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Antioxidant activity (2-2-diphenil-1-picrilhydrazil radical-scavenging assay) and phytochemicals of *Mimosa invisa* Colla and *Mimosa pigra* L. grown at different terrestrial habitats

Abstract. Giant false sensitive plants (*Mimosa invisa* Colla) and giant sensitive plants (*Mimosa pigra* L.) are invasive plant species in the tropics and native to America. They are widely distributed in different ecosystems and soil types. Apart from being considered woody shrub weeds, these plant species can be used as herbal medicine for their antioxidant activities. An experiment to study the antioxidant activities and phytochemicals of *M. invisa* and *M. pigra* grown at 200 m from the coastline and close to the riverbank (riparian abandoned land) was carried out from March to November 2023. Weed leaves were collected as purposive randomized sampling from different terrestrial habitats in the City of Padang, West Sumatra. Antioxidant activity was identified according to a 2-2-diphenyl-1-picrilhydrazil (DPPH) radical-scavenging assay, and phytochemical compounds were identified qualitatively. Results demonstrate that *M. invisa* had mild antioxidant activity and *M. pigra* had strong antioxidant activity. The IC_{50} values for *M. invisa* grown at 200 m from the coastline and at the riverbank were 121.3 ± 11.5 and 105.6 ± 2.90 ppm, respectively. The IC_{50} values of *M. pigra* grown at 200 m from the coastline and at the riverbank were 80.6 ± 15.9 and 85.1 ± 9.03 ppm, respectively. Phenolic, saponin, and steroid were detected in all weeds grown at different habitats. However, alkaloids and terpenoids were not detected. Interestingly, flavonoid was not detected in the leaves of *M. pigra* grown at the riverbank.

Keywords: Antioxidant · DPPH · Mimosa · Phytochemicals

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Introduction

The presence of weeds in agricultural lands has attracted serious attention and requires precautionary measures. Global agricultural production systems need to ensure feeding the ever-growing population (Bana et al., 2020; Cheriere et al., 2020; Dossou-Yovo and Saito, 2021; Kumar and Jagannath, 2021) , though weeds cannot be avoided. The reduction in crop growth and yield in the presence of weeds may result from resource competition (Dilliott et al., 2022; Landau et al., 2022; Satorre et al., 2020) and/or allelopathy (Mayerová et al., 2018; Sheldon et al., 2021).

Giant false sensitive plants (*Mimosa invisa* Colla) and giant sensitive tree (*Mimosa pigra* L.) are invasive plant species in tropical areas and widely distributed in different ecosystems and soil types. These species have a high diversity (Wan & Wang, 2018). Weeds of the members of Genus Mimosa have adverse effects on crops and animals that eat the leaves or fruits of these plants. Research has demonstrated that *M. pudica*-containing feed resulted in neurodegeneration in mice (Anderson et al., 2023). Another study revealed that *M. tenuiflora* has caused dysfunction in the reproductive system in some animals such as goat, cattle, and sheep. The calves of these animals experienced arthrogryposis, scoliosis, lordosis, and malformation in the rib bones (Riet-Correa et al., 2023).

Despite their negative impact on crops and animals, various weed species, including *Mimosa* spp., have demonstrated potential for medicinal plants for their antioxidant activities. Medicinal plants have been used for centuries in many countries, including Indonesia. *M. pigra* has been used for its antimicrobial activities against skin infections, diarrhea, malaria, tuberculosis, and persistent cough (Chinsembu et al., 2019). Another species, *M. malacophylla,* that is easily found in northern Mexico, has been used by local people as an ethnomedicinal plant for its diuretic effect and to treat kidney diseases (Guillén-Meléndez et al., 2022).

Various studies, mainly in pharmacology, on the antioxidant activities of (medicinal) plants have been reported, including those of *Mimosa* spp. Some secondary metabolite compounds have been isolated and identified from the weeds of the Genus Mimosa. For instance, *M. pudica* leaves have flavonoid-O-glycoside (Hawwal et al., 2021), tannin has been isolated from *M. tenuilora* (Hernandez et al., 2021), phenolics, tannin, phlobatannins, alkaloids, and saponin have been extracted from the leaves of *M. pigra* (Koodkaew et al., 2018). These compounds have been studied for their potential to cure human health problems. Another study has revealed the potential of the nanoflowers of *M. pudica* for the bioremediation of hazardous pollutants from industrial wastewater (Sharma & Basu, 2021). These works open the window of seeing weeds from different perspectives and change weeds' negative impacts into benefits.

The biosynthesis of plant secondary metabolites may be modified by some environmental factors such as soil salinity (Sivasamy et al., 2022), light spectrum (Peng et al., 2024), and drought, with the help of arbuscular mycorrhiza (Qian et al., 2024). Plant nutritional status plays a significant role in this biosynthesis, as well. *M. invisa* and *M. pigra* are found in many areas in the City of Padang, the Province of West Sumatra, Indonesia, especially on the riverbank (riparian abandoned land) and in places not so far from the coastline. The study was aimed at determining the antioxidant activities and phytochemical compounds of *Mimosa pigra* and *Mimosa invisa* grown at different terrestrial habitats, i.e, at 200 m from the coastline and the riverbank.

Materials and Methods

The experiment used a descriptive method and was conducted from March to November 2023 at the Laboratory of Plant Physiology, the Faculty of Agriculture, Universitas Andalas Padang, Indonesia. The leaves of *M. invisa* and *M. pudica* were collected from different habitats in the City of Padang following the purposive sampling method. The leaves were collected from three trees and composited within the same species and habitat. The weeds collected were triplicated. All sampling sites are located in the City of Padang with soil type of Ultisol, with more sand found in the coastal area. The average monthly rainfall during the experiment ranged from 44.80 mm to 624.00 mm, and the highest rainfall was recorded in June, which was 624.00 mm. However, plant samples were collected in April with an average monthly rainfall of 275.50 mm (BPS, 2025).

Preparation and measurement of antioxidant activity. The leaf antioxidant activity was measured following the DPPH free radical-

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scavenging assay method (Brand-Williams et al., 1995). This method is the most common for analyzing antioxidant activity (Gulcin & Alwasel, 2023). The leaves of the target plants were hot-air dried in an oven at 65°C for 12 h until the leaves were crispy and the water content reached approximately <10%. The dried leaves were then finely ground, called 'simplicial', stored in air-tight containers, and placed at ambient temperature for later use.

Preparation of the leaf extract for determining the phytochemical compounds and antioxidant activity. Leaf extraction was prepared with maceration in a methanol solution. Five g of simplicial was placed into a 125-mL reagent bottle, then 50 mL of methanol solution (60% methanol + water in 1:1 v/v) was added and macerated for 24 h at ambient temperature. The bottle was lightly shaken every 8 h. After 24 h of the maceration process, the solution was filtered through No. 1 Whatman filter paper. The extraction was triplicated, and the aliquots were pooled in one bottle and kept for 2 days at the laboratory to allow some parts of the liquid to solidify and sediment. The aliquot was then dried using a Rotary vacuum Evaporator Buchi® at 50°C to get a dark green and thick extract solution called "leaf extract".

Measuring the antioxidant activity. The antioxidant activity was assessed using a DPPH free radical-scavenging assay with ascorbic acid with minor modification (Brand-Williams et al., 1995). A 0.1 mM of DPPH solution was prepared and kept in a reagent bottle, which was tightly wrapped with aluminum foil to inhibit light interception. The leaf extract solution was prepared at 100, 200, 300, 400, and 500 ppm. Each concentration of the extract solution was taken for 0.2 mL and mixed with 3.8 mL of 0.1 mM DPPH solution before being thoroughly mixed in a vortex. The mixture was then incubated in the dark at ambient temperature for 30 min. The reduction in the absorption of DPPH solution following the exposure to an antioxidant was measured in a UV-VIS Spectrophotometer at 517 nm wavelength. A 4 mL of DPPH was used as the control sample. The ascorbic acid solution was used as a positive control. The measurement of each sample was carried out three times. The 50% DPPH inhibitory concentration (IC_{50}) was calculated with linear regression. The IC_{50} value is the leaf extract concentration necessary to inhibit 50% of DPPH free radicals and is determined through the linear regression equation formula. The percentage of inhibition was estimated using the following equation:

$$\text{inhibition (\%)} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \text{ x } 100$$

Assessing the phytochemical compounds (Harborne, 1998)

The presence of phytochemical compounds in the leaf extract were determined qualitatively.

Flavonoid. Forty mg of *M. invisa* or *M. pigra* leaf extract was put 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 until homogeneous. The appearance of red-orange color indicates the presence of flavonoids.

Phenolic. Forty mg of *M. invisa* or *M. pigra* leaf extract was put into a test tube, then added with ten drops of 10% $FeCl_3$, followed by homogenisation with a vortex for 15 seconds. The appearance of a greenish-blue color indicates the presence of phenolic compounds.

Alkaloid. Forty mg of M. *M. invisa* or *M. pigra* leaf extract was put into a test tube, then added with ten drops of concentrated H_2SO_4 followed by thorough mixing. The mixture was then added with five drops of Meyer's reagent. The formation of white precipitation indicates the presence of alkaloids.

Terpenoid and steroid. Forty mg of *M. invisa* or *M. pigra* leaf extract was put into a test tube, then 0.05 mL acetic acid anhydride and 2 mL of concentrated H_2SO_4 were added. The mixture was then thoroughly mixed in a vortex for 15 seconds and then settled for 1 minute. When green, blue, or purple are formed, the extract contains steroids. A similar procedure was repeated, and the presence of red or brown color confirmed the presence of terpenoids.

Saponin. Forty mg of *M. invisa* or *M. pigra* leaf extract was put into a test tube, then added with 1 mL of distilled water, and two drops of concentrated HCl. The mixture was then vertically shaken for approximately 30 seconds. The extract contains saponin when 1-10 cm height air bubbles were formed and stable for 10 minutes.

Data analysis and presentation. The data on antioxidant activity of each *Mimosa* sp grown at different terrestrial habitats were calculated for the mean values ± SD and are presented in a table. The presence or absence of phytochemical compounds is demonstrated in another table.

Results and Discussion

The antioxidant activity of *M. invisa* and *M. pigra* grown in different terrestrial habitats is demonstrated in Table 1. *M. invisa* grown at 200 m from the coastline and riverbank had mild antioxidant activity with IC_{50} values of 121.3 and 105.6 ppm, respectively. In contrast, antioxidant activity was found to be strong in *M. pigra*, with the corresponding IC_{50} values of *M. pigra* being 80.6 and 85.1 ppm, respectively.

Table 1. Antioxidant activity of *Mimosa invisa* and *M. pigra* grown at two terrestrial habitats

Species	Habitat	%inhibition (IC_{50}) (ppm)
Mimosa invisa	200 m from coastline	121.3 ± 11.5
	riverbank	105.6 ± 2.90
Mimosa pigra	200 m from coastline	80.6 ± 15.9
	riverbank	85.1 ± 9.03

Note: Data are presented as mean values ± SD

Plants belonging to the genus Mimosa are known for their ethno-herbal medicine to cure asthma, bronchitis, and fever (Bezerra et al., 2023). Different terrestrial habitats may result in various growth and physiological status of the plants. The plants grown near the coastline may be exposed to different soil nutrient content compared to other habitats. Areas close to the coastline may contain high salinity that affects the growth and production of plants. Salinity stress in soil may cause damage to root systems. Salt stress may also lead to an imbalance in the osmotic pressure, causing a variety of physiological changes (Shabbir et al., 2023), including photosynthetic and respiration rates (Habibi et al., 2021), leading to disturbance in primary or secondary metabolisms in plants.

Various plants have been used as herbal medicine for their free scavenging and antioxidant properties in countries such as *Euphrasiae stricta*, *Euphorbia platyphyllos* L., and *Epimedium brevicomum* Maxim. in Suadia Arabia for their phenolic and flavonoid content (Jafri et al., 2023), *Mimosa pudica* L. in Malaysia for its flavonoid and phenolic content (Baharuddin et al., 2021), and *Mimosa acutistipula* leaves in Brazil for the presence of tannins, flavonoids, phenol, alkaloids, and terpenes (Bezerra et al., 2023). Our finding is in accordance with previous research that *Mimosa* sp. produced various secondary metabolites, including flavonoids, phenolics, steroids, and saponin.

A phytochemical analysis of *M. invisa* and *M. pigra* leaves revealed that alkaloids and terpenoids were not detected in the samples (Table 2). The absence or presence of certain phytochemicals from various plant species is obvious and is affected by biotic such as soil microorganisms (Singh et al., 2021) and/or abiotic factors, such as seasonal temperatures (Mall et al., 2019). Interestingly, alkaloids and terpenoids were absent from the leaf extracts of both Mimosa species grown in different habitats. It is obvious that the synthesis of secondary metabolites in plants is regulated by certain gene(s). Flavonoids, for example, are major pigments that color most flowers, fruits, and seeds. Research in various plant species such as maize, petunia, and snapdragon has been the major experimental model in understanding the flavonoid-regulating gene(s) (Ferreyra et al., 2012). However, little is known about the regulatory genes for major phytochemicals in Mimosa.

Table 2. The Presence of phytochemical compounds of *Mimosa invisa* and *M. pigra* grown at two terrestrial habitats

Species	Habitat	Fla	Phe	Alk	Ter	Ste	Sap
Mimosa invisa	200 m from coastline	+	+	-	-	+	+
	riverbank	+	+	-	-	+	+
Mimosa pigra	200 m from coastline	+	+	-	-	+	+
	riverbank	-	+	-	-	+	+

Note: + : compound detected, - : compound not detected
Fla = Flavonoids, Phe = Phenolics, Alk = Alkaloids, Ter = Terpenoid, Ste = Steroid, Sap = Saponin

The sampling site of this experiment has different soil characteristics, with more sand found in Ultisol from the coastline than the riverbank. Different soil structures result in different responses in plants' production of phytochemicals. In Al-Jubail, Saudi Arabia, researchers found that soil texture affected plant phytochemical contents, including phenols and flavonoids, and most of the soil structures were loamy sand. They reported that different phytochemical content is related to the degree of genetic similarity (Alotaibi and Abd-Elgawad, 2023). Results of our study have also revealed the presence and absence of flavonoids in *Mimosa pigra* grown 200 m from the coastline and the riverbank, respectively.

Flavonoids, a class of natural products, have been applied for a wide range of purposes, including nutrition, pharmacy, and agrochemicals,

including pesticides. These flavonoid compounds have the potential to substitute synthetic chemicals for more environmentally friendly agriculture (Schnarr et al., 2024). The absence of flavonoids from the leaf extract of *M. pigra* grown at the riverbank may be result from the soil condition and other climatic factors. A recent study demonstrated that the concentration of flavonoids in *Phlomoides rotate* increased with altitude in the Tibet Plateau (Li et al., 2024). This finding has confirmed that climatic factors play role in the biosynthesis of secondary metabolites such as flavonoids. The flavonoid of citrus has the potential for biocontrol of pathogenic fungi causing cucumber wilt in seedlings (Wang et al., 2024). Through producing a secondary metabolite, this self-defense mechanism would help nature reduce its dependence on synthetic pesticides.

Phenolic, steroid, and saponin compounds from the leaf extracts were detected in both *Mimosa* sp. grown at different habitats with different soil nutrient content. Another study demonstrated an increase in total leaf phenolic content, flavonoid content, and antioxidant properties of *Echinacea purpurea* L. grown in salt-stress conditions (Ahmadi et al., 2022). The presence of saponin in our experiment is in accordance with another research that reported that saponin content has been reported to increase with low nitrogen supply in *Panax notoginseng* (Cun et al., 2024).

Terpenoids and alkaloids were not detected from the leaf extract in this experiment. The biosynthesis of terpenoid receives carbon supply from the mevalonic acid (MVA) pathway, and the 2-*C*-methyl-D-erythritol-4-phosphate (MEP) pathway, and the phenylalanine-derived phenylpropanoids are a large class (Bergman et al., 2024). Phenylalanine is one of the essential amino acids produced and utilise by plants in biochemical metabolism. Plants need nitrogen to biosynthesize the essential amino acids (Cebani et al., 2024), which can be used for the biosynthesis of secondary metabolites such as terpenoids and alkaloids. The absence of alkaloids and terpenoids from this experiment might be due to a lack of nutrient elements in the soil. The soil where the *Mimosa* sp grew was high in sand, with less in water holding capacity and less in mineral element. Various phytochemicals from the leaf extracts of *Mimosa* sp. correlate with the plants' antioxidant properties. This phenomenon has been reported in quinoa (*Chenopodium quinoa* Willd.) seeds to a certain degree (Yang et al., 2024).

Conclusion

Mimosa invisa and *Mimosa pigra* grown at different terrestrial habitats demonstrated antioxidant activity. *M pigra* had more potent antioxidant activity compared to that of *M. invisa*. Phytochemicals of flavonoids, phenolics, steroid, and saponin were present in both *Mimosa* species. However, alkaloids and terpenoids were not detected in both *Mimosa* species in both habitats.

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