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The potential of invasive species *Praxelis clematidea* extract as a bioherbicide for *Asystasia gangetica*

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Abstract. *Wardini TH, Afifa IN, Esyanti RR, Astutiningsih NT, Pujisiswanto AH. 2023. The potential of invasive species* Praxelis clematidea *extract as a bioherbicide for* Asystasia gangetica. *Biodiversitas 24:* 4738-4746. The occurrence of weeds in agriculture directly impacts crop productivity, and the reliance on synthetic herbicides to solve the problem has raised concerns about the increasing environmental damage. Using bioherbicides to control weeds is necessary to reduce the negative impact of synthetic herbicides. Bioactive compounds derived from plants can be utilized to develop eco-friendly herbicides. *Praxelis clematidea* extract was reported to exhibit allelopathic activity against weeds. This study aims to evaluate the potential of *P. clematidea* extract as a bioherbicide to control seed germination and seedling growth of *Asystasia gangetica* weed. *P. clematidea* water extract was screened for its chemical compounds and determined its phytotoxic effects on *Asystasia gangetica*. The results showed that *P. clematidea* water extract contained phenolics, alkaloids, and terpenoids. *P. clematidea* extract suppressed seed germination, the time needed for seed germination, sprout length, number of leaves, biomass, and total chlorophyll contents. LC_{50} values for the seed germination, seedling, root, and shoot growth were calculated at 9%, 25%, 11%, and 37% (v/v), respectively. Thus, *P. clematidea* could be used as an *A. gangetica* weed biocontrol agent and might be integrated into weed management programs.

Keywords: *Asystasia gangetica*, bioassay, *Praxelis clematidea*, secondary metabolites, toxicity

INTRODUCTION

Palm oil is a leading plantation commodity in Indonesia that contributes significantly to the country's economy. Palm oil and its derivatives are of high economic value as they can be used as raw materials in the food, cosmetic, pharmaceutical, and energy industries. These products are needed daily for the domestic and international community, and palm oil is the nation's highest foreign exchange income (Fauzi et al. 2014; Purnomo et al. 2020). Therefore, good management is needed to maintain the productivity of oil palm plantations.

Palm oil maintenance, including weed control, greatly affects the success of the growth and development of palm oil plants and the quality and quantity of crop yields (Fauzi et al. 2014). Weeds can reduce palm oil yields by up to 80% (Dadang et al. 2020) as they compete with the main crops for resources, such as nutrients, light, water, carbon dioxide, and space (Walia and Walia 2020). Meanwhile, chemical weed control using synthetic herbicides is farmers' main choice due to its effectiveness and efficiency in suppressing weeds, and the costs are relatively lower than other weed management methods (Dadang et al. 2020). However, the inappropriate use of synthetic herbicides, such as intensive application or continuous use of the same type of herbicide, causes environmental pollution (soil, aquatic, and atmosphere) (Morales et al. 2013; Velki et al. 2019), affects human health (Huovinen et al. 2015), weed resistance, residues in plants (Soltys et al. 2013), as well as growth and development disruption in non-target organisms (Filimon et al. 2021). The active ingredient of synthetic herbicides is not degraded easily in the soil, so their chemical structure is not eco-friendly, such as diuron, which can last up to 60 days (Muhamad et al. 2013; Soltys et al. 2013). Although no herbicide residues have been detected in any palm oil products (low category), such as paraquat (Nurulalia et al. 2022), glyphosate, glufosinate (Shinde et al. 2020), and metsulfuron-methyl (Khairuddin et al. 2021), there might be an impact to the cultivated plants (Nurulalia et al. 2022). Moreover, *Asystasia gangetica*, one of the dominant weeds in palm oil plantations, was reported as relatively resistant (sensitive category) to herbicides, which makes it difficult to control (Bilkis et al. 2022). Thus, sustainable weed management strategies based on eco-friendly herbicides are needed to reduce the use of synthetic herbicides in agricultural systems.

Bioherbicides can be an alternative solution to reduce synthetic herbicides in controlling weeds. The development of herbicides based on allelochemicals (secondary metabolites), especially on plants, does not cause the accumulation of compounds in the soil for a long time because its chemical compounds are easily and quickly degraded. Therefore, minimizing or avoiding further damage to human health and ecosystem sustainability is urgently needed. In addition, some allelochemicals are

water-soluble, making them easier to apply without added surfactants, and have a similar mode to synthetic herbicides (Soltys et al. 2013; Kremer 2019). Allelochemicals can be released into the environment through exudates, volatilization, leaching, transformation, or decomposition (Ilyas et al. 2017), thus disrupting various physiological processes in other plants such as photosynthesis, respiration, and hormonal balance (Soltys et al. 2013).

Praxelis clematidea is an invasive plant known as a bioherbicide candidate (Thepphakhun and Intanon 2020). Previous studies have shown that *P. clematidea* extract from plant shoots inhibited seed germination and growth of weeds, such as *Biden pilosa* (Thepphakhun et al. 2019; Intanon et al. 2020) and *Digitaria ciliaris* (Thepphakhun et al. 2019). That is due to the allelochemicals content in its extract, which comprises phenolic (Thepphakhun and Intanon 2020), terpenoid (Wang et al. 2006), and alkaloid groups (Permatasari et al. 2020). *Praxelis* allelochemicals are released into the environment through litter decomposition from plant organs (Thepphakhun et al. 2019); their toxic effects only last about 1 month in the soil (Thepphakhun and Intanon 2020). Therefore, this study aims to evaluate the impact of *P. clematidea* extract on seed germination and seedling growth of *A. gangetica* weed.

MATERIALS AND METHODS

Praxelis clematidea extract preparation

Praxelis clematidea plants were collected from palm oil plantations in Lampung Province, Indonesia. The plants were cleaned, and the shoot (stems, leaves, and flowers) and root (roots and basal stems) parts were separated. The samples were oven-dried at 80°C for 48 hours, ground into a fine powder using an electric grinder, sieved, and stored in sealed containers (Wang et al. 2021). *P. clematidea* extracts were prepared by macerating each fine powder using distilled water at a ratio of 1:10 (b/v) for 24 hours three times (3 x 24 hours), followed by filtering using two pieces of cheesecloth (Intanon et al. 2020; Thepphakhun and Intanon 2020). The filtrates were evaporated with a vacuum rotary evaporator at 40°C to obtain crude extracts for 4 hours/L (Falcão et al. 2013). Stock solution of whole plant extract was at 100% concentration (b/v). The stock was then diluted for the experiment to obtain 25%, 50%, and 75% (v/v) concentrations.

Phytochemical screening

Flavonoids

Two mL of crude extract was put into a test tube, added with 3 mL of methanol, and shaken. The solution was heated using a Bunsen burner and added with 0.1 g of Mg powder and 2 drops of 2N HCl. Then, 2 mL of amyl alcohol was added, shaken vigorously, and allowed to separate. The formation of yellow, red, or orange color indicates that the extract contains flavonoids (Marjoni 2022).

Alkaloids

Two mL of crude extract was put into a test tube, added with 3 mL of distilled water, 3 mL of chloroform, 2 mL of 10% ammonia, and shaken. Then, 2 mL of concentrated H_2SO_4 was added to the solution and shaken again. The organic phase (chloroform solution) was separated from the aqueous phase, while the Dragendorff reagent was added to the chloroform solution. The red or orange precipitate formation on the Dragendorff reagent indicates that the extract contains alkaloids (Marjoni 2022).

Terpenoids

Two mL of crude extract was put into a separatory funnel, added with 5 mL of distilled water and 5 mL of chloroform, then shaken. The chloroform fraction was separated into a porcelain cup, dried, and added with 2-3 drops of Lieberman Burchard reagent. The formation of brown, red, or purple indicates that the extract contains triterpenoids, whereas a blue or green hue indicates the presence of steroids (Oleszek et al. 2008; Marjoni 2022).

Saponins

The crude extract (0.5 mL) was put into a test tube, added with 10 mL of hot water, then shaken vigorously vertically for 1 minute. Stable foam formation indicates the presence of saponins and does not disappear after adding 2N HCl for 15 minutes (Marjoni 2022).

Data analysis

Color change and precipitate formation in each analysis were analyzed using ImageJ-RGB to obtain the mean and mode of RGB values based on color intensity. The mean RGB value represented sample color density, while the RGB mode values provided color codes, which were further converted into RAL codes to represent colors visually through RAL v.1.2.3 (Karapapa and Mcdonagh 2019). Meanwhile, foam formation in each treatment was observed by measuring the height of the foam formed (Marjoni 2022).

Thin Layer Chromatography (TLC)

TLC was used to confirm the secondary metabolite composition in the whole plant. Crude extract from the whole plant was dissolved in distilled water at a 1:100 (v/v) ratio to prepare the extract solution. The extract solution was hydrolyzed by adding ±20 mL of 2N HCl (acidic condition) in a water bath at 50°C for 1 hour. Hydrolysis was stopped by adding ±10 mL of concentrated ammonia (alkaline condition) and then reincubating the mixture in the same conditions. The extract solution was partitioned with chloroform and ethyl acetate solvents. The chloroform and ethyl acetate fractions were evaporated with a rotary evaporator at 40°C to obtain crude extracts. Chloroform and ethyl acetate solvents (with a ratio of 1:5 (g/mL, respectively) were added to the crude extracts (Falcão et al. 2013) to get specific concentrations. A 4 x 1 cm aluminum silica gel TLC plate (Merck Kieselgel 60 GF_{254}) separated the chemical compounds with varying eluent ratios and polarity to obtain the most optimum spot separation.

The TLC chamber was saturated with the eluent for 5 minutes. Each spot on the TLC plate was observed under visible light, 254 and 366 nm UV light, then the Rf value was calculated. The TLC plate was sprayed with the stained reagent to visualize the spots (Schmidt and Cheng 2017). 2% $AlCl_3$ reagent was used to visualize flavonoids, Dragendorff reagent for alkaloids, and Lieberman-Burchard reagent for terpenoids (triterpenoids, steroids, and saponins). The TLC plate was heated using a hair dryer at 100°C until the spots of the flavonoid (Xu et al. 2011) and terpenoid (Oleszek et al. 2008) were visible. The heat released was tested using a digital multitester Dekko DM-148C. However, alkaloids did not require this step (Gibbons 2006).

Seed preparation

Asystasia gangetica seeds were collected from palm oil plantations in Lampung Province, Indonesia. The seeds were soaked in 2.5% NaOCl for one minute to sterilize their surface, then washed three times for three minutes with distilled water (Samedani et al. 2013). Physical scarification was conducted by soaking the seeds in warm water at 40°C and cooling them to room temperature.

The effect of P. climatidea *extract on seed germination of* A. gangetica

A total of 20 *A. gangetica* seeds were placed in a 9-cm diameter petri dish lined with cotton (Samedani et al. 2013), then 2 mL whole plant extract at various concentrations, and 20 mL distilled water was poured on top. Water volume was adjusted to mimic field conditions, in which the seeds are usually planted in wet conditions. The Petri dishes were covered with the lids and stored in a germinator at 28°C at 12 hours light and 12 hours dark photoperiod (Ismail et al. 2015). The seeds were germinated when at least 1 mm radicles appeared from their surface (Samedani et al. 2013). Meanwhile, 2 mL of 3% (b/v) diuron solution and 20 mL of distilled water were added into the positive control planting medium. The negative control planting medium was given only 20 mL of distilled water. The parameters observed were seed germination, mean germination time (MGT), and morphology of *A. gangetica* seeds and sprouts. The observation was conducted daily for 3 weeks.

Seedling growth

Three weeks *A. gangetica* seedlings with 4 leaves were transplanted into 12 x 12 cm pots, stored in a greenhouse (Kumalasari et al. 2020), and acclimatized for 1 week. Each pot was filled with 80 g of planting medium and planted with one seedling. Seedlings were transferred in the morning, and the planting medium was watered before and after planting as much as 30 mL and 20 mL, respectively. Routine watering was done every two days (Taylor 2012) as much as 20 mL, representing a volume that could be applied to the soil before the water started to flow from the bottom hole of the pot (Ismail et al. 2015). After acclimatization, the sprouts were treated with 2 mL of whole plant extract at various concentrations and 20 mL of distilled water on the soil surface. The extract and the growth medium ratio was 1:40 (Thepphakhun et al. 2019). Routine watering was also done as described previously to keep the soil moist. Two mL of diuron solution and 20 mL of distilled water were added to the positive control planting medium, whereas the negative control was only given 20 mL of distilled water. The parameters observed were sprout length, number of leaves, biomass, chlorophyll content, and morphology of *A. gangetica* seedlings. The observation was conducted daily for one week.

Statistical analysis

The data were analyzed using Kruskal Wallis and pairwise comparisons in SPSS Statistics 26. Linear equations and log values for seed and sprout mortality rates were determined based on the probit model, and these were further used to calculate the values of LC_{50} using Microsoft Excel. LC_{50} values represent *P. clematidea* extract concentrations to inhibit 50% of the weed population during seed germination and seedling growth (Sandhiya et al. 2020).

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening showed that extracts from various parts of *P. clematidea* contained the phenolic, alkaloid, and terpenoid groups (Table 1). The root extract had moderate levels of flavonoids, alkaloids, and saponins and a low level of terpenoids. The shoot extract contained high levels of flavonoids and saponins, a medium level of terpenoids, and a low level of alkaloids. The whole plant extract had a high level of terpenoids and moderate levels of flavonoids, alkaloids, and saponins.

The TLC test results confirmed the phytochemical screening of the whole plant extract. The flavonoid and alkaloid profiles are presented in Figures 1 and 2. Flavonoids were found in the ethyl acetate fraction with Rf values of 0.67 (spot 1) and 0.60 (spot 2) using ethyl-acetate/methanol eluent (7:3). The spots appeared yellow-green (spot 1) and light blue (spot 2) under UV light at 254 nm and 366 nm, and turned red (spot 1) and yellow (spot 2) under visible light after spraying with $AlCl_3$ reagent. Alkaloids were found in the chloroform fraction with Rf values of 0.85 (spot 1) and 0.78 (spot 2) using chloroform/methanol eluent (9.5:0.5). The spots appeared dark blue under UV light at 366 nm UV. Spot 1 was purple under UV light at 254 nm and red-purple under visible light, while spot 2 was orange under UV light at 254 nm and orange-red under visible light after spraying with Dragendorff reagent.

Triterpenoids and steroids are presented in Figure 3. Triterpenoid was found in the ethyl acetate fraction with an Rf value of 0.80 using chloroform/methanol eluent (8:2). The spot appeared red-purple under visible light, violet at UV 254 nm, and purple-blue at UV 366 nm after spraying with Liberman-Burchard reagent. The steroid was obtained in the chloroform fraction using chloroform/methanol eluent (9.5:0.5) with an Rf value of 0.20. After spraying with Liberman-Burchard reagent, the spot appeared blue-

green under visible light, light-purple at UV 254 nm, and purple-blue at UV 366 nm.

Triterpene-saponin profiles can be observed in Figure 4. Triterpene-saponin was found in the chloroform fraction using n-hexane/ethyl-acetate eluent (1:1) with an Rf value of 0.20. The spot was purple under visible light, gray at 254 nm UV, and purple-blue at 366 nm UV after spraying with the Liberman-Burchard reagent.

Table 1. Phytochemical screening of *Praxelis clematidea* extracts

Extract source	Compounds tested	Reagent	Visible changes	Result	RGB value
Shoot part	Flavonoid	Mg + HCl 2N	Orange	+++	49.8
	Alkaloid	Dragendorff	Red-orange precipitate	+	82.1
	Terpenoid	Liberman-Burchard	Red-Purple	++	52.6
	Saponin	Hot water	Foam (1.9 cm)		-
		HCl 2N	Foam (1.1 cm)	+++	-
Root part	Flavonoid	Mg + HCl 2N	Orange	++	63.4
	Alkaloid	Dragendorff	Orange precipitate	++	71.4
	Terpenoid	Liberman-Burchard	Bright brown	+	58.3
	Saponin	Hot water	Foam (1.1 cm)		-
		HCl 2N	Foam (0.8 cm)	++	-
Whole plant	Flavonoid	Mg + HCl 2N	Orange	++	61.8
	Alkaloid	Dragendorff	Orange precipitate	++	74.2
	Terpenoid	Liberman-Burchard	Dark red	+++	42.6
	Saponin	Hot water	Foam (1.3 cm)	++	-
		HCl 2N	Foam (0.7 cm)		-

Note: (+++): high; (++): moderate; (+): low; (-): no changes

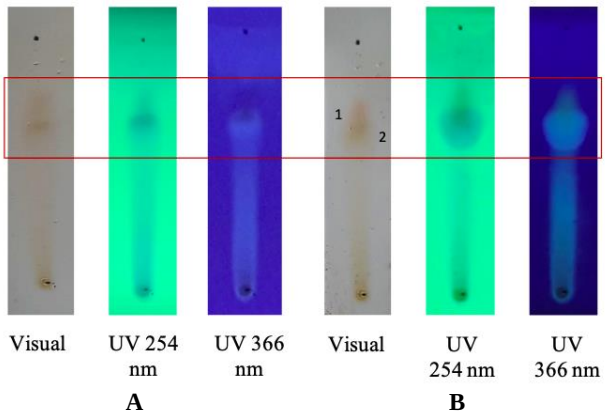


Figure 1. TLC profiles of flavonoids. A. Before spraying $AlCl_3$ reagent. B. After spraying $AlCl_3$ reagent

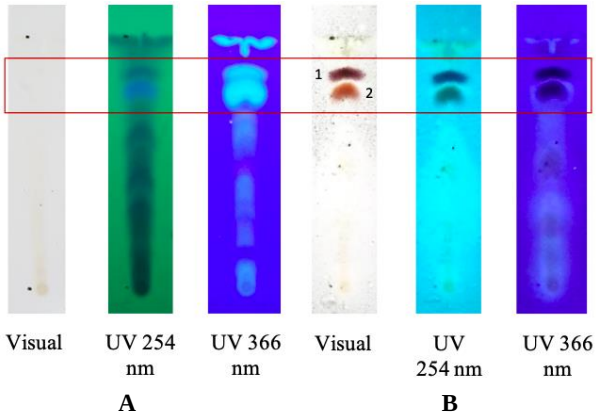


Figure 2. TLC profiles of alkaloids. A. Before Dragendorff reagent. B. After spraying Dragendorff reagent

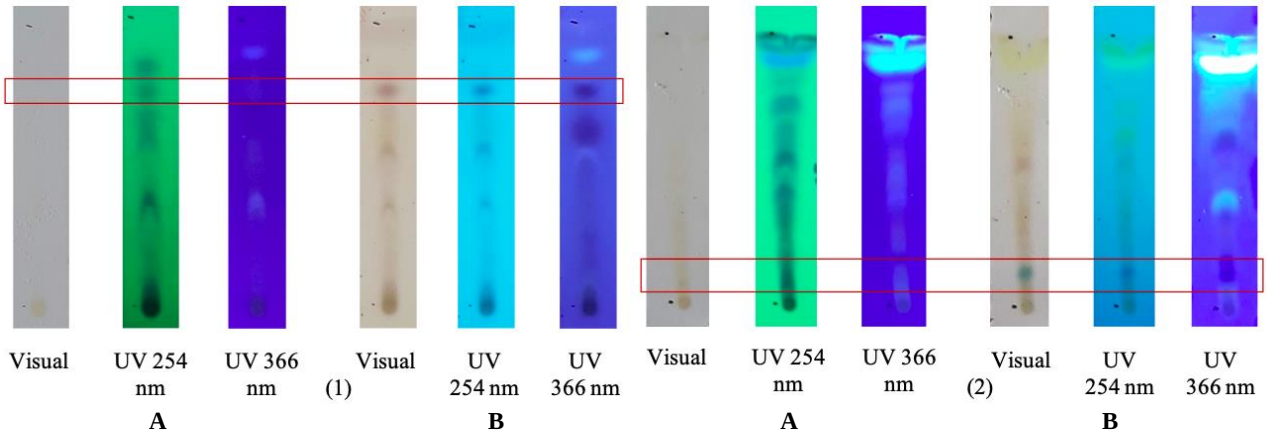


Figure 3. TLC profiles. A. Before Liberman-Burchard reagent. B. After spraying Liberman-Burchard reagent. 1: Triterpenoid, 2. Steroid

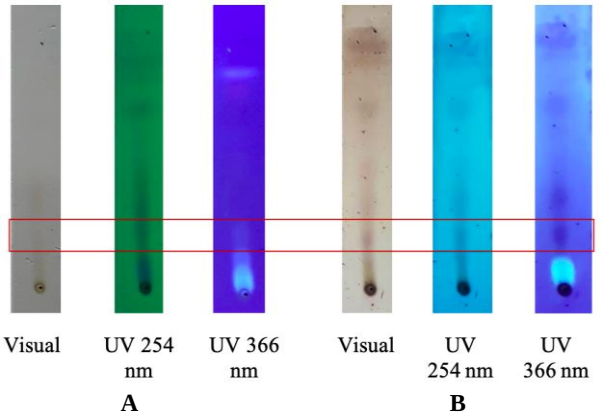


Figure 4. TLC profiles of triterpene-saponin. A. Before Liberman-Burchard reagent. B. After spraying Liberman-Burchard reagent

The effect of *P. clematidea* extract on seed germination and seedling growth of *A. gangetica*

Table 2 shows the effect of *P. clematidea* whole plant extract on seed germination. *P. clematidea* water extracts inhibited germination and prolonged the time for *A. gangetica* seeds to germinate (MGT). The lowest extract concentration (25%) suppressed germination up to 100% and delayed it up to three weeks. Meanwhile, the seed germination in positive control was 37% with an MGT of 11 days. The negative control had the highest seed germination and the fastest MGT.

Praxelis clematidea whole plant extract caused the failure of seeds to germinate and seed death, as indicated by no germination until the end of the observation, and some seeds have been overgrown by fungi on the seed coat (Table 3). In contrast, seeds that did not germinate in positive and negative controls showed no fungal growth. The seeds germinating in the positive control had sprout discoloration and did not have hairs on the radicle. The roots that were initially white became brownish or dark brown. Fresh sprouts in the positive control became wilted and ended up dying, whereas sprouts in the negative control grew well and had hairs on the radicle.

Table 4 shows the effect of *P. clematidea* whole plant extract on seedling growth. The water extract of *P. clematidea* inhibited the shoot, root, and leaf growth of *A. gangetica*, with extract concentrations that had a significant effect starting from 75%, 50%, and 25%, respectively. At the highest concentration (75%), there was still shoot elongation (13%), but the roots did not elongate at 50% and 75% concentrations. The number of leaves decreased with increasing concentration of treatment. Treatment of 50% and 75% concentration had fewer leaves than 25% and negative control. *P. clematidea* extract also reduced the biomass of shoots and roots of *A. gangetica*. The shoot and root biomass decreased with increasing extract concentration. At the treatment of 25% concentration, the shoot and root biomass were reduced significantly compared to the negative control, but it was not different from the positive control. *P. clematidea* extracts reduced the chlorophyll content of *A. gangetica*, which decreased with increasing extract concentration. At the treatment of 75% concentration, chlorophyll content was reduced significantly compared to negative control and 25% concentration. The reduction of chlorophyll in 75% concentration was not different from positive control.

Furthermore, *P. clematidea* whole plant extract caused sprout leaves discoloration (Table 5). Initially, the leaves were green and became yellow-brown. At 50% and 75% concentrations, the leaf edges were wrinkled, wilted, and dried. Morphological changes began to appear after 4th day of extract application.

The effect of *P. clematidea* extract on seed germination, root growth, shoot growth, and seedling growth of *A. gangetica*

Table 6 shows the concentration of extract of *P. clematidea* for suppressing 50% of the seed germination, root growth, shoot growth, and seedling growth of *A. gangetica* were 9%, 11%, 37%, and 25%, respectively.

Discussion

Phenolic, alkaloid, and terpenoid groups were contained in the shoot, root, and whole plant extracts of *P. clematidea*. Results of phytochemical screening were consistent with previous studies that *P. clematidea* plants contained phenolics, i.e., flavonoids (Thepphakhun and Intanon 2020); terpenoids, i.e., sesquiterpenes (Caryophyllene) and monoterpenes (Wang et al. 2006; Araniti et al. 2017; Zhi et al. 2018) and alkaloids, i.e., aurachin A and aurachin P (Permatasari et al. 2020). These secondary metabolites are found in all parts of the plant with various concentrations, but their presence depends on the plant species and environmental factors (Gomes et al. 2017). *P. climatidea* extract had a more significant effect on the inhibition of seed germination and seedling growth. The multi-target activities of *P. clematidea* extract provide better prospects as a pesticide since bioherbicides with various compounds are more desirable since their extracts have multi-target activities (Verdeguer et al. 2020).

The TLC results of *P. clematidea* extracts showed the presence of flavonoids and triterpenoids in the ethyl acetate fraction, while alkaloids, steroids, and saponins were found in the chloroform fraction. Flavonoids are characterized by yellow, orange, or red spots under visible light (Xu et al. 2011) and yellow, green, or blue after spraying with $AlCl_3$ reagent (Šarić et al. 2008). Alkaloids are characterized by yellow-orange, red-brown, or red-purple spots under visible light (Gibbons 2006; Svendsen and Verpoorte 2011) and blue after spraying with Dragendorff reagent (Mursiti et al. 2013). In contrast, terpenoids are characterized by red-purple spots (triterpenoid) or blue-green (steroid) under visible light and green, blue, red, or purple after spraying with Liberman-Burchard reagent (Oleszek et al. 2008). Previous studies reported flavonoids were isolated from *Praxelis* using the ethyl-acetate/methanol eluent (Falcão et al. 2013).

Identifying secondary metabolites in TLC using reagents and UV light only predicts the component groups of secondary metabolites in extracts. It cannot describe the activity or structural characteristics of a compound. Stain reagents were used to visualize color not visible under UV

light or visible light due to a lack of chemical reactivity to the structure of the compound (Gibbons 2006; Oleszek et al. 2008).

Whole plant extracts of *P. clematidea* inhibited seed germination, seedling growth, and leaf discoloration. The previous studies showed that *P. clematidea* extract inhibited the germination and growth of *Biden pilosa* (Intanon et al. 2020), *Brassica pekinensis* (Thepphakhun and Intanon 2020), and *Digitaria ciliaris* (Thepphakhun et al. 2019), followed by sprouts discoloration (Intanon et al. 2020). This study confirms that *P. clematidea* might be used as a bioherbicide controlling *Asystasia* weeds.

Praxelis clematidea whole plant extract also affected mean germination time (MGT) and seed morphology. *P. clematidea* extract delay germination is suspected to be due to the presence of triterpenoids and steroids (Ali et al. 2020), as well as saponins and Caryophyllene (Kocaçahçkan et al. 2009; Araniti et al. 2017), which act as inhibitors of the α- and β-amylase enzyme. These chemical compounds bind to the active site of α-amylase (Huang et al. 2021; Suresh et al. 2021), thereby preventing the enzyme from hydrolyzing starch in cotyledons into glucose, which is required for cell division and elongation during germination (Raven et al. 2017; Jain 2020). Therefore, this action will affect the average time for growing seeds (Pillai et al. 2020). Meanwhile, germination failure and seed death after applying *P. clematidea* extract may be associated with cell membrane damage. It might be due to the saponin content in the extract. Saponins can damage cell membranes by reducing surface tension and increasing cell membrane permeability. That, in turn, can cause loss of function of intracellular enzymes (H+-ATPase) that play a role in regulating ion transport, cell turgor, and osmoregulation, which ultimately triggers cell lysis and death (Böttger et al. 2012; Gomes et al. 2017).

Table 2. The effects of *Praxelis clematidea* extract on *Asystasia gangetica* seed germination

Concentration (%)	Germination (%)	Mean germination time (days)
Water	100+0a	6+0.8a
Diuron	37+20.6a	11+4.4a
Extracts (25%-75%)	0+0b	0+0b

Note: Values represent mean + standard deviation, and different notations [a;b] represent significant differences at a significance level of 5%

Table 3. Seed morphology during early germination

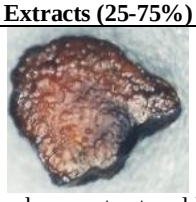
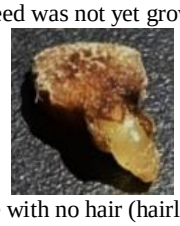
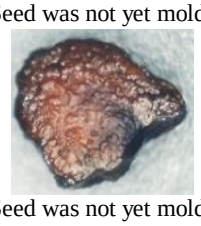
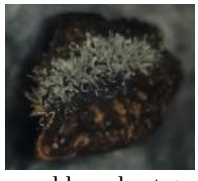
Days after application	Treatments		
	Water	Diuron	Extracts (25-75%)
Day 1st	 Seed testa began to break out	 Seed was not yet grown	 Seed was not yet moldy
Day 3rd	 Radicle with white hair	 Radicle with no hair (hairless) and brownish	 Seed was not yet moldy
Day 6th	 Radicle with white hair	 Radicle hairless and dark brown	 Seed was moldy and not germinated

Table 4. The effects of *Praxelis clematidea* extract on *Asystasia gangetica* seedling growth

Concentration (%)	Number of leaves	Length (%)		Biomass (%)		Reduction of chlorophyll content (%)
		Root	Shoot	Root	Shoot	
Water	6.0+0a	100+0a	100+a	100+0a	100+0a	10.8+5.5a
Diuron	4.0+0b	23.3+43.5b	0+0b	52.5+30.5b	57.6+15.9b	83.5+36.8b
Extract (25%)	4.8+1.1ab	6.7+14.9b	78.7+31.0a	83.5+95.9b	71.5+17.6b	10.1+3.2a
Extract (50%)	4.4+0.9b	0+0b	40.7+38.0ab	45.5+26.7b	67.4+19.6b	34.0+37.2ab
Extract (75%)	4.4+0.9b	0+0b	13.0+19.0b	42.0+20.4b	62.7+11.1b	53.2+43.1b

Note: Values represent mean + standard deviation, and different notations [a;b] represent significant differences at a significance level of 5%

Table 5. Morphology of *Asystasia gangetica* seedling shoot

Days after application	Treatments				
	Water	**Diuron**	**Extract (25%)**	**Extract (50%)**	**Extract (75%)**
Day 4th					
	Leaves were green, and leaf edges were not wrinkled, wilted, and dried	Leaves were yellow-brown, leaf edges were wrinkled and wilted	Leaves were green, and leaf edges were not wrinkled, wilted, and dried	Leaves were yellow-brown, leaf edges were wrinkled and wilted	Leaves were yellow-brown, leaf edges were wrinkled and wilted
Day 7th					
	Leaves were green, and leaf edges were not wrinkled, wilted, and dried	The plant dried up and died	Leaves were green, and leaf edges were not wrinkled, wilted, and dried	The plant dried up and died	The plant dried up and died

Table 6. LC_{50} values of *Praxelis clematidea* extract on *Asystasia gangetica* seed germination and seedling, root, and shoot growth

Bioassay	**Regression**	**LC_{50} value**	
		Extract (%)	**(v/v)**
Seed germination	y = 4.9316x + 0.3748	9	90 mL/L
Root growth	y = 4.7793x + 0.0043	11	110 mL/L
Shoot growth	y = 3.1509x - 0.0518	37	370 mL/L
Seedling growth	y = 3.5278x + 0.0877	25	250 mL/L

Whole plant extract of *P. clematidea* also affected sprout length, number of leaves, biomass, chlorophyll content, and leaf morphology. The mechanism of action of *P. clematidea* extract in suppressing shoot, root, and leaf growth is suspected to be due to flavonoids' role as auxin transport inhibitors (Yin et al. 2014; Abas et al. 2021). Auxin is required to control various physiological processes in plants' growth and development, such as cell division, elongation, enlargement, and differentiation (Majda and Robert 2018; Jain 2020). Inhibition of auxin transport can interfere with auxin redistribution by mimicking the binding form of the PINOID protein kinase (PID) so that the flavonoid compound molecule can bind to the PIN protein (Yin et al. 2014; Abas et al. 2021). The reduction in shoot and root biomass is likely due to alkaloids' role as photosynthetic electron transport inhibitors (Permatasari et al. 2020) by imitating the plastoquinone QB binding protein in photosystem II. The alkaloids bind to protein D1 and prevent electron transfer to photosystem I, which has a role in forming ATP and NADPH. These two forms of energy are required during the photosynthesis reaction (Calvin cycle) (Breton et al. 2007; Harris et al. 2009). Decreased levels of chlorophyll and morphological changes are suspected as a result of secondary effects of alkaloids. Blocking QB triggers ROS and photooxidation stress, damaging the lipid membrane and causing pigment degradation. Antenna pigment deficiency causes morphological changes that indicate poisoning symptoms (Rao 2014; Foyer and Mullineaux 2019).

The roots of *A. gangetica* were more sensitive to *P. clematidea* extract than its shoots. Previous research also showed that the LC_{50} value of the roots was lower than that of the shoots, indicating a higher toxic effect of the extract on the roots (Mergawi and Desoki 2018). It could kill as much as 50% of the plant population at low concentrations (Hartmann et al. 2014). It could be due to the soil application and regulation of the allelochemical supply. Applying *P. clematidea* extract through the soil surface causes the allelochemicals to be absorbed through the roots as the first organs exposed to growth regulatory allelochemicals (Hasan et al. 2021). Therefore, the application of *P. clematidea* extract to reduce 50% of the *A. gangetica* population should be administered through the roots.

In conclusion, the aqueous extract of *P. clematidea* whole plant contained phenolic, alkaloid, and terpenoid groups and significantly inhibited seed germination and seedling growth of *A. gangetica* weed. Terpenoids represented the highest compound content. The inhibition of *P. clematidea* extract on *A. gangetica* seed germination was suspected to be due to the role of terpenoids, and the growth of *A. gangetica* weed was thought to be affected by alkaloids and phenolics. The extract has a high phytotoxic effect on germination and root growth. Thus, *P. clematidea* aqueous extract could be an alternative for developing eco-friendly weed control strategies.

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