coming out of the seed coat were recorded as being germinated. The exact mechanism by which germination and seedling growth is affected by extracts is not known. However, it could be due to the inhibition of mitosis in the growing cells (Batish *et al*., 2004). Root elongation of sensitive plant species would have a dose-dependent response. Since root are the first target tissue to confront with excess concentration of pollutants, toxic symptoms seem to appear more in roots

rather than in shoots In this study, 10 mg/ml of aqueous extracts of CA, CH and 1mg/ml of methanol reduced the germination in plant seeds, and some also inhibitory to growth of their roots, stems and leaves. The exact mechanism by which germination and seedling growth was affected is not known. For germination, it might be caused from the inhibition of α-amylase activity. During germination, gibberellic acid is disrupted by phytotoxic chemicals. Gibberellic acid induces α-amylase production. The α-amylase is major enzyme responsible for conversion of reserve carbohydrates to soluble sugar. Inhibitions the enzyme activity of seeds has been reported to affect the mobility of reserve carbohydrates, leading to reduced germination. Furthermore, induction of α-amylase also relates to seedling growth until photosynthesis sufficiently supported growth. For seedling growth inhibition, phytotoxic compounds may inhibit mitosis which is highly active at meristematic areas of the growing root tip. The permeability of phytotoxic substances to root tissues might cause from directly explosion with phytotoxic substances. This reason might be one factor that causes greater inhibition of root length than shoot length (Sunmomu *et al*., 2014; Perata *et al*., 1997; Kato-Noguchi *et al*., 2004; Poonpaiboonpipat *et al*., 2013). The plant cell membrances are the first living structures that come in contact with the phenolic acids. Then, plant growth inhibitory effects of these compounds would be mainly related with membrance-associated disturbance (Sampietro *et al*., 2006). The citrus peels are very rich in phenolic compounds, such as phenolic acids and flavonoids (Tripoli *et al*., 2007).

At the concentration which we used the extractions of AG were observed to have no effect on seed germination. This phenomenon may have several explanations: first, it is necessary to consider the function undertaken by seed coats which constitute a barrier between the embryo and its immediate environment as reported by others. The extracts could be adsorbed by the seed coat, which would thus not affect the growth of the embryonic root: second, the protrusion of the root through the seed coat, a parameter used to measure germination percentage, does not necessarily mean growth by cell division, a phenomenon known to be inhibited by extracts but is due simply to cell elongation. Indeed, root or shoot growth is known to be more sensitive than germination to extracts (Salvatore *et al*., 2008). This observation is in agreement with earlier studies which reported that responses of receiver plants to allelochemicals occur in a concentration dependent manner. (Sunmonu *et al*., 2014)

4. Conclusions

The present study has identified several plant extracts, which could be candidates for the commercial botanical pesticides formulations. Moreover, the isolation, purification and investigation of the active principles responsible for the phy totoxic activity will be another landmark in the development of a verifiable application of these materials. The suitability of seed germination rate and root elongation method with *L. sativa* seeds as phytotoxicity test endpoints were evaluated and the phytotoxicity of selected extraction on *L.*

sativa seeds were determined using these methods. The methodology proposed in this research is applicable in the measurement of the phytotoxicity in lettuce exposed to extraction of some plant an alternative way to determine bioherbicide. The method is inexpensive, quick, and reproducible The 10 mg/ml of methanol extracts of *Citrus hystrix* and 10 mg/ml of dichloromethane extracts of *Alpinia galanga* had significant inhibitory effects on germination of lettuce seeds and plant growth inhibition which could be utilized as bioherbicide for future weed control. The study is worthy of further investigation since this could provide potential bioherbicide and may lead to the discovery of new effective and applicable bioherbicide.

In the present study, it could therefore be concluded that the methanol extracts of *Citrus hystrix* had significant inhibitory effects on germination of lettuce seeds and inhibition of root length and dichloromethane extracts of *Alpinia galanga* had significant inhibitory effects on inhibition of root and shoot length which could be utilized as bioherbicide for future.

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