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Evaluation of the Allelopathic Activity of *Albizia procera* (Roxb.) Benth. as a Potential Source of Bioherbicide to Control Weeds

Kawsar Hossen [1,2,3] and Hisashi Kato-Noguchi [1,2,*]

[1] Department of Applied Biological Science, Faculty of Agriculture, Kagawa University, Miki, Kagawa 761-0795, Japan
[2] The United Graduate School of Agricultural Sciences, Ehime University, Matsuyama 790-8566, Japan
[3] Department of Agriculture, Faculty of Science, Noakhali Science and Technology University, Noakhali 3814, Bangladesh
* Correspondence: kato.hisashi@kagawa-u.ac.jp

Abstract: Agricultural production depends heavily on the application of synthetic herbicides. Using these herbicides results in the development of herbicide-resistant weeds, poses hazards to human and animal health, and pollutes the environment. To solve these problems, developing and using bioherbicides must be increased. Although different uses of *Albizia procera* have been well reported, its allelopathic activity against weeds and crop species has not. Hence, we evaluated the allelopathic activity of the *A. procera* plant and isolated its allelopathic compounds. Extracts of *A. procera* significantly suppressed the seedling growth of the tested species (cabbage, alfalfa, lettuce, barnyard grass, timothy, and Italian ryegrass). The seedling growth decreased with increasing extract concentrations. The concentrations required for 50% growth inhibition (I_{50} value) of the tested plants were 0.0225–0.4935 mg/mL. The *A. procera* extracts were separated using different column chromatography, and two active fractions (AP-5 and AP-7) were isolated. Cress seedling growth was completely restricted by fraction AP-5, and fraction AP-7 restricted the cress shoots to 83.10% and roots to 85.65% of the control treatment. The findings of this study indicate that *A. procera* extracts have allelopathic activity and these fractions might contribute to the activity.

Keywords: sada koroi; allelopathic potential; allelopathic compounds; natural herbicides; weed management

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1. Introduction

Albizia procera (Roxb.) Benth. (known as sada koroi in Bangladesh) (Figure 1) from the family Fabaceae (sub-family Mimosaceae) is a quick-growing, relatively large leguminous plant with an open-type canopy. It usually grows to 7.0 to 15.0 m in height, but can reach up to 30 m. *A. procera* is a deciduous tree, losing its leaves in August to September (winter). The leaves of this tree are arranged bi-pinnately with a 10–30 cm rachis. Its light and smooth bark turns reddish as the tree ages. The racemes are 8 to 25 cm long and have yellowish-green sessile flowers. The reddish pods bear 6 to 12 tiny, brownish-green seeds [1,2]. This tree is indigenous to wettish deciduous and semi-evergreen hilly forests along with low-lying savanna woods in the southeast parts of Asia and the northern regions of Australia [3].

A. procera grows well in Nepal, Bangladesh, Pakistan, Vietnam, Thailand, Philippines, China, Indonesia, India (West Bengal, Assam, Nagpur), Burma, Andaman, Kenya, South Africa, and Uganda [4]. It is usually found in different regions of Bangladesh such as the Hill Tracts of Chittagong, Chittagong, Cox's Bazar, Dhaka, Sylhet, Dinajpur, and Mymensingh districts. *A. procera* also grows in the community or village forest areas of Bangladesh [5]. *A. procera* is widely planted in the homestead and road- and riverside areas of Bangladesh under public and private afforestation programs. It grows on flat, undulating, or steep slopes up to 60,000 cm above sea level, where the average annual rainfall is about

250 cm [6]. The tree prefers alluvial soil that has good drainage, but it may also grow in clayey, moderately alkaline, and salty soils. *A. procera* timber is valuable and durable for its interlocking wood used for manufacturing furniture, carts, wheels, boats, agricultural implements, posts, carvings, and boxes, as well as paper pulp for the production of good-quality paper [4]. *A. procera* is a common shading tree in tea gardens and streets [7]. This tree is attractive and grown for ornamental purposes in different regions (sub-tropical and tropical) of the world [8]. The young twigs and leaves are considered a good source of fodder for ruminant animals such as sheep, cattle, goats, deer, and elephants. *A. procera* is considered important in protecting degraded unused land because of its quick growth and nitrogen (N_2)-fixation ability [9], and also helps to improve soil fertility, conservation of water, control of soil erosion, and the environment [10,11]. The *A. procera* tree is also used medicinally to treat cancer [12], convulsions, pain, septicemia, and delirium [13], and to control disorders of the intestine and stomach. The seeds of the *A. procera* tree contain the toxic substance proceranin, used for killing rats and mice [14]. *A. procera* possesses several pharmacological properties including CNS depressants, cardiotonic, hepatoprotective, antioxidant, antidiarrheal, antihypoglycemic, analgesic, spermicidal, anti-inflammatory, hemolytic, antibacterial, anti-HIV, and immunomodulatory properties [15–18].

Figure 1. *Albizia procera* (Roxb.) Benth. tree.

It has been reported that this tree possesses various secondary metabolites such as carbohydrates, alkaloids, flavonoids, saponins, steroids, terpenoids, tannins, total phenol and glycosides. *A. procera* contains different compounds such as 5,2′,4′-trihydroxy-3,7,5′-trimethoxyflavonol-2′-O-β-D-galactopyranosyl- (1→4)-O-β D-glucopyranoside, 4-di-0-methyl-D-galactose, disaccharide, 3-O-(β-Dxylopyranosyl-(1→2)-α-L-arabinopyranosyl-(1→6)-2acetamido-2-deoxy-β- Dglucopyranosyl) echinocystic acid, machaerinic acid, Procera acide, proceraosides A−D, Perceragenin, and aldobiuronic acid [19,20]. A digestibility investigation of the leaves shows they are furnished with 65% acid detergent and 64% neutral fiber, 42% lignin, 4% ash, and 5.5% lipids [20]. Although it has been recorded that *A. procera* is used for different purposes, there is only one report about its allelopathic activity [21]. Perveen et al. [21] experimented with cress only, whereas we conducted our study with different crop and weed species. Therefore, this research was carried out to investigate the phytotoxic potential of the *A. procera* tree.

Weed species are considered the most important cause of the degradation of biodiversity because these species compete with crop species for nutrients and water. In addition, weed species have substantial agronomic influence, resulting in crop yield reductions [22], particularly when farmers must deal with herbicide resistance in weeds. Hence, weeds represent a significant economic cost in different countries, which may result in huge financial losses [23]. Because of this, farmers use a variety of control measures to achieve the highest production, including traditional methods (hand and mechanical weeding), the rotation of crops, and the application of herbicides [24]. The success of these various approaches is primarily influenced by the species of weed and the surrounding circumstances. For instance, it is believed that using contemporary synthetic herbicides is an efficient way to prevent weeds from growing and spreading, hence reducing their effect on the production of food and satisfying the rising demand for food [24]. Herbicides are used extensively and repeatedly by farmers and others responsible for maintaining public and private areas, and their use is gradually rising [25]. However, present studies have revealed that the widespread application of synthetic herbicides poses hazards to human and animal health [26] because some herbicides cause allergic reactions and can result in skin, digestive, neuromuscular, and ophthalmological diseases [27]. A number of herbicides have been identified as endocrine disruptors and likely or potential carcinogens [28]. Furthermore, different weed species are becoming increasingly resistant to synthetic herbicides [29]. It has been reported that 267 weeds throughout the world show resistance to synthetic herbicides [30], such as the common herbicide glyphosate [31]. Because of these factors, creating and applying bioherbicides has attracted the attention of scientists, many of whom have shown the allelopathic effects of natural compounds, primarily secondary substances, obtained from plant species, which use them in competition with harmful species [32].

Allelopathy is a biological process in which one component (organism or plant) releases certain kinds of secondary metabolites that are harmful to the growth, germination, reproduction, and survival of other plants or organisms. Various plant parts such as roots, leaves, stems, flowers, and fruits possess secondary metabolites (allelopathic compounds). These allelopathic compounds affect different physiological and chemical functions of surrounding plants or organisms [33]. The compounds are less harmful to humans and possess fewer negative effects than chemical herbicides, and scientists have been investigating their phytotoxic potential for many years [34]. This phytotoxic activity is regarded as a defensive activity of plant species against different organisms such as weeds [35]. Plant species possess different metabolites or compounds such as acids, alcohols, ketones, lactones, polyacetylenes, fatty acids, phenolics, quinones, cinnamic acid, flavonoids, coumarins, tannins, steroids, and terpenoids [36], which interfere with the growth and germination of weeds. The extracts of some plant species are used to control weeds as well as to reduce the proliferation of weeds without harming cultivated plants [37]. Therefore, plant-originated bioherbicides may be a safe and effective natural way to manage weed growth.

Bioherbicides or natural herbicides are produced from plants or other organisms and applied to manage weeds without harming other parts of the environment [38]. They were made available in the 1980s, but the farmers of Canada, USA, Europe, and Ukraine were the only users of these herbicides [38,39]. Applying bioherbicides in lieu of synthetic herbicides is gaining popularity all over the world. The extracts of plants that are usually used medicinally or nutritionally may also be used for the development of bioherbicides to control weeds in crop fields. Bioherbicides obtained from plant extracts or other organisms (natural sources) have shown significant activity against weed growth. Different plant extracts or compounds have a distinct inhibitory effect against weeds, but have no adverse effect on crops [40]. Bioherbicides typically do not remain active or persist for a long time in the environment, which means they tend not to contaminate water and soil, and do not negatively affect non-targeted components. Thus, bioherbicides produced from plant extracts or allelopathic compounds have very little or no harmful effect on the agroecosystem, or human and animal health [41]. Some allelopathic compounds are water soluble, which make them ready to use without mixing surfactants [36]. The molecular

structure of the compounds is much more complex and environmentally friendly than chemical herbicides. Allelochemicals or bioherbicides have multiple sites of action, which decrease the possibility of resistance to weeds [38]. Therefore, plant extracts or allelopathic compounds extracted from plant species represent promising prospects for producing or developing bioherbicides.

2. Materials and Methods

2.1. Plant Materials

Fresh leaves and twigs of *A. procera* were collected from different locations in the Gazipur district, Dhaka, Bangladesh (23°53′, 24°21′ N and 90°09′, 92°19′ E) during June and July, 2019. The gathered samples were washed and cleaned with running water. The samples were air dried in a shady location, and then ground into powder and stored in the refrigerator at 2 °C until extraction. To evaluate the allelopathic activity of *A. procera*, three dicot plant species (cabbage (*Brassica oleracea* var. *capitata*), alfalfa (*Medicago sativa* L.), and lettuce (*Lactuca sativa* L.)) and three monocot plant species (barnyard grass (*Echinochloa crus-galli* (L.) P. *Beauv*.), timothy (*Phleum pratense* L.), and Italian ryegrass (*Lolium multiflorum* Lam.)) were selected as test plant species. The representative test plants contained both crops and weeds.

2.2. Extract Preparation and Growth Bioassay

To obtain the methanolic extracts of *A. procera* and the bioassay, experiments were carried out by following the methodology described by Hossen et al. [42] with some modifications. Leaf powder (80 g) of *A. procera* was extracted with 600 mL of 70% (*v/v*) aqueous methanol for 48 h and filtered using filter paper (No. 2; Toyo Ltd., Tokyo, Japan). The residues were re-extracted with the same quantity of methanol for 24 h and filtered. The filtrates were combined and evaporated (at 40 °C) using a rotary evaporator to obtain crude extracts. The crude extracts of *A. procera* were dissolved in methanol to prepare six assay concentrations: 0.0187, 0.0562, 0.1875, 0.5625, 1.8750, and 5.6250 mg/mL. These concentrations were applied to a sheet of filter paper (No. 2; Toyo Ltd.) in Petri dishes (28 mm), which were kept in a laminar air flow chamber to dry out the methanol, and then 0.6 mL of a 0.05% (*v/v*) aqueous solution of polyoxyethylenesorbitan monolaurate (Tween 20; Nacalai, Kyoto, Japan) was added to the Petri dishes. Ten pre-germinated seeds of the monocots such as barnyard grass, timothy, and Italian ryegrass (the seeds were soaked with distilled water for 24 h and then allowed to emerge at 25 °C in the dark for 72, 48, and 60 h, respectively) and ten seeds of the dicots (cabbage, alfalfa, and lettuce) were placed on the Petri dishes. A control treatment was set without plant extracts. Finally, all of the Petri dishes were kept in a growth chamber for 48 h in dark conditions (at 25 °C), and the percentage of growth suppression was estimated by comparing with control treatment seedling length.

2.3. Isolation of A. procera Plant Extracts

The extracts of the *A. procera* leaves were accreted to yield an aqueous residue, and the pH was adjusted to 7.0 by adding 1 M phosphate buffer. The residues were partitioned five times with an equal quantity of ethyl acetate to obtain aqueous and ethyl acetate fractions. The ethyl acetate part was evaporated to dryness after soaking overnight with anhydrous Na_2SO_4. Using the aqueous part and the ethyl acetate part, a bioassay study was carried out as mentioned above. The ethyl acetate part (most active) was introduced to a silica gel column (60 g of silica gel 60, 70–230 mesh; Nacalai Tesque, Kyoto, Japan). The column was eluted with different percentages of ethyl acetate in n-hexane from 20% to 80% (amounts increased stepwise), ethyl acetate (150 mL), and two times in methanol (300 mL). From the bioassay result, the fraction (F7) eluted with 80% ethyl acetate showed the highest growth inhibition against cress. This fraction (F7) was subjected to a Sephadex LH-20 column (100 g; GE Healthcare, Uppsala, Sweden) loaded with 20, 40, 60, and 80% (*v/v*) aqueous methanol (150 mL per step) and methanol (300 mL). The highest inhibitory activity was shown by

the fraction (F2) eluted with 40% aqueous methanol, and the extracts were evaporated to dryness. The extract residues were dissolved in 20% (*v/v*) aqueous methanol and loaded onto a reverse-phase C18 cartridge (1.2 × 6.5 cm; YMC, Kyoto, Japan). The cartridge was eluted with 20, 30, 40, 50, 60, and 80% (*v/v*) aqueous methanol (15 mL per step) and methanol (30 mL). The highest inhibitory activity was shown by the fraction (F3) eluted with 40% aqueous methanol, and this residue was purified using reverse-phase HPLC (500 × 10 mm I.D. ODS AQ-325; YMC Ltd., Kyoto, Japan) at a flow rate of 1.5 mL/min with 40% aqueous methanol, and detected at the wavelength of 220 nm.

2.4. Statistical Analysis

The bioassay experiments were conducted using a CRD (completely randomized design), replicated three times and repeated two times (10 seedlings per replication, $n = 60$). The data were analyzed using SPSS software version 20.0 with one-way ANOVA and subsequent post hoc analysis with Tukey's HSD test at $p = 0.05$. The concentrations required for 50% growth inhibition (I_{50} value) of the tested plant species were determined using GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, CA, USA).

3. Results

3.1. Allelopathic Effect of A. procera Plant Extracts

The aqueous methanolic leaf extracts of *A. procera* markedly suppressed the growth of the seedlings of the test species (Figures 2 and 3). Significant growth inhibition of the shoots and roots of the tested species started at 0.0562 mg/mL. At the highest concentration (5.6250 mg/mL), the seedling growth of the test species was completely restricted, except the shoot growth of barnyard grass (94.5%) and the root growth of cabbage (97.3%). Notably, at the concentration of 1.8750 mg/mL, only the root growth of timothy was totally restricted, whereas the shoot and root growth of cabbage was inhibited by 79.7% and 95.5%, alfalfa 87.4% and 94%, lettuce 90% and 97.7%, barnyard grass 80.4% and 97.7%, timothy 98.9% (shoot), and Italian ryegrass 90.4% and 99.1%, respectively, compared with the control treatment. Furthermore, the shoot growth of the test species was inhibited by more than 50% at 0.5625 mg/mL, while the root growth showed the same activity at 0.1875 mg/mL compared with the control.

The I_{50} values of the *A. procera* leaf extracts for the shoot growth of the examined plants varied from 0.0862 to 0.4935 mg/mL (Table 1). On the other hand, for root growth, the I_{50} values varied from 0.0225 to 0.1087 mg/mL (Table 1). The barnyard grass and timothy seedlings showed more sensitivity to the extracts compared with the other test plant species. The timothy shoots and barnyard grass roots were the most sensitive. The I_{50} values also indicated that root growth was more sensitive than shoot growth to the *A. procera* leaf extracts.

3.2. Purification of Allelopathic Compounds from A. procera Extracts

The leaf extracts were isolated by partitioning into the aqueous phase and ethyl acetate phase. From the bioassay experiment, the ethyl acetate phase showed greater activity against cress and was thus chosen for the next purification step using a column of silica gel. In the silica gel column, the extracts were separated into different fractions, and the suppression activity of the isolated fractions was tested using a cress assay. Fraction 7 (F7) exhibited the highest suppression activity (shoot and root growth completely inhibited) against cress (Figure 4). The extracts (fraction 7) were again purified through a column of Sephadex LH-20, and fraction 2 (F2) showed the maximum inhibition compared with the other fractions (Figure 5). The shoot and root growth were limited to 89% and 94% of the control treatment, respectively. Fraction 2 (F2) was again purified through reverse-phase C_{18} cartridges, and finally the active compounds were purified using HPLC (high-performance liquid chromatography). The most active fractions (compounds) were detected at the retention times of 143–150 min (AP-5) and 163–170 min (AP-7) (Figure 6). The cress seedling growth was completely restricted by fraction (compound) AP-5, and fraction (compound)

AP-7 inhibited the cress shoots and roots to 83.10% and 85.65% of the control treatment, respectively. The other fractions (compounds) inhibited the cress seedling growth by less than 20% of the control.

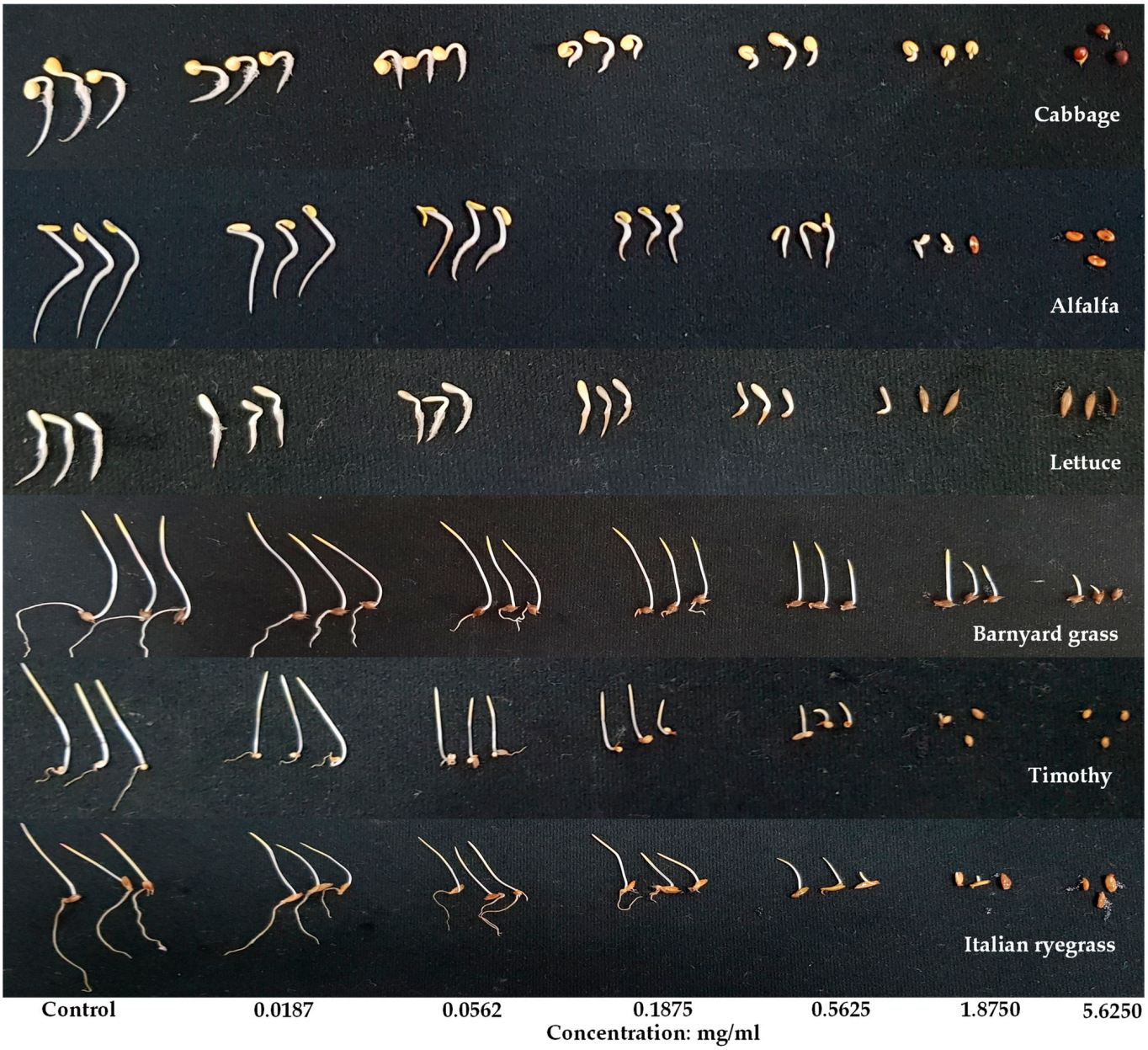

Figure 2. Allelopathic activity of the *A. procera* leaf extracts against the growth of the test plant species.

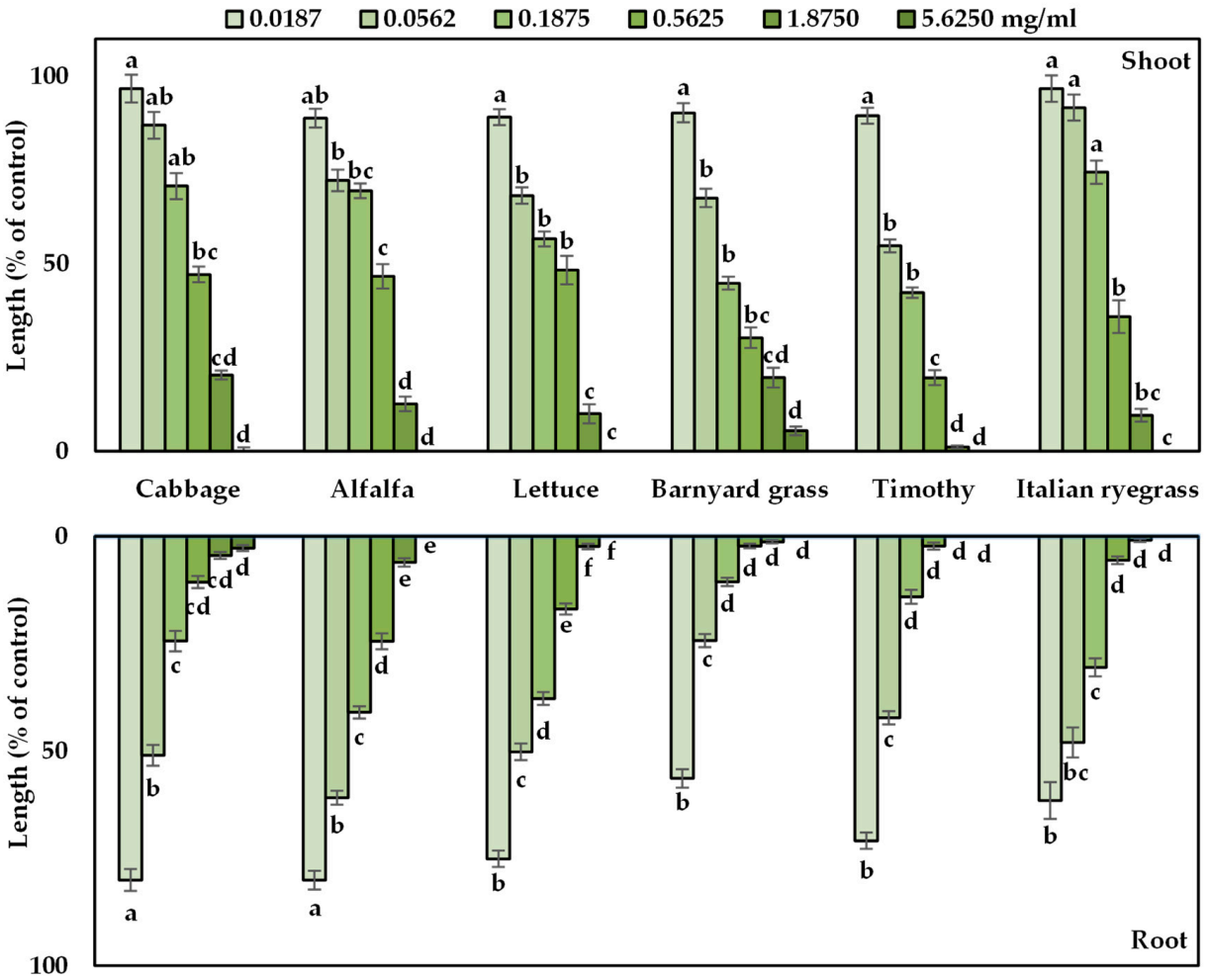

Figure 3. Inhibitory activity of the aqueous methanolic extracts of *A. procera* leaves against the growth of the tested species. The vertical bars on the treatments indicate mean ± SE with six replications ($n = 60$). Significant variations between the control and different treatments are represented by different letters (according to Tukey's HSD test at the 0.05 level of probability).

Table 1. I_{50} values of the aqueous methanolic extracts of *A. procera* leaves against the different test species.

Test Plant Species		I_{50} Value (mg/mL)	
		Shoot **	Root **
Dicot	Cabbage	0.4935	0.0823
	Alfalfa	0.4800	0.1087
	Lettuce	0.4500	0.0562
Monocot	Barnyard grass	0.1425	0.0225
	Timothy	0.0862	0.0412
	Italian ryegrass	0.3765	0.0476

Significant variations between shoot and root of the test species are indicated by ** $p < 0.01$ (paired *t*-test).

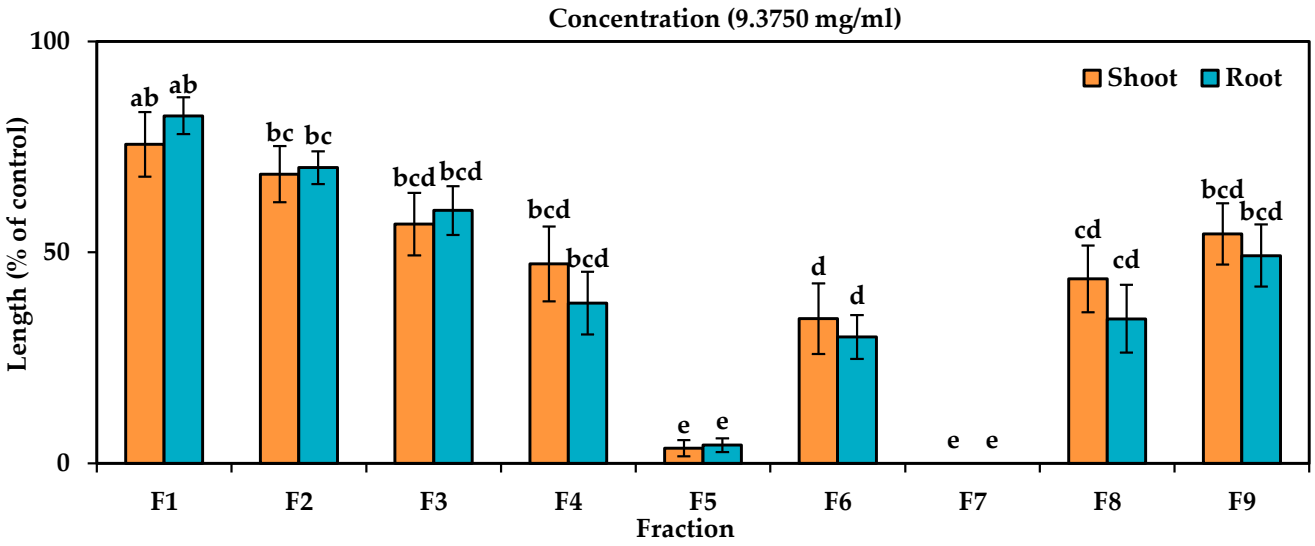

Figure 4. Allelopathic activity against cress growth of the different fractions of the *A. procera* extracts obtained from the column of silica gel. The tested species was treated with the concentration of 9.3750 mg/mL. The vertical bars on the treatments indicate mean ± SE with three replications (n = 60). Significant variations between the control and different treatments are represented by different letters (according to Tukey's HSD test at the 0.05 level of probability).

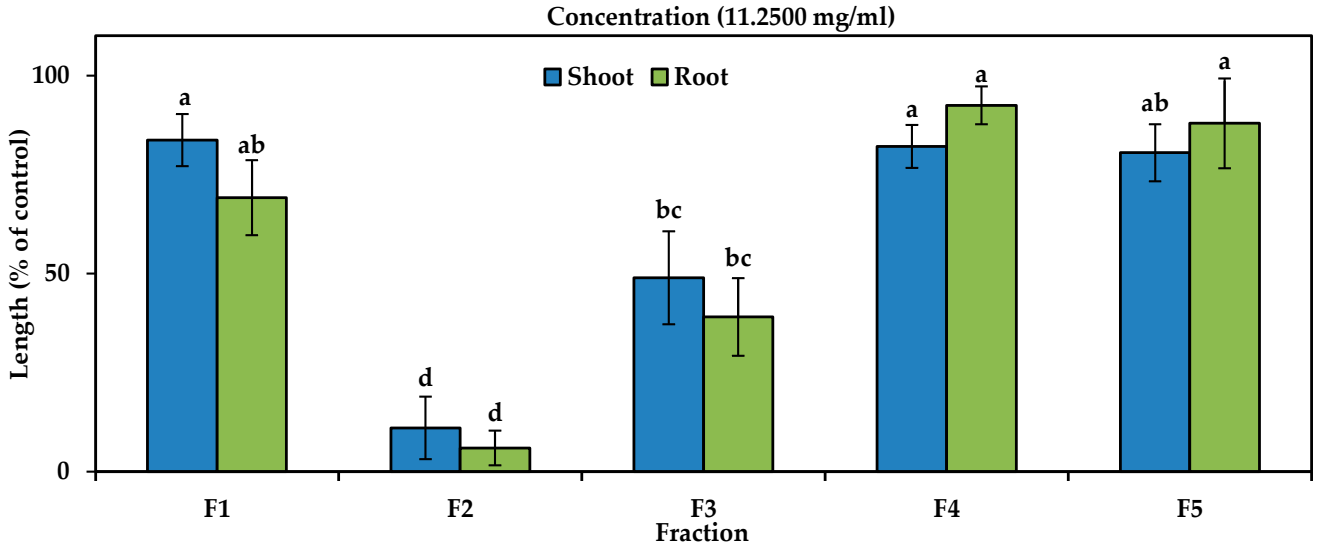

Figure 5. Allelopathic activity against cress growth of the different fractions of the *A. procera* extracts obtained from the column of Sephadex LH-20. The tested species was treated with the concentration of 11.2500 mg/mL. The vertical bars on the treatments indicate mean ± SE with three replications (n = 60). Significant variations between the control and different treatments are represented by different letters (according to Tukey's HSD test at the 0.05 level of probability).

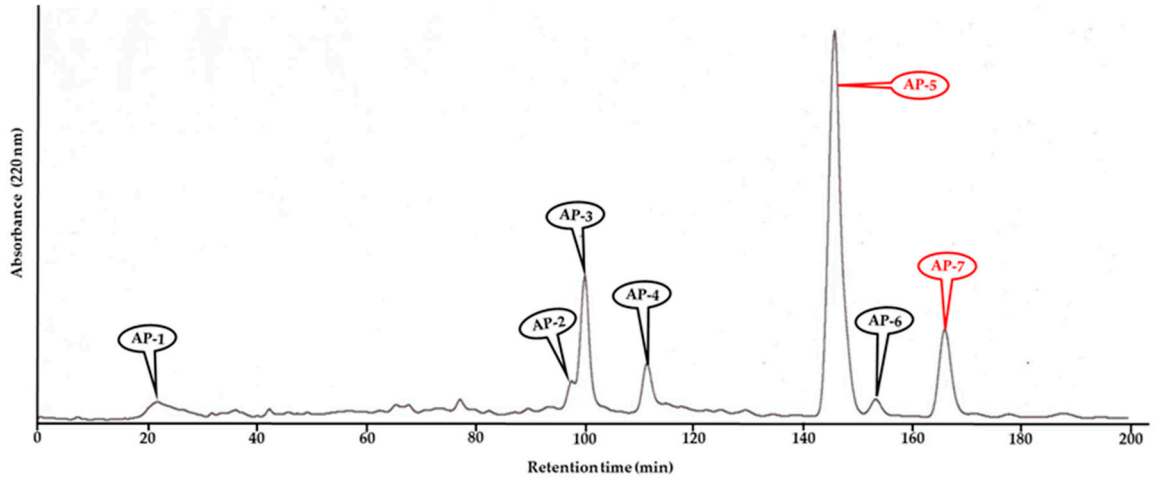

Figure 6. Chromatogram of the compounds obtained from the reverse-phase HPLC (the active compounds are marked in red).

4. Discussion

The *A. procera* leaf extracts significantly limited the growth of the tested species at various growth limitation percentages, as shown in Figure 3. The limitation of growth by the plant extracts increased when the extract concentration increased. Many researchers have reported such concentration-dependent growth limitations among extracts of different plant species against different dicot and monocot test plants, and the results of the present study support those findings [43–47]. The I_{50} values of the extracts against the tested species differed, indicating that the allelopathic effect depended on the species (Table 1). Different plant extracts such as *Fimbristylis dichotoma*, *Cyperus difformis*, *Ipomoea batatas*, *Garcinia pedunculata*, *Dischidia imbricata*, *Cyanotis axillaris*, *Acacia concinna*, *Swietenia mahagoni*, *Ricinus communis*, *Jatropha curcas*, *Leonurus sibiricus*, *Leucas aspera*, and *Ocimum tenuiflorum* showed variations in I_{50} values against different test species. For example, the I_{50} value of *Garcinia pedunculata* extracts on alfalfa was 0.0562 mg/mL for shoot and 0.0937 mg/mL for root, but in the case of *A. procera* plant extracts, the I_{50} values against the same species are 0.4800 and 0.1087 mg/mL. The variations in the susceptibility of the tested plants to plant extracts may result from the various biochemical and physiological characteristics of the plants. Our study findings also support other findings that showed the plant-dependent growth activity of many plant species extracts [48–51]. Moreover, the I_{50} values of the *A. procera* plant extracts showed that root growth inhibition was higher than shoot growth inhibition (Table 1). Various plant extracts such as *Acacia catechu*, *Garcinia pedunculata*, *Swietenia mahagoni*, *Marsilea crenata*, and *Cassia alata* displayed greater inhibition on the test plant root growth than shoots. Root growth showed higher susceptibility to the extracts because of the direct contact of the roots with the extracts or allelopathic compounds, and root cells are more easily penetrated than shoot cells [52,53]. The growth inhibitory activity of the *A. procera* extracts indicates that they might have an allelopathic effect and possess allelopathic compounds. To develop environmentally friendly bioherbicides from plant extracts, it is essential to evaluate the allelopathic activity and to isolate the allelopathic compounds. Hence, this research was carried out to determine the allelopathic activity of *A. procera* plant extract and to identify its allelochemicals.

The bioherbicides or plant extracts can suppress the germination of seeds by preventing the breakdown of nutritional deposits and the division of cells [54]. Bioherbicides restrict seed germination by osmotic effects on the imbibition qualities, which ultimately suppress germination as well as the elongation of cells [55]. The allelopathic activity of the plant part extracts, plant residues, or mulches may influence the germination, growth, and development of weeds. The plant processes (physiological and biochemical) underlying the suppression activity of the allelopathic compounds are essential to determine the mode

or site of the actions and the phytotoxic response of the plants. For instance, bioherbicides inhibit germination by inhibiting the elongation of hypocotyls and radicles, obstructing germination through synthesizing the reactive oxygen species (ROS), and destroying cellular constructions, protein metabolism, and plant hormones [56]. One important growth factor to take into account for plant development and growth is the shoots. In comparison to roots, shoots are typically less susceptive to the allelopathic plant species extracts [57]. The bioherbicides or allelopathic compounds can influence the genes responsible for the cellular depiction of the root endoderm and tissues by limiting their growth. It has been documented that several plant extracts have a direct or indirect influence on the content of chlorophyll. Extracts of M. polymorpha markedly reduce the content of chlorophyll and the photosynthetic agents of recipient plants [58]. The extracts of plant species have an adverse effect on chloroplast integrity and the membranes of thylakoid by inhibiting specific enzymes linked to chlorophyll. Bioherbicides have an impact on how proteins are metabolized in the plants, which causes a two-fold reduction in protein chaining in chlorophyll a or b. By inhibiting the synthesis of chlorophyll, they have an impact on photosynthesis. By lowering OEE1 production, the bioherbicides can influence the nutrition and gas exchange of the weed species [59]. They have the capacity to lower the magnesium accumulation in the weeds and this has a significant impact on the production of chlorophyll [60]. However, the bioherbicides are important for controlling weeds biologically, increasing farmers' earnings, and providing food for an expanding population. Despite recent advancements in the study of bioherbicides, researchers still have a lot of potential to investigate novel approaches and enhance current ones.

The assay-guided purifications of the *A. procera* extracts resulted in the separation of the two most active fractions (compounds) (AP-5 and AP-7) that showed allelopathic activity against cress. Hence, the phytotoxic activity of *A. procera* may suggest that this species can inhibit the seedling growth of the tested species (crop and weed species). This study is the first to document the allelopathic activity of *A. procera* leaf extracts against both crop and weed species and the allelopathic fractions (compounds) in the extracts. However, further research is needed to identify these allelopathic fractions (compounds) from *A. procera.*

5. Conclusions

The present research reported the concentration-dependent and species-specific allelopathic activity of *A. procera* leaf extracts, and the bioassay-guided separation led to the isolation of two active fractions (compounds) (AP-5 and AP-7), possessing very strong allelopathic activity against cress. However, further research is necessary to characterize these two fractions (compounds) and to understand their modes of action. Therefore, the demonstrated allelopathic potential might provide helpful information for the development of plant-based bioherbicides for weed control.

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