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RESEARCH ARTICLE

ANTIOXIDANT ACTIVITY AND ALLELOPATHICPOTENTIAL OF FIVE WILD PLANTS ON GERMINATION AND GROWTH OF *BIDENSPILOSA* L.

***Ahmed M. Abd El-Gawad, Ibrahim A. Mashaly andRasool I. Al-Nafie**

Department of Botany, Faculty of Science, Mansoura University, Mansoura, 35516, Egypt

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ABSTRACT

The present study aims to evaluate the antioxidant activity of *Cakilemaritima*, *Centaureaglomerata*, *Juncusbufonius*, *Lactucaserriola* and *Reichardiatingitana* collected from Egypt, as well as to characterize their allelopathic potential against *Bidenspilosa* L. Total phenolics, tannins, alkaloids, flavonoids and saponins were determined in the studied plants. The antioxidant activity was measured based on the reduction of DPPH. The allelopathic bioassays of germination and growth were assayed. *L. serriola* attained the highest values of phenolics, tannins and alkaloids compared. However, *C. glomerata* exhibited the highest values of flavonoids and saponins. The IC_{50}values of the antioxidant inhibition for *R. tingitana*, *J. bufonius*, *L. serriola*, *C. glomerata* and *C. maritima* were 663.98 ppm, 1026.62 ppm, 1029.45 ppm, 3783.05 ppm and 4964.20 ppm, respectively. The aqueous extracts of *L. serriola* was completely inhibited the germination of *B. pilosa* at 20 mg ml^{-1}. Moreover, the extracts of *C. glomerata*, *R. tingitana*, *C. maritima* and *J. bufonius* inhibited the germination of *B. pilosa* by about 79.80%, 64.65%, 49.49%and 39.39%, respectively.Similar inhibitory effects on radicle and plumule growth were observed. Although, *L. serriola* is commonly considered as a noxious weed, it may be used in controlling *B. pilosa*.

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INTRODUCTION

Natural products from plants are used as curative agents for various diseases since ancient times even although they did not precisely know the chemical composition of the nominated plant to be used for a specific disease. By the time, the development of screening, isolation and identification methods and techniques, has led to a whole new baseline in the medical sciences (Upadhyay and Ahmad, 2012). Secondary metabolites are not useless waste products by plants, but they actions as defense system and/or chemical signal compounds in other plant-plant, plant-animal and plant-microbe relationships (Wink, 2010). Chemical ecology means chemically mediated interactions between organisms and their biotic and abiotic environments (Hartmann, 2008). It includes a broad range of chemical interactions/communications and signaling processes as follows: a) chemical defenses of organisms, b) chemical communication with insects and plant-insect interactions (pheromones, ecdysones and pollination),c) Plant-microorganism interactions (phytoalexins) and d) Plant-plant interactions (allelopathy) (Talapatra and Talapatra, 2015).

Corresponding author: Ahmed M. Abd El-Gawad,
Department of Botany, Faculty of Science, Mansoura University, Mansoura, 35516, Egypt.

Rice (1984) defined allelopathy as the effects of one plant on another plant through the release of a chemical compounds into the environment. Allelopathy can play a beneficial role in agriculture systems. It used as a tool for weed control, pathogen prevention and soil enrichment (Kohli *et al*., 1998). Allelochemicals are non-nutritional secondary metabolites produced by plants that have stimulatory or inhibitory effects upon the growth, health, behavior, or population biology of neighboring organisms (plants, insects and microbes). The inhibition of plant due to allelochemical is more common, where it interfere with plant functions as respiration, photosynthesis, water balance, stomatal function, membrane permeability, cell division, protein synthesis and enzyme activities (El-Shora and Abd El-Gawad, 2014, 2015a, b; Rice, 1984). Allelochemicals are found in many organs such as seeds, flowers, pollen, leaves, stems, and roots.

Allelochemicals release by volatilization, residue decay, leaching, or root exudation (Zeng *et al*., 2008). On the other hand, antioxidants are substances that delay the oxidation process by inhibiting polymerization chains initiated by free radicals and other subsequent oxidizing reactions (Halliwell, 1999). The reactive oxygen species (ROS) have been responsible for about 100 diseases (Ali *et al*., 2001). ROS are generated through the metabolism of various biochemical compounds.

The amounts of ROS present in an organism can be regulated by the enzymatic antioxidant system such as catalase, superoxide dismutase and peroxidase, or by non-enzymatic antioxidants such as phenolics, flavonoids, carotenoids, ascorbic acid, tocopherol, and selenium, which are present in foods and medicinal plants (Atawodi, 2005, Ojo *et al*., 2006). These antioxidant compounds are produced as secondary metabolites and have evolved as the natural means by which plants survive in an aggressive environment. *Bidenspilosa L.* (Asteraceae) is a noxious annual weed native to tropical America. In Africa *B. pilosa* is recorded in many countries and it is likely to occur in all countries. This genus contains about 280 species worldwide and it is widespread in both field crops and wild areas because of its fast growth, strong invasive nature and its easy adaptation (Khanh *et al*., 2009). The present study aims to characterize the antioxidant activity of *Cakilemaritime* Scop., *Centaureaglomerata* Vahl., *Juncusbufonius* L., *Lactucaserriola* L., and *Reichardiatingitana* L. (Roth) as well as to evaluate their allelopathic potential against the nuisance weed *Bidenspilosa* L.

MATERIALS AND METHODS

Preparation of plant materials

C. maritima, *C. glomerata*, *J. bufonius*, *L. serriola* and *R. tingitana* aerial parts were collected during their vegetative stage. The plant aerial parts were washed with distilled water several times and let to dry at room temperature (25 °C) away from direct sun light for several days till complete dryness. The dried samples were ground into fine uniform texture andpreserved in a polyethylene bag in a refrigerator until use.

Phytochemical analysis

Total phenolics, tannins, alkaloids, flavonoids and saponins of *C. maritima*, *C. glomerata*, *J. bufonius*, *L. serriola* and *R. tingitana* samples were determined spectrophotometrically (Bohm and Kocipai-Abyazan, 1994; Obdoni and Ochuko, 2001; Sadasivam and Manickam, 2008; Van Burden and Robinson, 1969).

Determination of antioxidant activity using the 2, 2′-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method

The antioxidant activity of the prepared plant samples was measured in terms of radical scavenging activity, using the stable radical DPPH (Miguel, 2010). A reaction mixtures of 1 ml of a methanolic solution of each plant samples of different concentrations (200, 400, 600, 800 and 1000 ppm) and equal volume of the methanolic solution of 0.3 mM DPPH were prepared, mixed well and incubated in dark condition for 15 min at room temperature (25°C). Catechol was used as standard. The decrease in absorbance at 517 nm was determined using a spectrophotometer (spectronic 21 D model). The IC_{50} (the concentration ofspecimen required to reduce the absorbance of DPPH by 50%) was calculated graphically. The percentage inhibition of the DPPH radical was calculated as following:

$$\text{Inhibition (\%)} = \left(1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}}\right) \times 100$$

Allelopathicbioassay

Weed seed source

The seeds of *B. pilosa* were collected from the orchards habitat in Mansoura City, Al-Dakahlia Governorate, Egypt. Seeds were sterilized by 0.3% sodium hypochlorite for 3 minutes, rinsed several times by distilled water, dried at room temperature for 7 days and reserved in paper bag until further use (Sampietro *et al*., 2009; Uremis *et al*., 2005).

Preparation of plant extract

For bioassay tests, aqueous extracts of each plant samples were prepared to obtain concentrations of 2.5 mg ml^{-1}, 7.5 mg ml^{-1}, 10 mg ml^{-1} and 20 mg ml^{-1} (w/v). The extracts were filtered thought double layers of muslin cloth and by a Whatman No.1 filter paper, the pH values were adjusted to 7 and these were reserved in a refrigerator at 4^{o}C until further use (Rice, 1972).

Germination bioassay

For germination experiment, two layers of filter paper (Whatman No. 1) were placed in 90 mm diameter sterilized Petri dishes. In each dish, 20 seeds were settled and 10 ml of each plant extract was added in a concentration of 2.5 mg ml^{-1}, 7.5 mg ml^{-1}, 10 mg ml^{-1} and 20 mg ml^{-1} (w/v). Thecontrol 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 replicate. The experiment repeated three times and the inhibition percentage was calculated.

Seedling growth bioassay

The seeds of *B.pilosa* were germinated in the dark at room temperature for 2 days. Twenty germinated seeds were placedin Petri dishes lined with two layers of filter paper (Whatman No. 1) and 10 ml of different extractswere added in concentrations of 2.5 mg ml^{-1}, 7.5 mg ml^{-1}, 10 mg ml^{-1} and 20 mg ml^{-1}. Moreover, a control treatment was designed with distilled water. The design of the experiment was randomized complete block with three replicate. The experiment repeated twice, the radicle and plumulelengths of seedlings were measured on a tenth day and growth inhibition for radicle and plumulelengths were calculated.

Statistical analysis

Allvalues of phytochemistry, antioxidant and allelopathy experiments are the mean of three replicates ± standard error. The obtained data were subjected to ANOVA and the mean values were separated according to Ducan's test at 0.05 probability level using COSTAT 6.3 program.

RESULTS AND DISCUSSION

Phytochemical constituents of the studied plant species

The phytochemical constituents of the aerial parts of *C. maritima*, *C. glomerata*, *J. bufonius*, *L. serriola* and *R. tingitana* are presented in Table (1). *L. serriola* attained the highest significant values of phenolics, tannins and alkaloids

compared to the other plant species (*P≤0.05*). However, *C. glomerata* exhibited the highest values of flavonoids and saponins, while it contained very little content of phenolic compounds.*J. bufonius* and *R. tingitana* expressed relatively high contents of phenolics, tannins and saponins.

Table 1. The composition of the active secondary chemical constituents (mg/g dry weight) of the five studied plant species Different letters of each column indicate values significant variation (*P≤ 0.05*)

Plant species	Phenolics	Tannins	Alkaloids	Flavonoids	Saponins
Cakilemaritima	$31.43^{d\pm}$ 1.28	48.05^{c} ± 0.66	9.25^{c} ± 0.17	10.26^{c} ± 0.43	58.61^{b} ± 1.34
Juncusbufonius	81.32^{c} ± 0.92	55.33^{b} ± 1.19	1.21^{e} ± 0.05	16.80^{a} ± 0.67	38.66^{e} ± 1.23
Lactucaserriola	94.21^{a} ± 1.62	65.28^{a} ± 1.20	20.26^{a} ± 1.23	9.66^{d} ± 0.18	52.20^{c} ± 1.03
Centaureaglomerata	0.41^{e} ± 0.05	41.75^{d} ± 3.04	11.24^{b} ± 0.49	17.22^{a} ± 1.08	77.02^{a} ± 1.01
Reichardiatingitana	85.67^{b} ± 1.68	63.62^{a} ± 0.57	2.83^{d} ± 0.05	11.21^{b} ± 1.07	41.80^{d} ± 0.96
$LSD_{0.05}$	2.85	2.29	0.46	0.55	2.29

Table 2. Percentage of DPPH radical scavenging activity (mean value ± standard error) and IC_{50} values of methanolic extracts of the studied plant species and Catechol

Plant Species	Conc.(ppm)	Scavenging Activity %	IC_{50}(mg/ml)
Cakilemaritima	1000	9.72 ± 0.29	4964.20
	800	9.62 ± 0.74	
	600	7.67 ± 1.54	
	400	4.57 ± 0.20	
	200	2.35 ± 0.74	
Centaureaglomerata	1000	13.55 ± 0.82	3783.05
	800	11.71 ± 0.86	
	600	10.14 ± 1.01	
	400	5.67 ± 0.84	
	200	3.66 ± 1.85	
Lactucaserriola	1000	46.45 ± 2.00	1029.45
	800	39.47 ± 3.91	
	600	36.78 ± 3.30	
	400	23.47 ± 0.50	
	200	11.33 ± 1.82	
Juncusbufonius	1000	46.54 ± 2.32	1026.62
	800	41.66 ± 0.50	
	600	29.17 ± 2.56	
	400	20.19 ± 3.63	
	200	8.70 ± 1.25	
Reichardiatingitana	1000	64.96 ± 2.25	663.98
	800	57.81 ± 1.89	
	600	56.68 ± 0.91	
	400	28.32 ± 0.87	
	200	24.44 ± 3.45	
Catechol	50	59.08 ± 0.59	32.86
	40	53.33 ± 0.08	
	30	50.34 ± 0.45	
	20	45.88 ± 0.73	
	10	32.77 ± 1.31	

Antioxidant activity of the studied plant species

The antioxidant activities of different plant extracts under investigation are presented in Table (2). It is obvious that, the antioxidant activity of the extract increases by increasing the extract concentration. At 1000 ppm, the extracts of *R.tingitana*, *J.bufonius*, *L.serriola*, *C.glomerata* and *C.maritima* showed scavenging activities of 64.96%, 46.54%, 46.45%, 13.55 and 9.72%, respectively. However, the lowest concentration (200 ppm) shows the lowest antioxidant activity.The IC_{50} values of *R.tingitana*, *J.bufonius*, *L.serriola*, *C.glomerata* and *C.maritima* were 663.98 ppm, 1026.62 ppm, 1029.45 ppm, 3783.05 ppm and 4964.20 ppm, respectively (Fig. 1). The antioxidant capacity of *R.tingitana*may be ascribed to the high content of phenolics, tannins and saponins. lupeol, taraxasterol acetates, sesquiterpene lactones, glucosides (Abdel-Mogib *et al*., 1993), flavonoids, cinnamic derivatives and phenolic acids (Recio *et al*., 1992) and essential oils (El Alfy *et al*., 2015) were isolated from *R.tingitana*. This plant is characterized by various biological activities such as antiviral (Sassi *et al*., 20008), antimicrobial, cytotoxic activity (El Alfy *et al*., 2015), anti-inflammatory, antioxidant, antidiabetic, antifeedant and insecticidal activity (Stalińska *et al*., 2005). The present results showed that the antioxidant activity of the*R.tingitana* was higher than those reported by El-Amier*et al*. (2015).

Antioxidant effects of natural phenols are attributed to their ability to scavenge free radicals by becoming radicals themselves through formation of resonantly stabilized radical species.

By scavenging reactive radicals, phenols prevent celldamage and cell proliferation, which are often in the core of carcinogenesis (Švarc-Gajić, 2013).

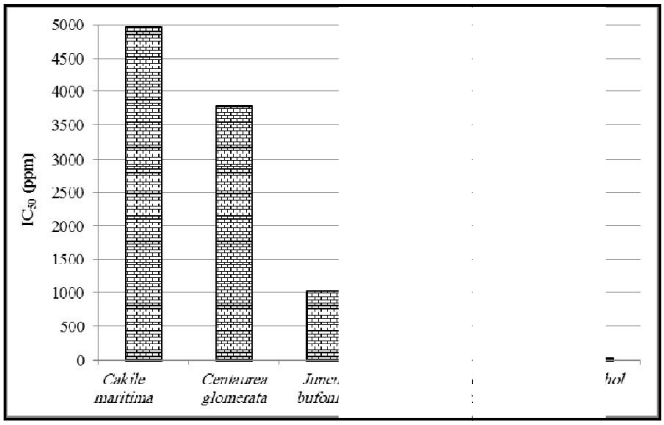

Figure 1. IC_{50} values (ppm) of the scavenging activity of different studied plant species and the catechol (standard)

Allelopathic effect of the aqueous extracts of the studied plantspecies on *Bidenspilosa*germination

The allelopathic effect of the aqueous extracts of *C.maritima*, *C.glomerata*, *J.bufonius*, *L.serriola* and *R.tingitana* on the germination of *B.pilosa*four days after treatment is showed in Table (3).

it was observed that the was no significant variation ($P \leq 0.05$) between the studied plants at the lowest concentration (2.5 mg ml^{-1}) The IC_{50} values (the concentration of a substance that is required for 50% inhibition of a specific biological or biochemical function) of the germination of *B. pilosa* were 8.73 mg ml^{-1}, 11.37 mg ml^{-1}, 14.95 mg ml^{-1}, 20.29 mg ml^{-1} and 26.83 mg ml^{-1}, respectively for *L.serriola*, *C.glomerata*, *R.tingitana*, *C.maritima* and *J.bufonius*.

The present study is coping with that reported by Obaid and Qasem (2005), where *L. serriola* inhibited the growth of vegetable crops.The previous phytochemical and biological investigation of *L. serriola* reported its analgesic, anti-inflammatory (Ahmad and Khan, 1992) and antioxidant activities, ascribed to the high phenolic contents which exhibited efficient free radical scavenging potential (Kim, 2001; Tepe and Sokmen, 2007; Nabavi *et al*., 2012). The present results showed the potent allelopathic effect of *L. serriola* on the nuisance weed *B. pilosa*, which could be ascribed to the high content of phenolics, tannins and alkaloids. Morever, arachidic, caproic, linoleic, oleic, palmitic, stearic acids, sitosterol, ascorbic acid, beta-carotene, deoxylactucin, lacticin, jacquilenin, lactupicrin and ubiquinone have been isolated from *L. serriola* (Khare, 2007).

Table 3. The allelopathic effect and IC_{50} of aqueous extracts of different studied plant species on the germination inhibition percentage (mean value ± standard error) of *Bidenspilosa* four days after treatment. Different letters in each column indicate values with significant variation ($P \leq 0.05$)

Plant species	Concentration of the extract (mg ml^{-1})				IC_{50} (mg ml^{-1})
	2.5	5	10	20	
Cakilemaritima	14.14^{a}±0.05	24.24^{ab}±0.05	29.29^{b}±0.02	49.49^{cd}±0.05	20.29
Juncusbufonius	9.09^{a}±0.01	14.14^{b}±0.02	29.29^{b}±0.02	39.39^{d}±0.02	26.83
Lactucaserriola	9.09^{a}±0.01	29.29^{a}±0.01	74.75^{a}±0.04	100.00^{a}±0.03	8.73
Centaureaglomerata	19.19^{a}±0.01	29.29^{a}±0.01	44.44^{b}±0.01	79.80^{b}±0.04	11.37
Reichardiatingitana	14.14^{a}±0.05	24.24^{ab}±0.05	34.34^{b}±0.02	64.65^{bc}±0.05	14.95
$LSD_{0.05}$	11.61	14.22	21.72	18.37	

Table 4. The allelopathic effect and IC_{50} of aqueous extracts of different studied plant species on the seedling growth inhibition percentage (mean value ± standard error) of *Bidenspilosa*ten days after treatment. Different letters in each column indicate values with significant variation ($P \leq 0.05$)

Plant species	Concentration of the extract (mg ml^{-1})				IC_{50} (mg ml^{-1})
	2.5	5	10	20	
	Radicle growth inhibition				
Cakilemaritima	27.27^{b}±0.79	47.83^{a}±1.84	69.17^{a}±1.84	80.63^{b}±1.43	7.15
Juncusbufonius	22.53^{c}±1.90	41.50^{c}±1.32	60.47^{c}±1.58	67.98^{c}±1.02	11.73
Lactucaserriola	35.57^{a}±1.88	43.87^{a}b±1.90	72.33^{a}±0.79	98.42^{a}±0.50	5.93
Centaureaglomerata	21.34^{bc}±2.48	27.27^{ab}±2.18	42.69^{b}±2.40	77.87^{d}±0.79	10.17
Reichardiatingitana	24.90^{bc}±1.32	38.34^{b}±3.30	47.04^{c}±3.71	64.82^{e}±0.73	12.33
$LSD_{0.05}$	5.16	6.43	6.65	2.75	
	Plumule growth inhibition				
Cakilemaritima	6.52^{d}±2.17	11.59^{c}±0.92	28.26^{b}±1.86	48.55^{b}±3.79	20.15
Juncusbufonius	9.55^{b}±1.45	16.14^{b}±2.08	18.99^{d}±2.43	21.70^{e}±3.32	65.92
Lactucaserriola	7.97^{c}±2.08	28.26^{a}±2.17	39.86^{a}±1.75	57.97^{a}±1.45	15.78
Centaureaglomerata	7.75^{c}±2.61	16.35^{b}±1.86	19.68^{c}±2.43	25.04^{d}±2.51	46.93
Reichardiatingitana	10.30^{a}±2.92	15.16^{b}±1.34	21.45^{c}±0.45	28.26^{c}±1.86	41.14
$LSD_{0.05}$	0.77	1.45	1.56	1.89	

The obtained data revealed that, the degree of inhibition was concentration-dependent. The aqueous extracts of *L.serriola*was completely inhibited the germination of *B. pilosa* at 20 mg ml^{-1}. Moreover, the aqueous extracts of *C.glomerata*, *R.tingitana*, *C.maritima* and *J.bufonius* at 20 mg ml^{-1} inhibited the germination of *B. pilosa* by about 79.80%, 64.65%, 49.49%and 39.39%, respectively. On the other hand, Some of these chemical compounds were reported as allelochemicals.

The allelopathic potential of *L. serriola* on the target weed (*B. pilosa*) could be incorporated in eco-friendly bio-control management program of weeds.

Allelopathic effect of the different aqueous extracts of the studied plant species on *Bidenspilosa* seedling growth

The allelopathic effect of the different aqueous extracts of the studied plant species on *B.pilosa* seedling growth after ten days of treatment revealed that, there was a significant variation between the studied plantspecies (*P≤0.05*). The degree of inhibition percentage of the plant extracts increased with the increase in its concentration (Table 4). *L.serriola* extract showed the highest inhibition (98.42%) of radicle growth at 20 mg ml^{-1}. However,*C. maritime C.glomerata*, *J.bufonius* and *R. tingitana* extracts showed 80.63%, 77.87%, 67.98% and 64.82%, respectively. At 2.5 mg ml^{-1} (lowest concentration), it was observed that, different extracts showed significant variations (*P≤0.05*). The IC_{50} values of *B.pilosa* radicle growth were 5.93 mg ml^{-1}, 7.15 mg ml^{-1}, 10.17 mg ml^{-1}, 11.73 mg ml^{-1} and 12.33 mg ml^{-1}, for *L.serriola*, *C.maritima*, *J.bufonius*, *C.glomerata* and *R.tingitana*, respectively (Table 4). On the other hand, the plumule growth of *B. pilosa* was reduced by about 57.97%, 48.55%, 28.26%, 25.04% and 21.70% under treatment of the highest concentration (20 mg ml^{-1}) from the extract of *L. serriola*, *C. maritime*, *R. tingitana*, *C. glomerata* and *J. bufonius*, respectively. The IC_{50} values of *B. pilosa*plumule growth were 15.78 mg ml^{-1}, 20.15 mg ml^{-1}, 41.14 mg ml^{-1}, 46.93 mg ml^{-1} and 65.92 mg ml^{-1}, for *L. serriola*, *C. maritima*, *R. tingitana*, *C. glomerata* and *J. bufonius*, respectively (Table 4). Thevariation in the allelopathic effects between the different plant species refers to that, the receiving plant growth may be stimulated below the threshold, with mild to severe growth reductions observed above the threshold; each depending on the sensitivity of the receiving species and the types of allele chemicals from the donor species (Cheema *et al*., 2013).

The present results are showed more sensitivity of radical to the allelochemicals than plumule, which could be attributed to the direct contact of radicle to the allelochemicals and the permeability of radicle cells (El-Shora and Abd El-Gawad 2014).*B. pilosa*seedling growth was inhibited by the allelochemicals from other plant species such as *Plantagolagopus*, *P. major* and *P. squarrosa* (Abd El-Gawad *et al*., 2015). Delayed seed germination and slow radicle growth under allelochemical treatment could be ascribed to the osmotic effects on rate of imbibition, delayed initiation of germination and especially cell elongation; the main factor that affects root growth before and after the tip penetrates the seed coat (Chon and Nelson, 2013).

Conclusion

R. tingitana collected from the Egyptian deserts shows a potent antioxidant capacity which may be ascribed to the high content of phenolics, tannins and saponins.However,*L. serriola* is commonly considered as an invasive serious weed of orchards, roadsides and field crops (Everitt*et al*. 2007), it may be used in controlling other weedy species through allelopathic application such as *B. pilosa*. Further study is needed to isolate and characterize the proper allelochemicalsfrom *L. serriola*, as well as to specify their modes of action.

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