Allelopathic Effect of *Barleria lupulina* Lindl.on Germination and Seedling Growth of Pigweed and Barnyardgrass

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Received: 18 April 2016; Accepted: 14 December 2016

Abstract

This study aimed to evaluate on an allelopathic potential and its chemical basis of *Barleria lupulina* Lindl., a traditional medicinal plant in Thailand. Pigweed (*Amaranthus spinosus* L.) and barnyard grass (*Echinochloa crus-galli* (L.) Beauv.) were used as test species. Aqueous extract of different parts at concentrations of 25, 50, 75 and 100 mg/mL was assayed on a seed germination and seedling growth of the test species. The leaf extract significantly showed the highest inhibitory activity of germination and seedling growth of the test species, followed by stems and roots respectively. The leaf selected on partially isolation of active compounds by acid-base partitioning method. Crude 70% aqueous-ethanol extract of leaf was further separated into acidic fraction (AE), neutral fraction (NE) and aqueous fraction (AQ). The fractions were assayed on the test species at the concentrations of 2,500, 5,000, 7,500 and 10,000 ppm. The inhibitory activity was significance depending on fractions and concentrations. NE fraction showed the most inhibition on germination and seedling growth of pigweed and barnyard grass followed by AE and AQ respectively. The inhibitory effect increased with increasing concentration. Pigweed were more susceptible to the fractions than barnyard grass. These results indicated that *B. lupilina* contained growth inhibitory substances and possess allelopathic activity. Then, partially isolation of active compounds showed NE fraction had the most effect. The results lead for further identification of allelochemicals and development into natural herbicides for sustainable agriculture.

Keywords: Allelopathy, *Barleria lupulina* Lindl, Inhibitory activity

Introduction

Weeds are a main factor causing yield loss by completing water, nutrients, light and carbon dioxide, etc. Nowadays, synthetic herbicides conveniently use for controlling weeds. However, the continuous use of synthetic herbicide had resulted in negative impacts for humans and environments, and also lead to increasing herbicide resistant on many weeds (Batish, Setia, Singh, & Kohli, 2004). Because of the realization on these impacts, researchers have investigated the other ways to controlling weeds and reducing the use of synthetic herbicides. Many plant species, including crop plants, are capable of producing and releasing biologically active compounds (allelochemicals) (Uddin, Li, Won, Park, & Pyon, 2012). Allelochemicals release from leaves, flowers, seeds, stem, and roots of living or decomposing plant materials (Weston, 1996). All of which enhance sustainability in agriculture, natural compounds released from allelopathic plant residues may help to reduce and replace the use of synthetic pesticides for pest management and therefore cause less pollution as well as being safer on humans, animals, and agricultural products (Khanh, Xuan, & Chung, 2007; Singh, Batish, Pandher, & Kohli, 2003; Sodaeizadeh, Rafieiolhossaini, & Van Damme, 2010). *Barleria lupulina* Lindl is a small shrub, distributed in the South-east Asia region. In Thai traditional medicine, the plant is externally used as an anti-inflammatory for insect bite, herpes simplex and herpes zoster (Kanchanapoom, Kasai, & Yamasaki,

2001). Several iridoid glucosides were found in aerial parts (Kanchanapoom et al., 2001; Suksamrarn, 1986; Tuntiwachwuttikul, Pancharoen, & Taylor, 1998). However, there has not been reported on herbicidal activity of *B. lupulina.* This research was aimed to investigate 1) allelopathic potential of different parts 2) partially isolation of active compounds and its activity.

Materials and Methods

General preparation

B. lupulina was planted at a garden field in Naresuan University, Phitsanulok Province, Thailand. Whole plant without pathogens and insects damage was harvested, cleaned with tap water, separated into leaf, stem and root, dried at 45 °C for 72 h and then ground into powder with electronic grinder.

Seeds of barnyardgrass and pigweed were collected from agricultural field in Phitsanulok province, Thailand. Barnyardgrass seeds were aired under full light for 72 h and incubated at 50°C for 24 h to break their dormancy and were used for the experiments.

Different part bioassay

Leaf, stem and root powder of *B. lupulina* was extracted with distilled water at ratio of 10:100 (w/v) at 14°C for 72 h. The extracted was filtered through cheesecloth and filter paper (Whatman NO.1), respectively which serving as stock solution of 100 mg/mL. These stocks were later diluted with distilled water to obtain concentrations 100, 50, 25 and 12.5 mg/mL. Five mL of aliquot extracts was added to petridish (9 cm diameter) containing two layers of filter papers. Twenty seeds of pigweed or barnyard grass were evenly placed in petridish. Distilled water was used as control. The petridishes were placed in laboratory room. Experiments were carried out in four replications for each concentration of each extract parts in completely randomized design manner Germination was deemed to have occurred only after the radicle had protruded beyond the seed coat by at least the dimension of the seed at 7 days after treatment. The seedling growth was measured as the root and shoot lengths at 7 days after treatment.

Solvent partitioning of active compounds and bioassay under laboratory

According to previously described methods of Laosinwattana, Phuwiwat, and Charoenying (2007); Teerarak, Laosinwattana, & Charoenying, (2010), dried leaf powder (100 g) was extracted with 70% ethanol in water for three times (1 L x 3) for 72 h at room temperature, followed by filtrated through three layers of cheese clothes to remove debris. Then, the combined supernatant was filtered through filter paper (Whatman No.1) and evaporated to dryness on the rotary evaporator at 45°C, leaving a sticky residue. It was diluted with 0.5 L of distilled water and stirred vigorously on a magnet at 45°C for 20 minutes, resulting in aqueous solution and acidified to a pH 3 by 6 N HCl. The filtrate was extracted with ethyl acetate for three times (0.5 L×3). The ethyl acetate solution was mixed with anhydrous magnesium sulfate and concentrated to 0.5 L and then extracted for three times with saturated aqueous $NaHCO_3$ (0.5 L×3). It was dried with anhydrous magnesium sulfate and evaporated by reducing pressure which an ethyl acetate-soluble neutral fraction (NE fraction) was obtained. The combined sodium bicarbonate phase was concentrated to 0.5 L, adjusted to a pH 3 by 6 N HCl, and then extracted with ethyl acetate (0.5 L×3). The ethyl acetate solution was combined, dried with $MgSO_4$, and then evaporated to obtain the ethyl acetate-soluble acidic fraction (AE fraction), and the remains of the aqueous phase were discarded (Figure 1). Each fraction (OR, AQ, NE and AE fractions) was weighed and tested on inhibitory activities.

All fractions (OR, AQ, NE and AE) were again dissolved in original solvent to compare their phytotoxic effects. 5 mL of each fraction solutions (2,500, 5,000, 7,500 and 10,000 ppm) was added in a 9-cm Petri dish lined with two germination papers and evaporated to dryness under a fume hood. After evaporation, Five mL of distilled water was added onto the germination paper and then 20 seeds of each species were evenly placed on the paper. Distilled water was used as control. The petridishes were placed in laboratory room. Experiments were carried out in four replications for each concentration of each extract parts in completely randomized design manner Seven days after treatment germination, shoot and root lengths were measured.

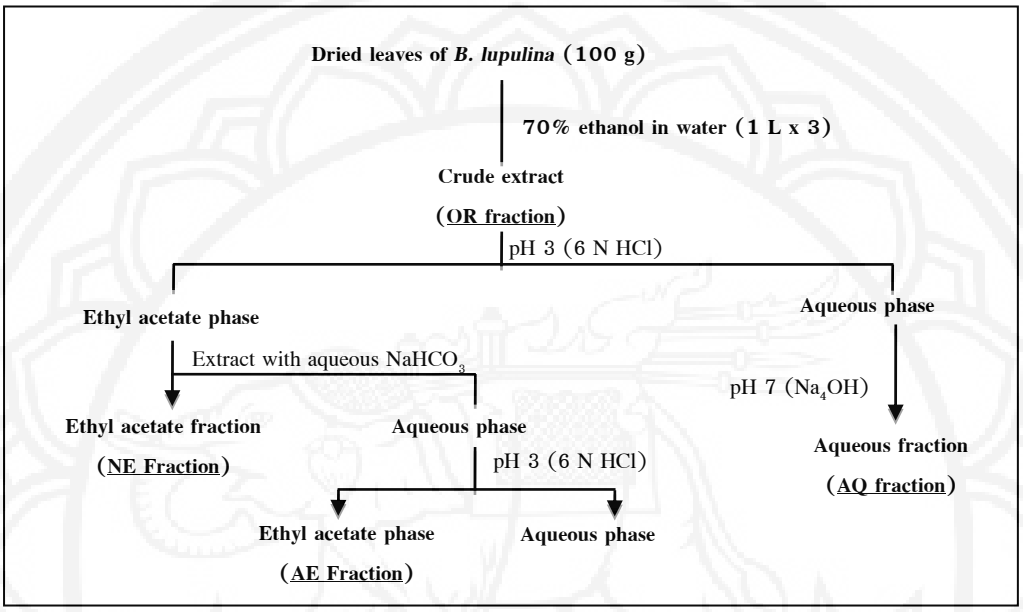

Figure 1 Flow chart for extraction and partial separation of active compounds by acid-base solvent partitioning from *B. lipulina* dried leaf.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA). Whenever ANOVA indicated significant effects ($p < 0.05$), a pairwise comparison of means by Tukey's studentized range test at was carried out.

Results

Different parts bioassay

The data in figure 2 showed that an inhibitory effect of aqueous extract from leaf of *B. lupulina* had significantly higher than stem and root extract. For example, at the concentrations of 100 mg/mL leaf extract inhibited seed germination of pigweed and barnyard grass by 100% and 48% respectively, while stem extract inhibited by 80% and 11%, respectively and root extract inhibited by 11% and 8%, respectively. Barnyard grass seemed more tolerant on the extracts than pigweed suggesting that selective activity. For example, the leaf extract at the concentrations of 12.5, 25, 50 and 100 mg/mL inhibited seed germination of barnyard grass by 29%, 43%, 45% and 47%, respectively while pigweed by 20%, 60%, 80% and 100%, respectively. The results also showed the stimulation effect on shoot length of pigweed at the low concentration (12.5 and 25 mg/mL).

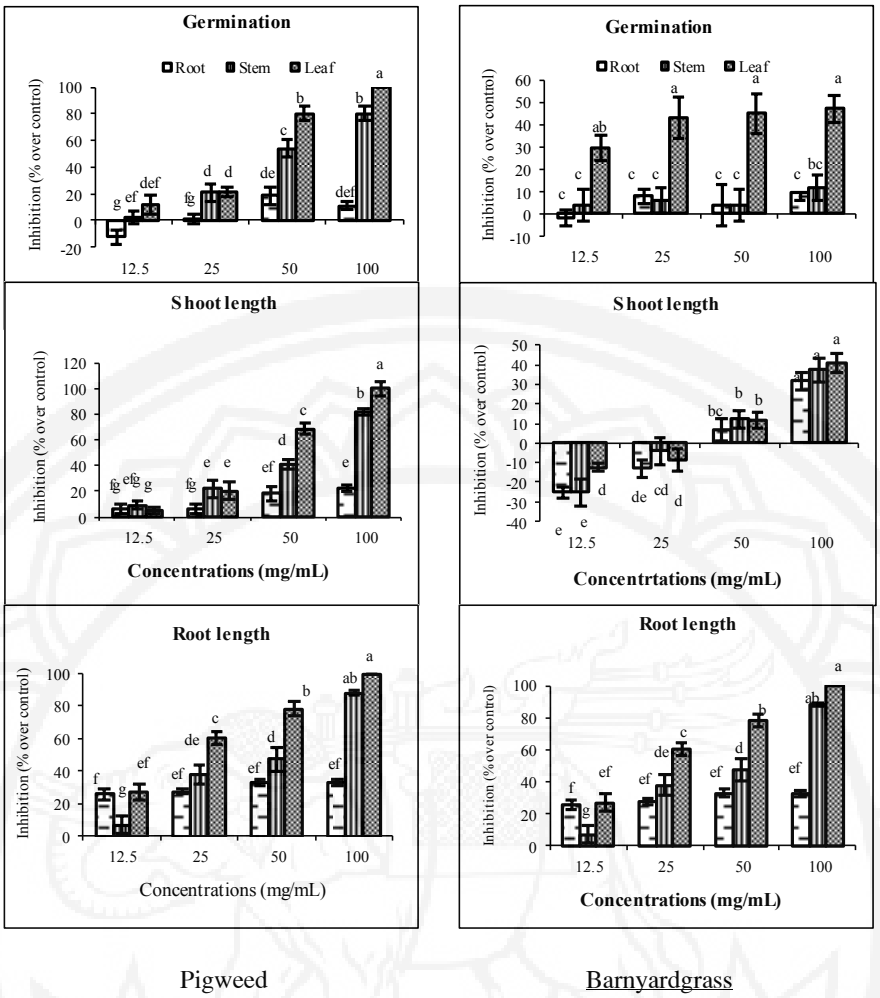

Figure 2 Effects of leaf, stem and root aqueous extract from *B. lupulina* on germination, shoot and root seedling length of pigweed and barnyardgrass. Means with same letters is not significantly different at $p<0.05$.

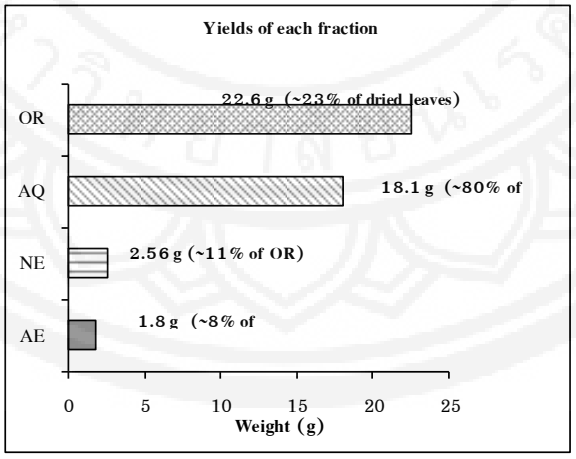

Figure 3 Percentage of yields of each fraction separated by acid-base solvent partitioning from *B. lupulina* dried leaves.

Solvent partitioning

Crude extract of leaves was fractionated by a simple partitioning procedure. OR (70% ethanol crude extract) was separated into AQ (Aqueous fraction), NE (neutral fraction) and AE (acidic fraction) (Figure 1). The yields of each fraction was represented in Figure 3. The allelopathic activities of AE, AQ and NE was compared with OR and indicated by the inhibition of germination and growth of bioassay weeds. Although, the concentrations for bioassays varied from 2,500 to 10,000 ppm, resulted that the most fractions inhibited germination and seedling growth of pigweed and barnyard grass (Figure 4). NE fraction had the greatest inhibition of pigweed and barnyard grass. AQ fractions showed a weak inhibitory effect on germination, shoot length and root length of pigweed and barnyard grass. All fraction completely inhibited the germination of pigweed at 10,000 ppm. NE, AE, OR and AQ fractions at 10,000 ppm inhibited the germination of barnyard grass 66.25%, 23.75%, 15% and 11.25%, respectively. NE fraction at all concentrations completely inhibited germination of pigweed except at 2,500 ppm.

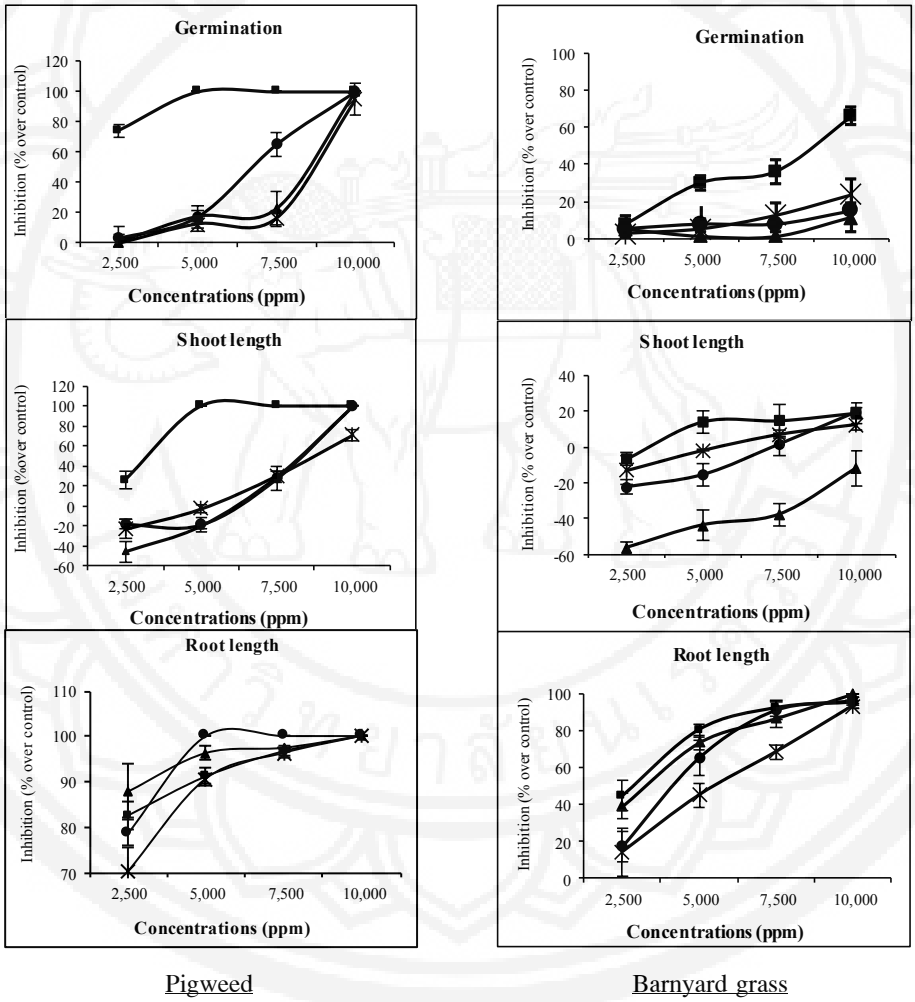

Figure 4 The inhibitory effect of crude extraction yield obtained by acid-base solvent partitioning from *B. lupulina* leaves on germination, shoot length and root length of pigweed and barnyard grass. Note; Original fraction (●); neutral fraction (■); acidic fraction (X); aqueous fraction (▲).

Discussions

The result showed that allelopathic effect of aqueous extract *B. lupulina* differed depending on plant parts and concentration. Leaf extract significantly showed the most inhibitory effect on germination, shoot and root growth of pigweed and barnyard grass followed by stem and root respectively. This indicated that inhibitory substances were in leaf. The result agreed with the leaf extract from *Aglaia odorata* Lour which had more inhibitory effect than branch (Laosinwattana et al., 2009). This reason might be that leaves are a main metabolism site of plant where secondary metabolites are found more than other parts (Xuan, Shinkichi, Hong, Khanh, & Min, 2004; Sisodia & Siddiqui, 2010). Besides, the results indicated that the inhibitory effect was increased by increasing the concentrations. The stimulation effect of *B. lupulina* was found at 12.5 and 25 mg/mL. Allelochemicals always showed stimulatory effect at lower concentration and inhibitory effect at higher concentration (Einhellig, 1986). The inhibitory effect of *B. lupulina* on pigweed was higher than barnyard grass at an equal concentration indicated that the extract varied with weed species which no-agree with the *Aglaia odorata* Lour extract more inhibitory on barnyard grass than wild pea (*Phaseolus lathyroides* L.). This reason may be caused from the smaller size of pigweed than barnyard grass (Laosinwattana et al., 2009).

For partially isolation of active compounds by acid-base solvent portioning method. NE fraction showed more inhibitory effect than other fractions suggested that most allelochemical compounds produced by *B. lupulina* could be presented in NE fraction. It was different results to Teerarak et al. (2010), who reported that a secoiridoid glucoside named oleuropine which identified as an allelopathic compound from AE fraction of a related *Jasminum officinale* var. *grandiforum*. Previous reports were found four iridoid glucosides from *B. lupulina* have been identified as 6-*O*-*p*-methoxy-cis-cinnamoyl-8-*O*-acetylshanzhiside methyl ester, 6-*O*-*p*-methoxy-*trans*-cinnamoyl-8-*O*-acetylshanzhiside methyl ester, 6-*O*-*p*-*cis*-coumaroyl-8-*O*-acetylshanzhiside methyl ester and 6-*O*-*p*-*trans*-coumaroyl-8-*O*-acetylshanzhiside methyl ester (Tuntiwachwuttikul et al., 1998). However, it was not concluded that the four iridoid glucosides were in NE or AE fraction..

The importance of allelochemicals mixtures is recognized both in herbicide research and exploring plant allelochemicals (Inderjit, Streibig, & Olofsdotter, 2002). It is suggested that the mixture compound in NE fractions gave significantly inhibited the tested weed species. For the yield of fractions, NE fraction yield had less than OR. The OR yield was about 23% of dried leaf. After partially separation that giving AE and NE. The NE yield was about 2.56% of dried leaf. The hypothesis of crude extract of *B. lupulina* was the joint action of AE, NE and AQ.

In conclusion, the allelopathic activity of *B. lupulina* was difference depending on plant parts and concentrations. Leaf extract showed the highest inhibitory activity followed by stems and roots respectively, which indicated that *B. lupilina* contains growth inhibitory substances and possess allelopathic activity. The crude extract of leaf was further separated by solvent partition base on acid-base. Neutral fraction (NE) showed the highest activity followed by AE and AQ respectively, which lead for identification of allelochemicals, and develop to natural-herbicides used for sustainable agriculture in the future.

Acknowledgement

This research was supported by Naresuan University Research Found in Science and Technology (No. R2558C217).

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