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Bioactive constituents and allelopathic activities of the invasive weed Ranunculus sceleratus L. Nile Delta, Egypt

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Abstract: *Ranunculus sceleratus* L. (celery-leaved buttercup) is a herbaceous plant grows in moist habitats and is native to temperate and boreal North America and Eurasia. Moreover, it is listed as an invasive weed in northern Africa, Europe and Asia. This study aimed to determine some secondary products in *R. sceleratus* (Shoot and Root system) collected from canal banks of drains (3 stands), Nile Delta, Egypt and to demonstrate their antioxidant and allelopathic potential. Results revealed that, methanolic extract of *R. sceleratus* rich in phenols, saponins and tannins. The antioxidant activity of the *R. sceleratus* has IC_{50} value of 0.37 mg/ml and 0.34 mg/ml for shoot and root, respectively, ml, *Chenopodium. murale* germination / compared to 0.15 mg/ml for catechol. At 400 mg was inhibited by 79.74% and 92.64 for shoot and root extract, respectively, compared to control. However, the shoot growth was reduced by 76.06 % and 87.96 %, with the same sequence. The root growth was more sensitive to the allelopathic effect compared to the shoot, where it was inhibited by 82.68% and 98.67%, respectively, compared to control at the highest concentration. The obtained results on this invasive weed *R. sceleratus* could be a source of eco-friendly bioherbicides against *C. murale* and as a source of antioxidants.

keywords: Ranunculus, Hydrophytes, Phytotoxicity, Antioxidant, Phytochemical, Nile Delta.

1. Introduction

Allelopathy is the biochemical interactions between plants that results from the activity of different phytochemicals synthesized by higher plants. Many plants show pronounced allelopathic activity [1-4] due to their capability to synthesize variable allelochemicals that release into the environment by leaching from leaves, degradation of plant residues, volatilization and root exudation [5] and could influence the life of some surrounding plants and animals [6]. There are several classes of allelochemicals include phenolics, flavonoids, alkaloids, tannins, terpenoids and steroids [7]. Such allelochemicals influence plant growth and development and could be used to reduce weed pathogens and enhance crops yield [8].

Ranunculus genus comprises about 600 species of flowering plants in the buttercups family Ranunculaceae. *Ranunculus sceleratus* L. (celery-leaved buttercup) is a herbaceous plant that grows annually with 20–60 centimeter height, branches frequently, grows in moist habitats and tolerates occasional droughts [9, 10]. The root system of *R. sceleratus* is located in the upper sedimentary layer, about 10-25 centimeter depth. Plant stems are light green, robust and smooth. The aerial parts have an abundance of trichomes [10]. It has a circumpolar distribution in the northern hemisphere, native to temperate and boreal North America and Eurasia. It is listed as an invasive weed in northern Africa, Europe, western and northern Asia [11, 12].

Ranunculus species have been reported to synthesize several allelochemicals like phenolics [13], flavonoids [14,15], alkaloids [15, 16], triterpene saponins [17, 18], fatty acids, organic acids [19, 20] and essential oils [21] that could help human in protection against chronic diseases. It has also been reported that *Ranunculus* species possess anti-inflammatory and antioxidant properties [22, 23], allelochemical [24], antimicrobial, cytotoxic potentiality [25, 26] and pharmacological activities [15, 27].

Despite of the biological activities and bioactive compounds present in *Ranunculus*

species, there is no available knowledge about allelopathic activity of *Ranunculus sceleratus*. This study aimed to determine some secondary metabolites and to investigate their antioxidant and allelopathic activity.

2. Materials and methods

1.Plant material and extraction

Shoot and root system of *Ranunculus sceleratus* L. were collected during vegetative stage in March from canal banks of drains, Nile Delta, Egypt. The species were identified according to Boulos [10]. The samples were air dried then ground into a fine powder using a grinder (IKA®MF 10-Basic Microfine-Grinder Drive, Breisgau, Germany) and stored in paper bags. Voucher specimen was kept in the herbarium of Botany Department, Faculty of Science, Damietta University.

1. Bioactive metabolites

Total phenolics, flavonoids and alkaloids were determined using spectrophotometric techniques adapted by Harborne [28], Sadasivam and Manickam [29], and Boham and Kocipai-Abyazan [30], respectively. Tannins were determined according to Van-Buren and Robinson [31], while saponin content was determined by the method adopted by Obadoni and Ochuko [32].

2.DPPH radical scavenging assay

The extracts radical scavenging activity was determined according to the reaction with a 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) then compared to the standard catechol. Antioxidant activity was determined as described by Lim and Quah [33] where two ml of 0.15 mM DPPH was added to 1 ml of the studied extracts in different concentrations (50 - 400 mg ml^{-1}). The solvent was used instead of the extract to prepare blank. The contents were incubated for 30 minutes in dark, the absorbance (A) was measured at 517 nm. The antioxidant activity was calculated as:

% antioxidant scavenging activity = $[1–(A_{sample}/A_{control})] \times 100$

IC_{50} was calculated as the concentration of the antioxidants of the extract required to decrease initial concentration of DPPH radicals by 50%. The antioxidant activity of catechol was also assayed for comparison.

4.Allelopathic assay

Chenopodium murale seeds were gathered from maize cultivated fields in the north delta coast in Gamasa city, Al-Dakahlia Governorate, Egypt. Seeds were sterilized by 0.3% calcium hypochlorite, rinsed by distilled water and dried again using filter papers at room temperature for 7 days [34].

Concentrations of 50, 100, 200, 300 and 400 mg ml^{-1} extracts were prepared using stock extract of 0.1 g / 100ml. The osmotic concentrations were less than 0.1 Mpa that are not determining factor influencing germination [35]. The pH values were adjusted to 7 using 1M hydrochloric acid, then kept in refrigerator at 4 ºC for any further use [36].

3. Results and Discussion

1.Bioactive metabolites

The concentration of the biologically active phytoconstituents in the shoot and root systems of *R. sceleratus* are presented in Table 1. The methanolic extract (70%) was used for determination of the *R. sceleratus* active ingredients. The concentration of total phenolics in root and shoot (27.54 and 15.33 mg / g dried weight) were higher than those of saponins (16.87 and 15.98 mg g^{-1} dry weight), tannins (12.06 and 8.63 mg / g dried weight), and flavonoids (9.96 and 6.87 mg / g dried weight). However, alkaloids expressed the lowest contents in roots and shoots (3.88 and 2.57 mg / g dried weight).

Table 1: Concentrations of the active secondary metabolites (mg / g dried weight) determined in *Ranunculus sceleratus*

Item	*Ranunculus sceleratus*	
	Shoot	Root
Tannins	12.06±0.63	8.63±0.45
Saponins	16.87±0.89	15.98±0.84
Flavonoids	9.96±0.68	6.87±0.36
Alkaloids	3.88±0.20	2.57±0.14
Total Phenol	27.54±1.45	15.33±0.81

2 Antioxidant activity

1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals were used for evaluation of the antioxidant scavenging activity of the methanolic extracts of the *Ranunculus sceleratus* by measuring the concentration of an antioxidant needed to decrease the initial DPPH

concentration by 50% (IC_{50}). The IC_{50} is inversely proportional to the antioxidant power where the lower the IC_{50}, the higher the antioxidant activity. The evaluation of the antioxidantactivity of the *Ranunculus sceleratus* extract is presented in Table 2. By increasing the plant extract concentration, their scavenging activity increased. In case of shoot and root of *R. sceleratus* extracts the increase was up to 500 µg / ml where the scavenging activity was 54.92% and 57.50% respectively. Moreover, the IC_{50} value of the *R. sceleratus* extract was 0.37 mg / ml and 0.34 mg / ml for shoot and root, respectively, compared to 0.15 mg / ml for catechol. The antioxidant potentiality of *R. sceleratus* was reported by many researchers [25, 37- 39]. The antioxidant activity of *R. sceleratus* may be ascribed to the high content of phenolics [13], flavonoids [15], alkaloids [15], triterpene saponins [17] and essential oils [21]. The obtained results demonstrated that *R. sceleratus* antioxidant activity was higher than the results recorded by Neag *et al*. [39], but lower than those recorded by Shahid *et al*. [38].

Table 2: DPPH radical scavenging activity and IC_{50} values of methanolic extracts of the *Ranunculus sceleratus*

Plant organ	Concentration (µg ml^{-1})	Scavenging activity (%)	IC_{50}(mg ml^{-1})
Shoot	500	54.92±2.48	0.37
	400	51.44±2.39	
	300	48.03±1.30	
	200	40.93±1.11	
	100	34.04±0.92	
	50	19.65±0.53	
Root	500	57.50±2.88	0.34
	400	53.35±3.11	
	300	50.87±2.37	
	200	44.71±1.24	
	100	37.88±1.02	
	50	21.89±0.75	
Catechol asacontrol	500	84.35±2.66	0.15
	400	71.67±3.41	
	300	65.00±1.89	
	200	56.33±1.27	
	100	32.47±0.98	
	50	26.47±0.54	

3.Allelopathic activity

The induced changes in germination and growth of seedlings under the influence of allelochemicals could be demonstrated using cell ultrastructure, molecular biology, in addition to biochemical and physiological characteristics [4, 40]. The allelopathic potentiality of methanolic extracts (70%) from *R. sceleratus* (shoot and root) on germination and seedling growth of *Chenopodium murale* was evaluated using five different concentrations, and the results are presented in Figure 1. The inhibition was concentration dependent; meanwhile, root extracts more inhibition than shoot extract.

At 400 mg / ml, the germination of *C. murale* was inhibited by 79.74% and 92.64 for shoot and root extract, respectively, compared to control. However, the shoot growth was reduced by 76.06 % and 87.96 %, with the same sequence. The root growth was more sensitive to the allelopathic effect compared to the shoot, where it was inhibited by 82.68% and 98.67%, respectively, compared to control at the highest concentration (Figure 1). The obtained data indicated that *C. murale* root growth was increasingly sensitive toward the allelopathic effect caused by *R. sceleratus* than shoot growth (Figure 1) and can be attributed to the permeability of the root membrane as well as the direct touch with the allelochemicals [41- 43]. Moreover, the roots exposed to the extract for longer periods and were the first to emerge [44].

This plant is distinguished by various biological activities such as analgesic, anti-inflammatory activity [25], antioxidant properties [22, 23], allelochemical [24], antimicrobial and cytotoxic activities [25, 26] and pharmacological activities [15, 27]. The obtained results illustrated the potential of *R. sceleratus* allelopathic influence on the targeted *C. murale* weed, that could be related to the high content of phenolics, tannins, flavonoids, saponins, terpenoids, alkaloids and essential oils that have been isolated from *R. sceleratus* [17, 21, 45].

Conclusion

In this study, the crude extract of *Ranunculus sceleratus* expressed more pronounced antioxidant capacity compared to the commercial antioxidant, which may be attributed to their high content of phenolics, tannins and saponins. However, *Ranunculus sceleratus* is considered as an invasive weed of orchards, roadsides and field crops [12], it may

be used in controlling other weedy species through allelopathic application such as *Chenopodium murale*. These results support more studies to be carried for evaluation of the effect of extracts and fractions from *Ranunculus sceleratus* as natural antioxidants and bioherbicides.

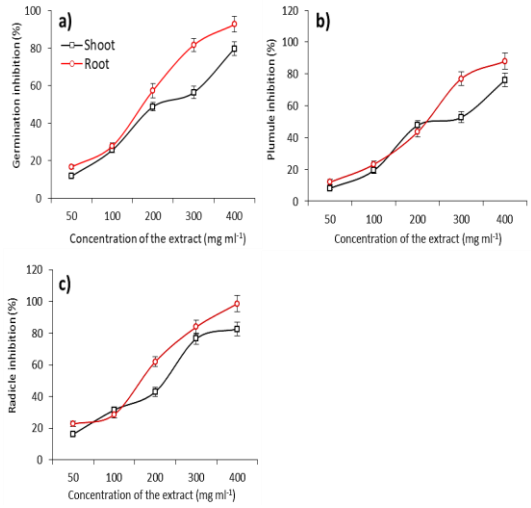

Figure 1: Allelopathic effect of methanolic extract from *Ranunculus sceleratus* shoot and root on a) germination, b) plumule growth and c) radicle growth of *Chenopodium murale*. The bars indicate a standard error, n = 3.

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