Exploring the Potential of Touch-Me-Not (*Mimosa pudica* L.) to Suppress the Germination and Early Growth of Spiny Amaranth

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Abstract. Touch-me-not plant (*Mimosa pudica* L.) is known to be a weed of pantropical distribution and is native to the Caribbean and South and Central America. This weed has been used for herbal medicine. However, little is known for its use to control other weeds. An experiment to study the effect of touch-me-not plant litter on the germination and early growth of spiny amaranth (*Amaranthus spinosus* L.) has been conducted from May to August 2024 at the Plant Physiology Laboratory, Universitas Andalas Padang. Plant extract was prepared through maceration in methanol before phytochemical compound identification. The experiment used a completely randomized design with four concentrations of touch-me-not plant litter (0, 10, 20, and 30%) and four replicates. The seeds of spiny amaranth were germinated for 14 days in Petri dishes. Data were analysed with analysis of variance. Results demonstrated that the touch-me-not plant litter reduced germination and early growth of spiny amaranth. The reduction occurred concentration-dependently. The 30% extract showed the most inhibition. The phytochemicals found were flavonoids, phenolics, saponins, alkaloids, and triterpenoids. Future research should be directed to quantify the amounts of phytochemicals in touch-me-not extract. This finding opens a window to use *Mimosa pudica* as an environmentally friendly bio-herbicide.

1 Introduction

Yield lost in agricultural crop production could not have been completely avoided. The lost resulted from weeds interference in crops due to resource competition [1, 2] and/or allelopathy [3-5]. Crop loss for weed presence have been extensively reported including loss in rice [6, 7], maize [8, 9], soybean [10, 11], and some horticultural crops [12-15]. One of plant species considered as weed is *Mimosa pudica* L. and has different common name such as sensitive plant, touch-me-not, shame-plants, and sleepy grass, and others. This creeping flowering plants, native to South America, are invasive in tropical areas and are found in various terrestrial ecosystems.

Some studies reported touch-me-not plants may result in various adverse effects in animal. Having grazed on a paddock invaded by these plants for three month during the dry season, horses experienced gradual hair loss of the mane and tail. A touch-me-not containing feed administered to mice caused neurodegeneration. Despite of their negative effect to crop plants and some animals, weeds such as touch-me-not plants have demonstrated potential for medicinal plants that protect rats against colitis, prevent or treat diarrhoea, dysentery, diabetes, and urinary tract infection in vivo and in vitro. Touch-me-not and various plant species have been used for medicinal purposes for centuries, mainly for their antioxidant activities, in many countries and have been parts of the local wisdom. The antioxidant activities result from the phytochemical compounds and often become a unique character of the producing plants.

Despite its use as ethnomedicinal plants in many parts of the world, *Mimosa pudica* has been recorded as one of invasive weed species found abundantly in one of active mining site in Nasarawa State, Nigeria. Other weed species found were *Urena lobata* and *Sida linifolia*. This demonstrates that *Mimosa pudica* plays ecological importance and reduced-nutrient soil due to mining. Under the experimental condition, *Mimosa pudica*'s growth responded differently to photoperiod and light spectrum. Growth parameter's such as fresh weight, chlorophyll fluorescence antioxidant, lipid peroxidation, and carbohydrate content. These advantages make Mimosa species among the most suitable choice for ecological restoration. The phytochemical compounds in Mimosa pudica may contribute to its competition and/or allelopathy to other species.

Various phytochemical compounds have been isolated and identified from plants of the *Mimosa* spp. including touch-me-not. The compounds have been studied for their medicinal properties including flavonoids, tannins, phenolics, alkaloids, and saponin. These phytochemical compounds are produced through secondary metabolite pathways and often as a response to changes in the environmental factors. The secondary metabolites are also known as allelochemicals and are responsible for weed interferences in crop plants. Despite their negative effects on other plants, allelochemicals are potential to be used as biocontrol agents. The experiments reported here was aimed at determining the phytochemical compounds of touch-me-not (*Mimosa pudica* L.) plant extract and the effect of touch-me-not plant simplicial on the germination and early growth of spiny amaranth (*Amaranthus spinosus* L.).

2 Materials and methods

2.1 Preparation and assessment of phytochemicals in touch-me-not plants

Plant samples of touch-me-not were collected from around the university dormitory of Universitas Andalas in the City of Padang, West Sumatra, Indonesia (0°53'9.600" S 100°30'10.800" E). The experiment used a descriptive method for phytochemical and antioxidant analysis. Bioassays of spiny amaranth seeds was conducted according to a completely randomised design (CRD) with four concentrations of the touch-me-not simplicial (0, 10, 20, and 30% v/v) and was replicated four times. The experiments were carried out from May to August 2024. The presence of phytochemical compounds from the extract of aerial parts of the touch-me-not plants were determined qualitatively.

The aerial parts of the touch-me-not plants were used for this experiment. The plants were washed in running tap water to clean the soil and derbies then air-dried at room temperature for 12 h. The plants were then cut into pieces of approximately 3 cm long and were put in envelopes prior to being hot-air dried in an oven at 65°C for 12 h until the leaves were crispy. The dried plants were then finely ground, called 'simplicial', stored in air-tight containers, and placed at room temperature for later use.

2.2 Preparation of the plant extract

Plant extract was prepared with maceration in 60% methanol solution. Ten g of simplicial was placed into a 250-mL reagent bottle, then 100 mL of methanol solution was added. The mixture was then macerated for 72 h at room temperature. The bottle was lightly shaken every 24h. After 72 h of maceration process, the solution was filtered through No. 1 Whatman filter paper. The extraction was triplicated, and the aliquots were collected in one bottle and kept at the laboratory for 48 h to allow some parts of the liquid to solidify and sediment. The aliquot was then dried in a rotary vacuum evaporator Buchi® (Heildolph Laborota 4000) at 50°C. The final extract of the plants was dark green and thick and then called "plant extract".

2.3 Phytochemical assay of the plant extract

The presence of phytochemicals in the touch-me-not plant extract was determined as follows:

2.3.1 Flavonoids

The plant extract of touch-me-not was weighed for forty mg and placed into a test tube. Then 0.05 mg of Magnesium and 1 mL of concentrated HCl were added. The mixture was then thoroughly mixed in a vortex for 15 seconds until homogenous. The appearance of red-orange color indicates the presence of flavonoids.

2.3.2 Phenolics

Forty mg of the plant extract was placed into a test tube prior to the addition of ten drops of 10% $FeCl_3$. The mixture was then homogenised in a vortex for 15 seconds. The appearance of a greenish-blue color indicates the presence of phenolic compounds.

2.3.3 Alkaloids

Forty mg of the touch-me-not extract was put into a test tube, then ten drops of concentrated H_2SO_4 were added and followed by thorough mixing. Five drops of Meyer's reagent were added into the mixture. The presence of alkaloids was indicated by the formation of white precipitation.

2.3.4 Saponins

Forty mg of the touch-me-not extract was put into a test tube, then 1 mL of distilled water was added followed by two drops of 0.1 N HCl. The mixture was then vertically shaken for approximately 30 seconds. When the extract form 1-10 cm air bubbles that stable for 10 minutes, the extracts contain saponins.

2.3.5 Terpenoids and Steroids

Forty mg of the touch-me-not extract was put into a test tube, then 0.05 mL acetic acid anhydride and 2 mL of concentrated H_2SO_4 were added to the extract. The mixture was then thoroughly mixed for 15 seconds in a vortex and let settled for 1 minute. The appearance of green, blue, or purple color of the solution indicates the steroids content of the extract. A similar procedure was repeated and the appearance of red or brown color solution confirmed the presence of terpenoids.

2.4 Measuring the antioxidant activity

The antioxidant activity of the touch-me-not plant extract was determined using a DPPH free radical-scavenging assay with ascorbic acid with minor modification. A 0.1 mM of DPPH solution in methanol was prepared and homogenised in a vortex for 30 seconds. The mixture was then kept in a reagent bottle that was tightly wrapped with aluminium foil to prevent light interception. The touch-me-not plant extract was dissolved in distilled water to make a series of solution with the concentration of 100, 200, 300, 400, and 500 ppm. Each solution concentration was taken for 0.2 mL and mixed with 3.8 mL of 0.1 mM DPPH solution prior to thoroughly mixed in a vortex for 30 seconds. The mixture was then incubated in the dark for 30 minutes at ambient temperature.

The mixture of the plant extract with the reagent solution was then ready for a measurement through a UV-VIS Spectrophotometer (Hitachi®) at 517 nm wavelength. A 4 mL of DPPH solution was used as the control sample. The ascorbic acid solution was used as a positive control. The reading and recording of sample solution absorbance via the spectrophotometer was carried out three times. The 50% DPPH inhibitory concentration (IC_{50}) was calculated with linear regression. The IC_{50} value indicates the leaf extract concentration necessary to cause a 50% inhibition of DPPH free radicals. The percentage of inhibition was calculated using the following equation:

$$inhibition\ (\%) = \frac{(control\ absorbance - sample\ absorbance)}{control\ absorbance} \times 100\% \tag{1}$$

2.5 Bioassays of the touch-me-not simplicial on spiny amaranth germination

This part of the experiments was aimed at studying the effect of touch-me-not plant simplicial on the germination and early growth of spiny amaranth. Four concentrations of the simplicial were prepared by dissolving the simplicial in distilled water (0, 10, 20, and 30%; w/v). The experiment used a randomised completely design with four replicates.

The seeds of spiny amaranth were surfaced sterilised by dipping them into 70% ethanol solution for 30 seconds then followed by 5 minutes soaking in 10% of sodium hypochlorite solution. The seeds were then rinsed three times with sterile distilled water. Fifty sterile seeds of spiny amaranth were placed on Whatman No. 1 filter paper in Petri dish (size 100 x 15 mm). Then five mL of the simplicial solution was added. The similar volume of the treatment solution was then added at 7 and 12 days later. The Petri dishes were kept at germinating chamber at ambient temperature. Variable responses for the bioassay are as follow:

2.5.1 Seed growth rate

The germination of spiny amaranth was observed and recorded every 24 h for seven days. All normal seedlings were recorded during the observation period. The seed growth rate of spiny amaranth was calculated using the following formula:

$$\mathrm{SGR} = \frac{\Sigma(\mathrm{d.t})}{\Sigma \mathrm{t}} \tag{2}$$

Notes:
SGR = seed growth rate (%/etmal)
d = % of normal seedling at time of observation
t = time of germination

2.5.2 Germination percentage

The germination percentage of spiny amaranth was observed at day 14 after the onset of the germination. The value of germination percentage was calculated using the following formula:

$$GP = \frac{\sum GS}{\sum SS} \, x \, 100\% \qquad (3)$$

Notes:
GP = germination percentage
GS = number of seeds germinated
SS = total number of seeds sown

2.5.3 Radicle and hypocotyl length, and seedling dry weight

Data on radicle and hypocotyl length, and seedling dry weight was recorded at 14 days after the onset of the germination. The radicle and hypocotyl of spiny amaranth seedlings were measured using cotton thread then converted to a ruler. Following the measurement of the radicle and hypocotyl length, the seedlings were then put into brown paper envelopes and air-dried in an oven for 6 h at 50°C. The dried seedlings were then weighed and recorded as dry weight per Petri dish.

2.6 Data analysis

Data of spiny amaranth bioassay were analysed with an analysis of variance at 5% and mean comparisons were calculated according to Duncan's new multiple range test (DNMRT) at 5%. Data analysed was conducted using Statistical Tools for Agriculture Research (STAR®) software. The phytochemical and antioxidant activity of the touch-me-not plant extract are descriptive data. All data are presented in tables accordingly.

3 Results and discussion

A phytochemical analysis revealed that the plant extract of touch-me-not contained flavonoids, alkaloids, phenolics, saponin, and triterpenoids (Table 1). The absence or presence of phytochemicals in plants is obvious and is affected by various factors including biotic and a-biotic. Our finding is in accordance with the work of who uncovered some advantageous phytochemicals from the touch-me-not plants such as alkaloids, flavonoids, tannins, and saponins.

Table 1. The phytochemical compounds found in the touch-me-not plant extract

Phytochemicals	Reagent	Indicators	Presence
Alkaloids	Dragendorff	Yellow precipitation	+
Flavonoids	Concentrated HCl + Mg	Red or orange solution	+
Phenolics	FeCl 10%	Dark blue solution	+
Saponins	0.1 N HCl	Bubbles formation	+
Steroids	CH3COOH + H2SO4	Blue or green solution	-
Triterpenoids	CH3COOH + H2SO4	Red or orange solution	+

Although this research has not yet quantitatively measured the phytochemicals, their presence demonstrated their negative effects on spiny amaranth seed germination (Table 3). Plants belonging to the Genus Mimosa including *Mimosa pudica* have long been known for their ethno-herbal medicine to cure various diseases such as fever, asthma, and bronchitis for their flavonoid and phenolic content. Yet their allelopathic effects on other weed species has not much been reported. Many plants species, known as weeds in agricultural crop production, have been used by local people as herbal medicines. The presence of phytochemical compounds is responsible for both properties, allelochemicals and antioxidant activities, in the producing plants.

Flavonoids and phenolics, both uncovered through this study, are of the major phytochemicals in plants and have been reported for their inhibition effect on the germination and growth of other neighbouring plants species such as wild mustard [3], lettuce and popping pod, and *Apocynum pictum* and *A. venetum*. These various finding is in accordance with our results that the phytochemicals from touch-me-not plant simplicial reduced germination and seedling growth of spiny amaranth. Alkaloids and saponins have also been reported for their inhibition effects due to an increase in susceptibility to grey mold fungus *Botrytis cinerea*.

The synthesis of phytochemical compounds is regulated by certain gene(s). Flavonoids, for instance, are major pigments that play major roles in coloring most flowers, fruits, and seeds. Phenolic compounds showed an inhibition properties in Fusarium growth and mycotoxin production, inhibition of *Pseudomonas aeruginosa* biofilm [40], and function as antibacterial activity against *Pseudomonas fragi*. Phytochemical compounds are potential to control weed and disease in agricultural crop production to support environmentally-friendly agriculture [3]. This practice opens a window to further exploration of natural products to substitute the commercial chemicals for agriculture.

Touch-me-not plants have been used for herbal medicine in many parts of the globe including Asia, Africa, and Southern America. This experiment found that the antioxidant activity of the touch-me-not plant extract is very weak with the value of IC_{50} 339.52 ppm (Table 2). Although the antioxidant activity under current experimental condition is very weak, the potential of touch-me-not plants as herbal medicine has been widely used.

Table 2. Antioxidant activity of the touch-me-not plant extract

Sample	IC50 value (ppm)	Remarks
Touch-me-not	339.52	Very weak
Ascorbic acid (control)	59.67	strong

Various factors such as plant age, soil properties, environmental condition, and soil water status may affect phytochemical compounds responsible for antioxidant activity. Previous study demonstrated that *Mimosa invisa* grew 200 m from the coastal line had higher IC50 value than that of grew at the riverbank.

The administration of touch-me-not plant simplicial significantly ($P < 0.001$) affected the germination and early growth of spiny amaranth across all tested concentrations when compared to the control treatment group (0% of the touch-me-not simplicial) under the experimental condition (Table 3). A general trend of decreasing germination and early growth with increasing simplicial concentration was observed in all treatments, showing a dose-dependent inhibition. The presence of phytochemicals in touch-me-not plant extract (Table 1) may contribute to allelopathy of this species to other plant species. The methanolic extract of touch-me-not plants inhibited root and shoot growth of lettuce (*Lactuca sativa* L.) and popping rod (*Ruellia tuberosa* L.). The inhibition occurred in a concentration-dependent manner. Root growth inhibition resulted from inhibition in mitosis. Another research found that ethyl acetate extract of the leaf of *Mimosa pigra* inhibited germination and growth of barnyardgrass (*Echinochloa cruss-gallii*) a common weed found in rice plants.

Germination process is initiated by water uptake by the seeds to swell the seeds and soften the seed coat. The water reactivates enzymes needed to breakdown and mobilise the food stored reserves leading to the production of energy needed for the germination.

Table 3. Germination and early growth of spiny amaranth with touch-me-not plant simplicial

Extract (v/v)	Germination (%)	Growth speed (%/etmal)	Radicle length (cm)	Hypocotyl length (cm)	Dry weight (mg)
0%	89.50 a	90.87 a	2.48 a	3.48 a	100 a
10%	70.50 b	67.90 b	1.51 b	3.30 b	50 b
20%	50.50 c	31.80 c	1.20 c	2.21 c	30 c
30%	28.00 d	16.31 d	0.43 d	1.62 d	10 d
CV	9.88%	14.76%	13.80%	9.66%	19.77%

The delayed germination (82% from the highest concentration treatment group compared to 0% control group) and decreased in seedling early growth were positively correlated with the concentration of touch-me-not plant simplicial. The presence of the simplicial contributed to a disruption of germination process as the imbibed water contained plant materials having some phytochemicals (Table 1). The presence of touch-me-not simplicial resulted in 68.71% germination inhibition of spiny amaranth seeds. Phytochemical compounds may affect seed germination in a negative way demonstrated that phenolic compounds showed inhibitory effects on microbial biomass associated with the germination of sphagnum. On the other hand, reported that environmental stress reduced the germination and seedling growth of choy sum. The reduced germination was indicated by the accumulation of proline and soluble protein and upregulated the activities of certain enzymes such as superoxide dismutase and peroxidase. In natural condition, the germination process cannot avoid the effect of other environmental factors. Studied the effect of cold-water imbibition by cotton seeds affected overall germination performances. The cold-water stress significantly increased phosphatidic acid (PA) levels while significantly reduced the content of other membrane lipids such as phosphatidylethanolamine, phosphatidylglycerol, and phosphatidylinositol in the cotton seeds.

The water absorbed by the spiny amaranth seeds, under the experimental condition, contained the dissolved simplicial part entering the seeds. Ideally, the imbibition of water will boost the process of seed germination. However, if the water contained other compounds may negatively affect the germination. For instance, the presence of salt in water as salt stressor, 150 $mmolL^{-1}$ resulted in significant reduction in the radicle length, radicle fresh weight, and total fresh weight. Salt stress had various effects on starch accumulation in cotyledons and radicles.

4 Conclusion

Phytochemicals of flavonoids, phenolics, saponins, alkaloids, and triterpenoids were present in the touch-me-not plant (*Mimosa pudica* L.) extract. The extract showed an antioxidant activity with the IC_{50} value of 339.52 ppm. The touch-me-not plant simplicial reduced the germination and seedling growth of spiny amaranth in concentration-dependent pattern. The 30% simplicial concentration showed the most inhibition. This finding may lead to future research of using phytochemicals from touch-me-not plants as potential bioherbicide. Moreover, naturally decaying touch-me-not plant in the field may act allelopathically to suppress other surrounding weeds. A near future research should be directed to quantify phytochemical compounds in touch-me-not plants.

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