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Total phenolics, flavonoids, antioxidant activity, and allelopathic potential of praxelis

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Abstract

Praxelis (*Praxelis clematidea* (Griseb.) is an invasive weed in many crops. Praxelis extract has been shown to exhibit allelopathic activity inhibiting seed germination and growth of other weed species. This research aimed to investigate phytochemicals of praxelis, evaluate the allelopathic potential of crude extract, and determine allelopathic longevity in praxelis amended soil. Praxelis was extracted with methanol, 50% (*v/v*) aqueous solution. A series of dilutions of the crude extract were carried out and used in the determination of the phytochemicals. Chinese cabbage (*Brassica pekinensis* L.) and lettuce (*Lactuca sativa* L.) were used as test species in the bioassay of allelopathic studies and allelopathic longevity in praxelis amended soil, respectively. The results showed that praxelis extracts at higher concentrations had greater levels of phytochemicals including phenolics, flavonoids, and antioxidant activity. In a bioassay study, the praxelis extracts at 100% completely inhibited growth and germination of Chinese cabbage. The concentrations of 25% and 50% slightly inhibited Chinese cabbage germination but suppressed root and shoot growth. Praxelis amended soil reduced lettuce growth compared to untreated control, except 14 days after incubation. Based on toxicity evaluation, the praxelis allelochemicals in soil could last up to 7 days. The praxelis extracts exhibited the suppression of germination and growth of tested species. The results suggest praxelis extracts or praxelis amended soil contained potent allelochemicals which can lead to future use as a weed control option.

Keyword: *allelochemicals, Chinese cabbage (Brassica pekinensis L.), lettuce (Lactuca sativa L.), Praxelis clematidea, soil incorporation*

1. Introduction

Controlling weeds in crop production is crucial. Weeds reduce crop yield and quality because they compete with the crops for limited resources such as light, space, CO_2, nutrients, and water (Abouziena & Haggag, 2016). Herbicides are most conventional weed management in field crops; however, negative impacts of herbicides on the environment can occur through accumulation in soil and water, decreased in biological diversity, and development of herbicide-resistant weeds (Al-Samarai, Mahdi, & Al-Hilali, 2018). Therefore, it is necessary to explore alternative weed management systems which is effective and eco-friendly. Allelopathy is a natural mechanism in which plants (including microorganisms) influence the growth and development of each other through the release of allelochemicals into the environment (Rice, 1984). Allelochemical compounds are usually extracted from plant structures placed in contact using water or an organic solvent (Pereira et al., 2019). Allelopathic influence may result in positive outcomes, but generally it is considered to have negative effects on the other plants (Eljarrat & Barcelo, 2001). Allelopathic chemicals are known to have a potential role in sustainable weed management (Won, Uddin, Park, Pyon, & Park, 2013).

Praxelis (*Praxelis clematidea* (Griseb.) (King & Rob, 1970) is an invasive weed in many cropping systems in Thailand. It is an annual weed in Asteraceae. Praxelis has been found to have allelopathic effects in suppression of germination and growth of many plants. Patsai (2011) reported that praxelis extracts inhibited germination and growth of leaf mustard (*Brassica juncea* L.) and rice (*Oryza sativa* L.) and soil incorporation with dry praxelis leaves inhibited emergence and growth of field mustard (*Brassica campestris* L.). Only a few studies have identified the bioactive allelochemicals in praxelis, which were terpenes from volatile oil extracts (Wang et al., 2006) and flavonoids from aerial parts (Falcão et al., 2013; Maia et al., 2011). Thepphakhun, Phimsri, and

Intanon (2019) reported phenolic content of praxelis with respect to incubation durations and allelopathic activities of different praxelis concentrations amended soil. Phenolic content in plants has been reported to play an important role in allelopathy (Li, Wang, Ruan, Pan, & Jiang, 2010), however, for praxelis there has been a lack of association of bioassay activities with phytochemical content. To our knowledge, this is the first study to report the association of bioassay activities with phytochemical contents of praxelis extracts and the use of praxelis amended soil on plant injury. The basic knowledge gained from this study on the allelopathic response of praxelis in extract form or soil incorporation can be used as an alternative weed control option in crop production systems.

2. Objectives

This study aimed to 1) determine the phytochemicals in praxelis extract by measuring total phenolic content, total flavonoid content, and free radical scavenging activity (DPPH), 2) evaluate the allelopathic potential of praxelis extract, and 3) determine allelopathic longevity in praxelis-amended soil.

3. Materials and methods

3.1 Plant materials

Aboveground tissues of praxelis (including leaves, stems, and flowers) were collected from a rubber tree plantation in Wang Thong District, Phitsanulok Province, Thailand in July to September, 2018 (16° 54' 21.384" N 100° 32' 31.596" E). Praxelis tissues were cleaned, oven-dried at 45 ºC for 72 hours, ground, and sieved through 0.5-mm mesh before storing in sealed container. Praxelis powders were used for extraction and soil incorporation studies.

3.2 Preparation of praxelis extract

The praxelis extraction (stock solution) was prepared at 1:10 *w/v* (plant weight: amount of 50% (*v/v*) aqueous methanol). The extract was filtered using a filter cloth and Whatman No.1 filter paper and evaporated in a rotary evaporator to collect the aqueous extract. The extracts of praxelis were diluted with 50% (*v/v*) aqueous methanol to achieve praxelis extract concentrations of 12.5%, 25%, 50%, and 100% (stock solution) for phytochemical and allelopathic activity studies. All studies were conducted in the laboratory and greenhouse at Department of Agricultural Science, Naresuan University, Phitsanulok, Thailand.

3.3 Total phenolic content

The total phenolic content was determined colorimetrically using the Folin-Ciocalteu method as described by Singleton and Rossi (1965). For each extract concentration, 2 mL of 20-fold diluted extract were added to the test tubes. Then, 5 ml of ten-fold diluted Folin-Ciocalteu reagent and 4 mL of 7.5% Na_2CO_3 were added to the tubes and mixed for 10 min at 25 °C. The samples were then incubated at 45 °C for 15 min. Untreated control was prepared in the same way, 50% (*v/v*) aqueous methanol was added instead of the extract. The absorbance of the samples and the blank was measured on the spectrophotometer at 765 nm. The total phenolic content was determined from the linear equation of a standard curve prepared with gallic acid and expressed as mg GAE /g of extract were expressed in terms of gallic acid equivalent (standard curve equation: $0.0202x + 0.0104$, $R^2 = 0.999$). The experiment was a completely randomized design with three replications. The experiment was conducted twice, and the means were averaged ($n = 6$).

3.4 Total flavonoid content

Total flavonoid content was determined using a method reported by Ordonez, Gomez, Vattuone, and Isla (2006). For each extract concentration, 1 mL of 20-fold diluted extracts was prepared in test tubes and 2 mL of 2% $AlCl_3$ in methanol were added. Untreated control was prepared in the same way, 50% (*v/v*) aqueous methanol was added instead of the extract. The absorbance of the samples and the blank was measured on the spectrophotometer at 415 nm after 1 hour at 25 °C. The total flavonoid content was determined from the linear equation of a standard curve prepared with apigenin and expressed as mg API /100 g of extract. Total flavonoid content was expressed in terms of apigenin (standard curve equation: $0.0142x - 0.0096$, $R^2 = 0.991$). The experiment was a completely randomized design with three replications. The experiment was conducted twice, and the means were averaged ($n = 6$).

3.5 *In vitro* antioxidant activity assays: DPPH free radical scavenging assay

The free radical scavenging activity of the fractions was measured by a 2,2′-diphenyl-1-picrylhydrazyl (DPPH) assay according to the method described by Tan, Osman, Wong, Boey, and Padzilah (2009). For each extract concentration, 1 mL of 20-fold diluted extracts was added to test tubes and mixed with 2 mL of 0.1 mM DPPH solution in methanol. The mixture was vortexed for 1 min and incubated at 25° C for 60 min in the dark. The absorbance was measured at 765 nm. Methanol (50%) (*v/v*) aqueous was used as the untreated control and trolox was used as a standard reference. The DPPH radical scavenging effect (%) was calculated by using the following: Scavenging effect (%) = [(A sample -A sample blank)/A control] × 100. Antioxidant activity of praxelis was measured via DPPH free radical scavenging and expressed in terms of trolox (the standard curve equation: 1.6085X + 29.568, R^2 = 0.9962). The experiment was a completely randomized design with three replications. The experiment was conducted twice, and the means were averaged (n = 6).

3.6 Effects of praxelis extract on germination and growth of Chinese cabbage

Praxelis concentrations of 12.5%, 25%, 50%, and 100% (stock solution) were used in the bioassay. For each extract concentration, 5 mL of praxelis extract were added to a petri dish. Control plates received 50% (*v/v*) aqueous methanol. Plate was placed in a fume hood for a 30-min evaporative period to remove methanol. After 30 min, each plate received 5 mL of distilled water. Sixteen seeds of Chinese cabbage (*Brassica pekinensis* L.) were placed in a 9 cm diameter petri-dish lined with a germination paper. The petri dish was placed in a growth chamber at 25/25 °C day/night temperatures with a 12 h photoperiod**.** On day 7, germinated seeds were counted when the radical and/or hypocotyl length were at least 2 mm. The experiment was a completely randomized design with three replications. The experiment was conducted twice, and the means were averaged (n = 6).

3.7 Effects of soil amended with praxelis on growth of lettuce and its allelopathic longevity

There were seven incorporation times of soil amendment with praxelis: 3, 5, 7, 10, 14, 28 and 35 days. No praxelis amendment was used as untreated control (day 0). Praxelis at 4 kg/m^2 was incorporated into the soil (Kamphaeng Phet series, Fine-silty, mixed, active, isohyperthermic oxyaquic (ultic) haplustalfs) at various incorporation times to a 4 cm depth in a 6 cm diameter pot. The soil was classified as a clay soil (67.9% clay, 6.4% sand, and 25.7% silt) with chemical properties of 3.3% organic matter, 0.2% total nitrogen, 18.2 ppm phosphorus, 87.9 ppm potassium, pH of 5.6, and electrical conductivity of 0.26 ds/m. Soil was passed through a 2-mm sieve before use.

Lettuce (*Lactuca sativa* L.) seeds were sown in trays. A 20 days old lettuce seedling was transplanted into the pot at the various times of praxelis amendment. Pots were placed in the greenhouse and plants were watered daily with 10 mL. Toxicity of praxelis on lettuce was observed 14 days after transplanting using a visual evaluation score for toxicity of 0-10 (Plant Protection Research and Development, 2011). Lettuce aboveground biomass was measured 14 days after transplanting by harvesting and drying at 60 °C for 48 hours. The study was structured as a randomized complete block design (RCBD) with four replications. The experiment was conducted twice, and the means were averaged (n = 8).

3.8 Statistical analyses

There were no significant differences based on the Levene's ANOVA test for homogeneity of variances for the phytochemical amount, bioassay, and soil amended with praxelis using a one-way ANOVA; therefore, data were pooled across studies. Data of phytochemical amount, number of germinated seeds, radical and hypocotyl length of Chinese cabbage, toxicity level of lettuce, and lettuce biomass were analysed using ANOVA. Means were separated using a least significant difference ($P \leq 0.05$). Statistical analyses were performed using the program R version 3.5.1 (R Core Team, 2018). Inhibition percentage (IP) was obtained by using the inhibition percentage equation:

$$IP = 100 - (T \times 100/C) \quad (1)$$

where T represents response of treatment and C is response of untreated control.

4. Results and discussion

4.1 Total phenolics, flavonoids and antioxidant activity of praxelis

The total phenolics, flavonoids, and antioxidant activity of praxelis differed in various concentrations of praxelis (Table 1). No total phenolics, flavonoids and antioxidant activity was found in untreated control. The greatest phenolic content was 70 mg GAE/g of praxelis extract in concentration at 100% followed by 40.7, 22.3, and 11.2 mg GAE/g in the 50%, 25%, and 12.5% concentrations, respectively. The greatest flavonoid content was 263.9 mg API/ 100 g of praxelis extract in concentration at 100% followed by 123.3, 70.4, and 61.5 mg API/100 g in the 50%, 25%, and 12.5% concentrations, respectively. The greatest antioxidant activity was 184.8 mg trolox/ 100 g of praxelis extract in concentration at 100% followed by 133.4, 109.3, and 77.3 mg trolox/100 g in the 50%, 25%, and 12.5% concentrations, respectively.

The greater the praxelis concentration, the higher the amounts of total phenolic, total flavonoid, and antioxidant activity were present. Total phenolic content in praxelis was similar or greater when compared to other allelopathic plants in the Asteraceae family. In hairy beggarticks (*Bidens pilosa* L.), total phenolics in leaves, stems, and roots were 47.6, 76.1, and 30.7 mg GAE/g extract, respectively (Deba, Xuan, Yasuda, & Tawata, 2007). However, the concentration of total phenolic, flavonoids and radical scavenging activity by DPPH method in plant extracts depends on the solvent concentration used in the extract preparation, extraction technique, and other conditions (i.e. extraction time and temperature) (Škrovánková, Mišurcová, & Machů, 2012; Rafińska et al., 2019). Different plant species contain different types of allelochemicals at various concentrations (Alías, Sosa, Escudero, & Chaves, 2006; Cheng & Cheng, 2015).

4.2 Effects of praxelis extract on germination and growth of Chinese cabbage

Effects of praxelis extract on germination and growth of Chinese cabbage differed among various concentrations of praxelis extract (Table 2). Praxelis concentration of 100% completely inhibited seed germination and growth of Chinese cabbage. Praxelis concentrations of 50%, 25%, and 12.5% inhibited seed germination for 75%, 53%, and 8%, respectively, compared to the untreated control. Praxelis extract concentrations of 25% and 50% inhibited 69% and 100% of radical length and 69% and 100% of radical length, respectively, when compared to the untreated control. Praxelis extracts had the greater effect on Chinese cabbage growth than germination. Germination is less sensitive to secondary metabolites than growth development of seedlings (Ferreira & Aquila, 2000). The inhibitory effects on germination and growth of Chinese cabbage may possibly be related to the presence of allelochemicals including phenolics and flavonoids. Allelopathic toxicity might be due to synergistic effect of the compounds rather than single toxicity (Fagg & Stewart, 1994).

Table 1 Total phenolic content, total flavonoid content, and antioxidant activity derived from DPPH assay at various extract concentrations of *Praxelis clematidea*

Conc. (%*w/v*)[1]	Total phenolic content[2] (mg GAE/g of extract)	Total flavonoid content (mg API/100 g of extract)	DPPH assay (mg trolox/100 g of extract)
12.5	11.2 ± 0.69 d	61.5 ± 0.43 c	77.3 ± 1.73 d
25	22.3 ± 0.94 c	70.4 ± 0.30 c	109.3 ± 6.16 c
50	40.7 ± 0.08 b	123.3 ± 2.84 b	133.4 ± 1.83 b
100	70.0 ± 0.14 a	263.9 ± 1.25 a	184.8 ± 1.47 a

[1]Conc: concentration of aerial extracts of *P. clematidea* by 50% (*v/v*) aqueous methanol
[2]Means in the same column with the same letters are not significantly different by least significant difference at 95%, means ± SE (standard error).

Table 2 Effects of praxelis extracts on germination and growth of Chinese cabbage

Conc. (%*w/v*)[1]	Germination (seedling/plate)[2]	Hypocotyl length (cm)	Radical length (cm)
control	12.0 ± 0.58 a	1.7 ± 0.09 a	1.3 ± 0.08 a
12.5	11.0 ± 1.15 ab	1.5 ± 0.26 a	1.0 ± 0.15 a
25	5.7 ± 2.19 bc	0.5 ± 0.18 b	0.4 ± 0.16 b
50	3.0 ± 1.00 c	0.1 ± 0.03 b	0.0 ± 0.01 b
100	0.0 ± 0.00 c	0.0 ± 0.00 b	0.0 ± 0.00 b

[1]Conc: concentration of aerial extracts of *P. clematidea* by 50% (*v/v*) aqueous methanol
[2]Means in the same column with the same letters are not significantly different by least significant difference at 95%, means ± SE (standard error)

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4.3 Effects of soil amended with praxelis on growth of lettuce and its allelopathic longevity

Allelochemical effects of praxelis in soil were greater on 3, 5 and 7 days after incorporation (DAI) and decreased after 10, 14, 28, and 35 DAI (Figure 1A). Biomass of lettuce was reduced after transplanting in soil incorporated with praxelis compared to untreated control, except 14 days after soil incorporation with praxelis (Figure 1B). According to toxicity evaluation, allelochemicals from praxelis possibly decomposed when incorporated in the soil for 10, 14, 28, and 35 DAI suggesting that allelopathic potential of soil incorporated with praxelis could last up to 7 days. However, the praxelis amended soil did not affect aboveground biomass of lettuce in similar to the level of toxicity which was confirmed by visual evaluation. This could be because other soil factors such as high organic matter content (3.3%) might have weaken the allelopathic strength. Thepphakhun et al. (2019) showed the toxicity of soil (Uttaradit series with 1.7% organic matter) amended with praxelis lasted up to 7 days after soil incorporation and reported negative correlation between level of toxicity and biomass observations. Matthiessen and Shackleton (2005) reported the toxicity of isothiocynate allelochemical was lower in rich organic matter soils than less organic matter soil, which was presumably because the sorbed allelochemical was not bioactive. Moreover, longevity of the allelopathic effect of soil amendment can depend on type of residues and other soil factors such as reactive mineral surfaces, ion-exchange capacity, inorganic ions, and biotic (Inderjit, 2001). Allelochemicals in some plants have been reported to loss their inhibitory effect on plant growth and their phytoxic effect within 14 to 24 days (Khalid, Ahmad, & Shad, 2002). In other studies of allelochemical longevity in soil, Intanon, Reed, Stevens, Hulting, and Mallory-Smith (2014) reported that allelochemicals in soil amended with meadowfoam (*Limnanthes alba* Hartw. ex Benth) seed meal lasted at least 6 days. Xuan, Tawata, Khanh, and Chung (2005) stated that allelochemicals in soil amended with kava (*Piper methysticum* L.) and alfalfa (*Medicago sativa* L. cv. Rasen) lasted up to 10 days.

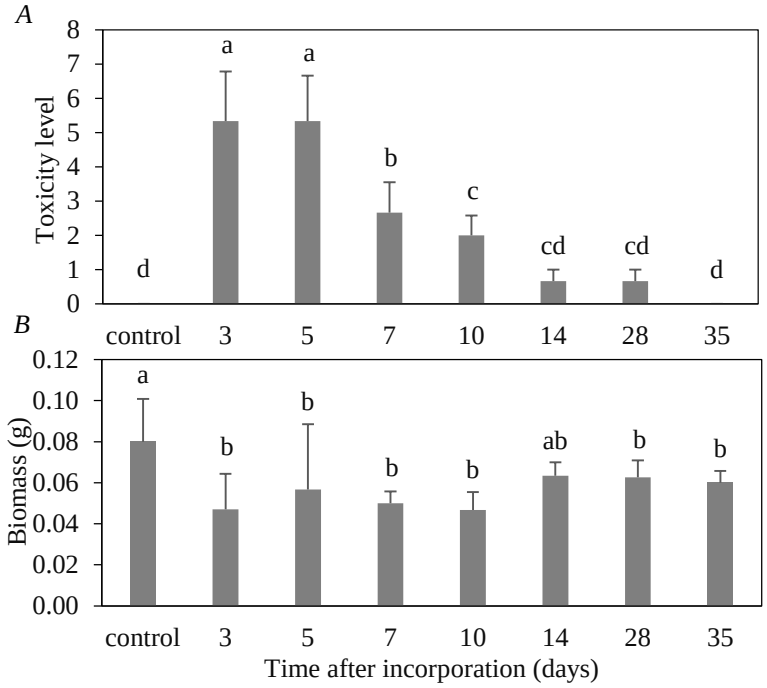

Figure 1 Effects of soil amended with praxelis at various incorporation times on toxicity (A) and dry weight (B) of lettuce. Toxicity 1evel of 0-10; 0 = normal, 1-3 = slightly toxic, 4-6 = moderately toxic, 7-9 = severely toxic, and 10 = killed. Control represents no praxelis amendment as untreated control on day 0. Vertical bars represent SE of the means, $n = 8$. Different letters indicate significant differences (LSD, $P < 0.05$).

5. Conclusion

Praxelis extracts at higher concentrations had greater levels of phytochemicals including phenolics, flavonoids, and antioxidant activity. Praxelis extract concentration of 100% completely inhibited Chinese cabbage germination and growth. The concentrations of 25% and 50% slightly inhibited Chinese cabbage germination but suppressed root and shoot growth. The effects of praxelis allelochemicals in the soil was greater within 7 days after praxelis incorporation. The growers could amend soil with praxelis at 4 kg/m^2 for plant suppression (i.e. weeds) without lettuce injury up to 10 days before transplanting lettuce. Therefore, the results suggest praxelis extracts or praxelis amended soil contained potent allelochemicals which can lead to future use as a weed control option.

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