Chapter

Phytotoxicity of *Plantago major* Extracts on Germination and Seedling Growth of Purslane (*Portulaca oleracea*)

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Abstract

Plantago major L. (Plantaginaceae family) has been used as herbal remedies for centuries in almost all over the world and in the treatment of a number of diseases. This study aims to assess the allelopathic potential of *Plantago major* extracts on the germination and early seedling growth of purslane. Total phenols, tannins, saponins, flavonoids, and alkaloids were determined in *P. major*. Furthermore, concentrations of 2.5, 5, 10, 20, and 40 mg.ml^{-1} of both alcoholic and aqueous extracts were prepared to study their phytotoxic effect on the germination and seedling growth of *Portulaca oleracea* weed. In our study, showing the germination of *P. oleracea* was completely inhibited (96.30 mg.ml^{-1}) under treatment of *P. major* methanolic extracts at 40 mg ml^{-1}. Moreover, both radicle and plumule were strongly inhibited (87.20 and 74.29 mg.ml^{-1}, respectively) under the same treatment. This could be attributed to the high content of bioactive constituents. Therefore, this species can be used in the method of biological control of weeds. In addition, further studies are required to identify and characterize the proper allelochemicals and demonstrate their modes of action.

Keywords: allelopathy, *Plantago major*, *Portulaca oleracea*, phytochemical

1. Introduction

At present there is a lot of emphasis on finding new methods to fight weeds, and concept of competition between plant species has been improved with that of plant allelopathy [1, 2]. Allelopathy involves the effects of one plant on another because of the chemicals it releases or the breakdown products of their metabolites [3]. There are some examples of plant toxins among the plant secondary compound classes of alkaloids, terpenes, and especially phenolics [4]. Phytotoxicity assays have been reported to be an important approach for identifying plants that are likely to be a source of vital herbivorous compounds [5, 6].

The allelopathic effects of crop plants or crop residues on weeds benefit farmers, which can cause significant economic losses [7]. There is competition for weed crops for moisture, nutrients, space, and light, which negatively affects crop yield [8]. It has been reported that the predominant species of weed allelochemicals stop crop production but sometimes also stimulate seed growth, germination, and crop production [9, 10].

Management methods that reduce the requirement for herbicides are needed to reduce adverse environmental impacts. Herbicides can cause crop injury [11]. Moreover, there is a keen interest in developing alternative methods of natural weed control in organically grown crops [12], as weed control remains one of the most significant agronomic challenges in the production of organic crops. Weed management is often the most troublesome technical problem to be solved in organic farming, especially in poorly competitive crops like vegetables [6, 13]. Cultivation and hand hoeing are common practices used in organically grown leek crops.

Portulaca oleracea L. (purslane) is a common troublesome weed worldwide. Despite being considered a poor competitor, it can quickly establish and easily regenerate by vegetative reproduction method [14]. *Plantago* is the largest genus within the Plantaginaceae family comprising approximately 275 annual and perennial species distributed all over the world [9]. *Plantago major* L. (*Plantago major ssp. major* L.) is a perennial plant that belongs to the Plantaginaceae family and is found in fields, lawns, and on the roadsides. It can become about 10–60 cm high, but the size varies a lot depending on the growth habitats. The leaves grow in rosettes, and they are ovate to elliptical with parallel venation (5–9) [15]. In Asia and Europe, the aerial parts of *P. major* is often used as herbal remedies in the treatment of a number of diseases related to the skin, respiratory and digestive organs, reproduction, and against infections [16].

Phytochemical investigation of the genus revealed the presence of polysaccharides, phenylpropanoid glycosides, alkaloids, triterpenes, flavonoids, and phenolic acids as the main bioactive compounds present in the aerial parts [17–20]. The aim of the present study was to evaluate the allelopathic potential of *Plantago major* extracts on the germination and early seedling growth of purslane.

2. Materials and methods

2.1 Plant material

Plantago major L. was collected from canal banks in Al Anbar city (Iraq) during their vegetative stage (February 2018). The identification of species was done according to Boulos [15]. The plant material was handily cleaned, washed several times with distilled water to remove dust and other residues, dried in room temperature in shaded place for several days till complete dryness and ground into powder, and then preserved in well-stopped bottles [21].

2.2 Phytochemical analysis

Plantago major was collected and prepared as previously mentioned. Total phenolics, flavonoids, and alkaloids were estimated using spectrophotometric techniques adapted by Harborne [22], Sadasivam and Manickam [23], and Boham and Kocipai-Abyazan [24], respectively. Tannins were determined according to Van-Buren and Robinson [25], while saponin content was estimated by the method adopted by Obadoni and Ochuko [26].

2.3 Allelopathy bioassay weed seed source

The seeds of *Portulaca oleracea* were collected from cultivated land from Al Anbar, Iraq. Seeds were sterilized by 0.3% sodium hypochlorite for 3 minutes, washed several times by distilled water, dried at room temperature for 7 days, and reserved in paper bag until further use [27, 28].

2.4 Preparation of extracts

For bioassay tests, aqueous and methanol extracts were prepared to obtain various concentrations of 2.5, 5, 10, 20, and 40 $mg.ml^{-1}$ (w/v). The solutions were filtered through double layers of muslin cloth followed by Whatman No. 1 filter paper. The pH of the mixtures was adjusted to 7 with 1 M HCl, and then mixtures were stored in a refrigerator at 4°C until further use [29].

2.5 Germination bioassay

For germination experiment, 25 seeds were placed in each filter paper in addition to 10 ml of tested extract for each Petri dish (90 mm diameter). The control treatment was designed with distilled water. Germinated seeds were counted daily starting from the first day of treatment. The design of the experiment was randomized complete block with three replicates. The experiment was repeated three times, and the inhibition percentage was calculated.

2.6 Seedling growth bioassay

The seeds of *Portulaca oleracea* were germinated in the dark at room temperature for 2 days. Twenty-five germinated seeds were placed in Petri dishes lined with two layers of filter paper (Whatman No. 1), and 10 ml of different extracts (2.5, 5, 10, 20, and 40 $mg.ml^{-1}$) were added. Moreover, a control treatment was designed with distilled water. The design of the experiment was randomized complete block with three replicates. The experiment was repeated twice, the radicle and plumule lengths of seedlings were measured on the tenth day, and growth inhibition for radicle and plumule lengths were calculated.

3. Results and discussion

3.1 Phytochemical constituents

Several phytotoxic substances causing germination and/or growth inhibitions have been isolated from plant tissues [30, 31]. The phytochemical constituents of *Plantago major* are presented in **Table 1**. *Plantago major* contained high contents of phenolics (132.2 mg/g dry weight) and tannins (28.7 mg/g dry weight), while it contained relatively alkaloids (10.6 mg/g dry weight), saponins (15.8 mg/g dry weight), and flavonoids (14.8 mg/g dry weight).

These results are supported with the study of Kolak et al. [32] and Miser-Salihoglu et al. [33]. In addition, this results relatively comparable to those reported in *Senecio glaucus* as described by El-Amier et al. [34] with the exception of phenols less, but higher than those reported by Kobeasy et al. [35] on same species and

Plant species	Active organic compounds ($mg.g^{-1}$ dry weight)				
	Phenolics	Tannins	Alkaloids	Flavonoids	Saponins
Plantago major	132.2 ± 2.35	28.7 ± 0.89	10.6 ± 0.05	14.8 ± 0.21	15.8 ± 0.06

Table 1.
Concentrations of the active organic compounds estimated in Plantago major.

El-Amier et al. [36] on *Euphorbia terracina* as well as El-Amier and Abdullah [37] on some wild plants (*Calligonum polygonoides*, *Cakile maritima*, and *Senecio glaucus*).

3.2 Allelopathic effect of *P. major* extracts on *P. oleracea* germination

Allelopathy is a phenomenon by which some plants affect the others, either positively or negatively, by exuding chemicals [38]. In the present study, the allelopathic effect of shoot extracts (aqueous and methanol) on the germination percentage of *Portulaca oleracea* at 4 DAT was shown in **Figure 1**. It is observed from the figure that the methanolic extract of *Plantago major* exhibited higher germination inhibition of *Portulaca oleracea* than the aqueous extract. This could be attributed to the methanol polarity that has the ability to extract a wide variety of active components compared to water [39]. The degree of inhibition was significantly increased in a concentration-dependent manner. The aqueous extract of *P. major* at 40 mg ml^{-1} inhibited the germination of *P. oleracea* by about 30.24%, while the lowest concentration (2.5 mg ml^{-1}) inhibited the germination by 4.60%. On the other hand, *P. major* methanolic extract showed a highest inhibition of germination at 40 mg ml^{-1}, while at 2.5 mg ml^{-1}, it exhibited lowest inhibition percentage (20.37%).

Many plant species showed inhibitory effects on *P. oleracea* germination such as *Medicago sativa* and *Vicia cracca* [40], *Salvia macrochlamys* [41], wheat, and rye straw [42]. Aqueous extract of some plant species may contain some toxic substances [43]. These substances probably inhibit the germination and seedling growth of other plant species [44], which was due to their interference with indole acetic acid metabolism, or synthesis of protein and ion uptake by the plants [45].

3.3 Allelopathic effect of *P. major* extracts on *P. oleracea* seedling growth

Allelopathy offers potential for biorational weed control through the production and release of allelochemics from leaves, flowers, seeds, stems, and roots of living or decomposing plant materials. Under appropriate conditions, allelochemics often exhibit selectivity, similar to synthetic herbicides [46].

The allelopathic effect of both aqueous and methanolic extracts on *Portulaca oleracea* radicle growth after 10 days of treatment revealed that there was significant variation between different extracts. However, the degree of inhibition significantly

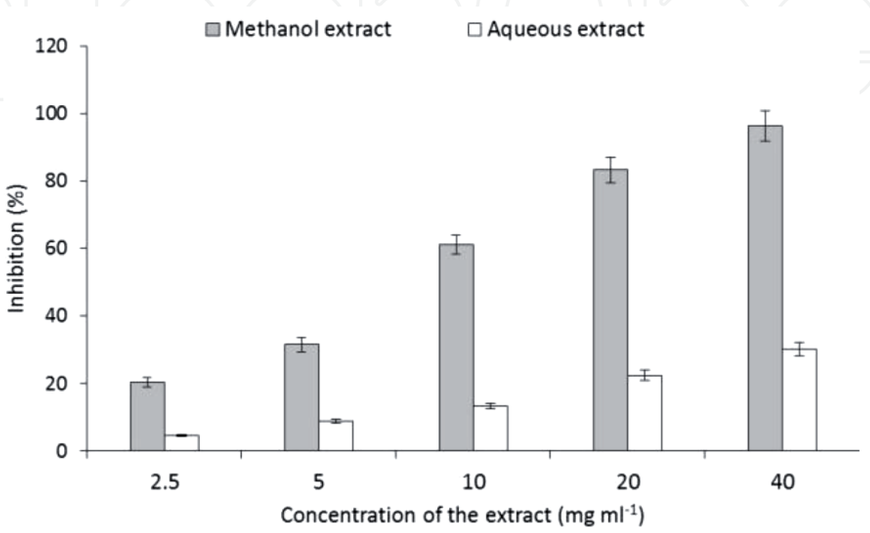

Figure 1.
The allelopathic effect of both aqueous and methanolic Plantago major extracts on the germination inhibition percentage (mean value) with the error bars of Portulaca oleracea 10 days after treatment.

increased in a dose-dependent manner (**Figure 2**). The aqueous extract of *P. major* showed 52.34% at 40 mg ml^{-1}, while it showed the lowest inhibition percentage of radicle growth (3.5%) at 2.5 mg ml^{-1} (**Figure 2**). On the other side, the methanolic extracts from *P. major* at 40 mg ml^{-1} inhibited the radicle growth of *Portulaca oleracea* by 87.20%, while at the lowest concentration (2.5 mg ml^{-1}), *P. major* extract showed the lowest inhibition percentage (19.21%) of radicle growth (**Figure 2**).

The phytotoxic effect of both methanolic and aqueous extracts from the studied *Plantago* species on *Portulaca oleracea* plumule growth revealed slight significant variation between two extracts. However, there was a very large difference between different concentrations (**Figure 3**). The aqueous extract from *P. major* showed the highest inhibition percentage of *Portulaca oleracea* plumule growth (48.69%) at 40 mg ml^{-1}, while at 2.5 mg ml^{-1}, *P. major* extract inhibited the plumule growth by 4.11%. On the other hand, the methanolic extract of *P. major* exhibited high inhibition (74.29%) of *P. oleracea* plumule growth at 40 mg ml^{-1}. The lowest concentration (2.5 mg ml^{-1}) of *P. major* extract inhibited the plumule growth by 60.95% (**Figure 3**). Phytochemical investigation of the genus revealed the presence of

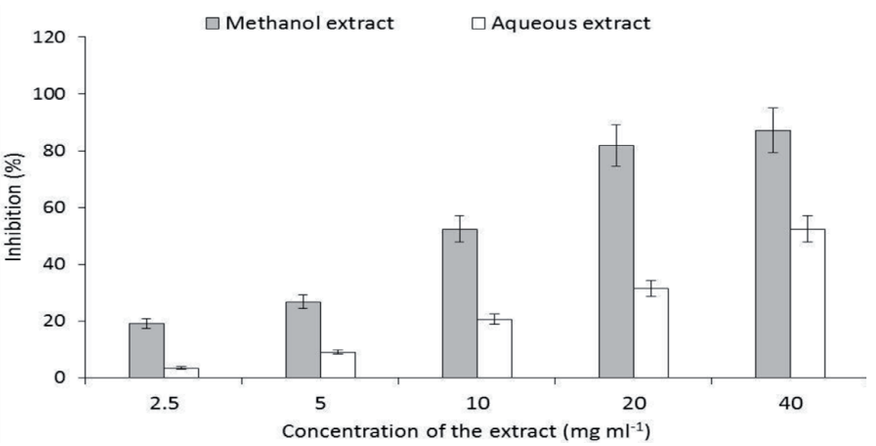

Figure 2.
The allelopathic effect of both aqueous and methanolic Plantago major extracts on the radicle growth inhibition percentage (mean value) with the error bars of Portulaca oleracea 10 days after treatment.

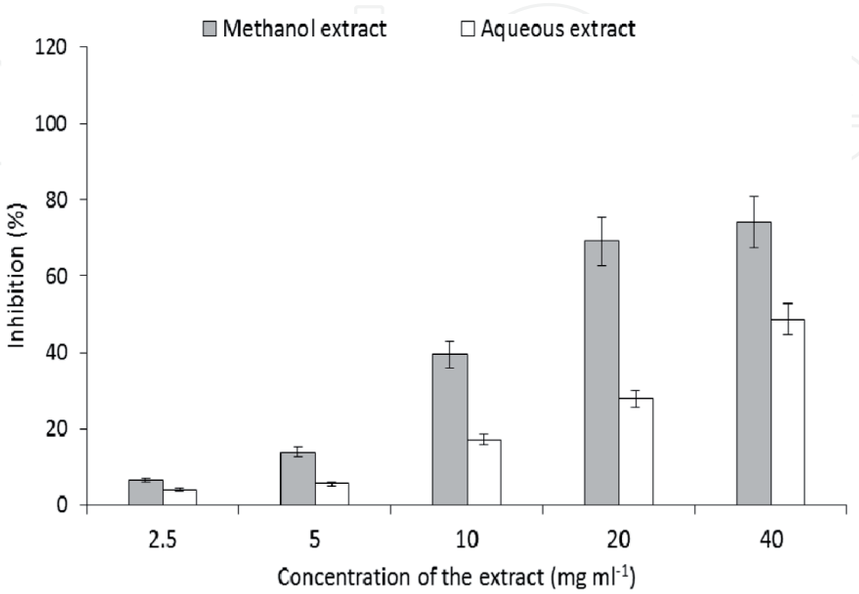

Figure 3.
The allelopathic effect of both aqueous and methanolic Plantago major extracts on the plumule growth inhibition percentage (mean value) with the error bars of Portulaca oleracea 10 days after treatment.

phenylpropanoid glycosides, alkaloids, triterpenes, flavonoids, and phenolic acids as the main bioactive compounds present in the aerial parts [17, 19, 20].

The allelopathic effect of *P. major* could be attributed to several bioactive compounds that act in a synergistic manner or to compounds which regulate one another such as flavonoid, phenolic acids, saponin, alkaloids and tannins. *Plantago* species was reported to contain several bioactive secondary metabolites such as vanillic acid, iridoid glycoside (aucubin), caffeic acid derivatives, chlorogenic acid, ferulic acid, *p*-coumaric acid, and triterpenes (oleanolic acid, ursolic acid) [16, 47, 48]. Many of these compounds were reported as allelochemicals [49]. Generally, the reduction in the seedling growth of *P. oleracea* in this study may be attributed to reduction in cell division of the seedlings, altering the ultrastructure of the cells as well as leading to the alteration of the ion uptake, water balance, phytohormone balance, photosynthesis, respiration, and inactivate several enzymes [50, 51].

4. Conclusion

In conclusion, the aim of this study was to assess the allelopathic potential of *Plantago major* extracts on the germination and early seedling growth of purslane. In our study, showing the germination of *Portulaca oleracea* was completely inhibited under treatment of *P. major* methanolic extracts at 40 mg ml^{-1}. Moreover, both radicle and plumule were strongly inhibited under the same treatment. This could be attributed to the high content of bioactive constituents. Therefore, this species can be used in the method of biological control of weeds. In addition, further studies are required to identify and characterize the proper allelochemicals and demonstrate their modes of action.

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