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PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND ALLELOPATHIC ACTIVITIES OF *DIPLOTAXIS HARRA* CRUDE EXTRACTS

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Abstract

Diplotaxis harra (Harra, family Brassicaceae) is a common annual species in the desert of Egypt with a 20-30 cm height. The present study aimed to screen the phytochemical compounds and evaluate the antioxidant activity of *D. harra* collected from North Eastern Desert, Egypt, as well as to characterize its allelopathic potential against *Chenopodium murale* L. Standard methods were used for the qualitative and quantitative of tannins, saponins, flavonoids, alkaloid, phenolics, steroids, terpenoids, and glycosides. Antioxidant activity was analyzed by using 2, 2-diphenyl-1-picryl-hydrazyl-hydrate assay. *D. harra* attained the highest values of alkaloids, flavonoids, and phenolics compared. In the DPPH test system, the IC_{50} value of the antioxidant inhibition for *D. harra* was 2.11 mg ml^{-1}. The data obtained indicated that, the root system of *C. murale* was more affected than shoot system to the inhibitory allelopathic activity induced by *D. harra.* At higher concentration (20 g l^{-1}) the germination of the tested seed was highly inhibited by 96.25 g l^{-1}, while the shoot and root were reduced by 82.14% and 93.50%, respectively. However, the different response was observed at the lower concentration (2.5 g l^{-1}). In conclusion, the incorporation of allelopathic substances into agricultural management might scale back the development of pesticides and reduce environmental deterioration.

Key words: *Diplotaxis harra, Chenopodium murale,* phytochemical, phytotoxicity, antioxidant

Introduction

In the process of using wild plants over thousands of year's humans have influenced plant evolution. The early days of agriculture began approximately 10,000 years ago (Zohary *et al.*, 2012; Shelef *et al.*, 2017) when people used native species and selected for desirable traits for human consumption. Such plants have been cultivated in diverse areas; in USA, Mexico, India and many other countries, and produced a considerable economic return. Native species can reduce the negative impacts of introduced species. Invasive species often spread and damage the environment, threatening biodiversity, agriculture, and human health (Schmitz and Simberloff, 1997). In the arid climate, primary and secondary compounds increase when plants are mildly stressed due to less availability of nutrients and water, but decrease when agricultural practices emphasize productivity and growth (Zahran and El-Amier, 2013; Kamoun, 2018). On the other hand, the desert vegetation in Egypt is by far the most essential and characteristic type of natural plant life. It covers about 95% of the total area of the country and is mainly formed of xerophytic shrubs and sub-shrubs as well as, ephemeral (or annual) plant growth depending on the amount of precipitation in a given year (El-Amier and Abdul-Kader, 2015).

Brassicaceae (Cruciferae or Mustard family) is one of the largest families in the plant kingdom and is rich in medicinal species, containing about 338 genera and 3350 species as well as are distributed worldwide. In Egypt, it represents about 53 genera and 107 species (Boulos, 1999). Rizk (1986) stated that plants of this family were used as anti diabetic, anti bacterial, anti fungal, anti cancer, and anti rheumatic; also, it showed potent insecticidal activity. Many previous studies (Guarrera, 2003; Pieroni *et al*., 2004) referred to *Diplotaxis* as a traditionally used plant with therapeutic properties and as an alimentary plant usually consumed as salads (Guarrera, 2003; Falleh *et al.*, 2013). Moreover, Hashem and Saleh (1999) stated that some species of *Diplotaxis* contain high percentages

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of antimicrobial components.

Diplotaxis harra (Forssk.) Boiss, is a common annual species in the desert of Egypt with 20-30 cm height. The plant is found in sandy stony desert valleys and locally known as *Harra* and spreading white-hispid with thick, woody root (Hegazy, 2001). It has been reported to have an analgesic effect and has moderate toxicity (Mohammed *et al.,* 2013). The phytochemical analysis of aerial parts which carried out by Atta *et al.,* (2011) has shown the presence of flavonoids, tannins, glucosinolates and sterols.

Allelopathy is a well-studied phenomenon due to its multi-dimensional impacts and has been identified as a potential tool for sustainable crop production (Jabran *et al.,* 2015). Allelopathy involves the endogenous synthesis of biochemicals known as allelochemicals, which affect the plant growth and development, plant diversity, dominance, succession, and climax of natural vegetation of neighboring plants and other species (Sangeetha and Baskar 2015; Abd El Gawad *et al.,* 2018 a). Moreover, weeds are one of the major constraints on crop production because it competes with crops for vital resources such as light, air, water, nutrients, and space, leading to a significant reduction in crop growth, productivity and quality (Rehman *et al.,* 2019). The present research aimed to investigation the phytochemical compounds, antioxidant and allelopathic activities of crude plant extract from *Diplotaxis harra* (Forssk.) Boiss.

Materials and methods

Plant material

The aerial parts of *Diplotaxis harra* (Forssk) Boiss were collected from populations growing naturally in the North sector of Eastern desert (wadi Hagoul), Egypt during the flowering period (March 2018). The plant species were identified, according to Boulos (1999). Immediately after collection of the plant samples, the shoot system of species was handily cleaned, washed several times with distilled water, dried in an oven at 50°C, ground to a fine powder and preserved in well-stopped bottles which were directly used for different investigations. Aqueous and methanolic extracts were obtained by soaking the plant materials in large two flasks for 48h.

Preliminary phytochemical screening

Qualitative phytochemical analysis of *D. harra* aerial parts were carried out on the aqueous extract and on the dry powdered for the presence of some phytochemicals including: tannins, saponins, flavonoids, alkaloid, phenolics, steroids, terpenoids and glycosides as described by Trease and Evans (1989), Sofowara (1993), Evans (1996) and Joshi *et al.,* (2013).

Quantitative Estimation of some secondary compounds

The total phenolics content was quantitatively estimated spectrophotometrically according to Chlopicka *et al.,* (2012) methods. The alkaloids were extracted with 10% acetic acid in ethanol and determined based on Joshi *et al.,* (2013) method, while the total flavonoid content was determined according to Stankovic (2011).

Antioxidant Activity

Antioxidant activity was determined by using a stable free radical (1, 1-diphenyl-2-picrylhydrazyl) DPPH (Miguel, 2010). Briefly, two mL of 0.15 mM freshly prepared DPPH was added to 2 mL of both plant extracts in different concentrations ranging from 100 to 1000 µg ml^{-1}, and catechol as standard. A control was prepared by adding 2 mL of DPPH to 2 mL solvent. The mixture was incubated in the dark at the room temperature for 30 min. The absorbance was recorded at 517 nm, and the IC_{50} (The sample concentration providing 50% free radical scavenging activity) was calculated graphically. The antioxidant activity was calculated using the following equation:

$$\text{Scavenging activity \%} = \frac{A_{Control} - A_{sample}}{A_{Control}} \times 100$$

Allelopathic bioassay

The seeds of *Chenopodium murale* were collected from the cultivated land in Mansoura City, Al-Dakahlia Governorate, Egypt. Seeds were sterilized in 0.3% sodium hypochlorite for 3 minutes, washed several times by distilled water, dried at room temperature for seven days and preserved in a paper bag until further use.

In order to test the phytotoxic activity, methanol extracts were prepared at various concentrations (2.5, 5, 10, 15, 20% w/v). The concentrations used were selected based on the preliminary tests. 5 mL of each concentration was transferred in sterilized Petri dish (9 cm) contained sterilized Whatman No. 1 filter paper as well as 20 seeds of the tested plant. The control treatment was designed with distilled water. The germinated seeds, with 2 mm radicle were counted daily. The inhibition percentage and the length of radicle and plumule were calculated according to Abd El-Gawad and El-Amier (2015).

Statistical analysis

All assays were in triplicate analyses and the data were recorded as mean values ± standard error. One-way analysis of variance (ANOVA) was performed in a randomized complete block design using COSTAT software program to assess the differences between treatments based on Duncan's test at 0.05 probability level.

Results and Discussion

Phytochemical analysis

There is a growing interest in using bioactive phytochemicals from natural origin. Phytochemicals obtained from a different part of plants have different effects on human health (Avato and Argentieri, 2018). In the present study, the qualitatively identified phytochemicals of aerial parts of wild *D. harra* have been tabulated in Table1. In the identification process, the extracts showed many types of chemical constituent in different solvents. The extraction efficiency of biologically active compounds depended on a large number of parameters - extraction method such as; type of solvents, temperatures and extraction times (Lincheva *et al.,* 2017). The results obtained for the quantitative estimation of some secondary metabolites of wild *D. harra* are presented in Fig. 1. The results showed that the aerial parts contained mainly phenolics compound (22.71 mg g^{-1}, respectively). While, alkaloids and flavonoids attained values of 8.37 and 6.93mg g^{-1} dry weight, respectively.

Table 1: Qualitative phytochemical screening of various extracts of *Diplotaxis harra* aerial parts.

Phytochemical screened	Aqueous extract	Methanolic extract
Alkaloid	-	+++
Saponins	++	-
Tannins	+	+
Flavonoids	++	+++
Phenolics	++	+++
Glycosides	++	++
Steroids	+	+
Terpenoids	+	++

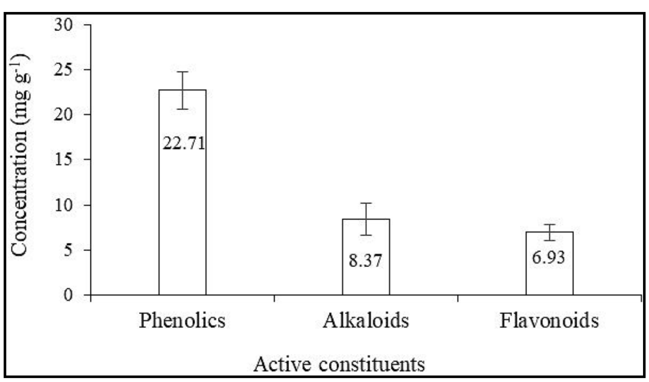

Fig. 1: The concentration of different active constituents in mg g^{-1} dry weight in *Diplotaxis harra*.

The phytochemical analysis of *D. harra* in this study relatively agreed with those mentioned by Akrout *et al.,* (2010) on *Diplotaxis harra* and *D. simplex* from Tunisia, except alkaloids not recorded and Attia *et al.,* (2006) on *Diplotaxis acris* from Egypt. Comparing the obtained results to that for other plant species of the Egyptian flora revealed that the phytochemical analysis of *D. harra* agreed with that obtained by Alghanem and El-Amier (2017) on *Pergularia tomentosa* (Solanaceae) and El-Amier *et al.,* (2014) on *Senecio glaucus* growing in the arid region.

Antioxidant Activity

Antioxidants are entities that reduce oxidative stress by reacting with a free radical early in the oxidation process and are exogenous (natural or synthetic) or endogenous compounds (Carocho *et al.,* 2018). Plant antioxidants are important for human health and include numerous compounds such as ascorbic acid, tocopherols, polyphenolic compounds, and terpenes (Hartsel *et al.,* 2016). The evaluation of the antioxidant activity of *D. harra* extract is shown in Fig. 2. It is obvious that by increasing the plant extract concentration; there was a corresponding continuous increase in scavenging activity. At 4000 µg ml^{-1}, the extract showed scavenging activities of 54.89% while, the lowest concentration (250 µg ml^{-1}) showed the lowest antioxidant activity (17.68%). In the DPPH test system, the free radical-scavenging activity of the crude extract of *D. harra*, with an IC_{50} value of 2.11 mg ml^{-1}, but higher than that of the positive control catechol (0.15 mg ml^{-1}).

The powerful antioxidant activity of shoot extract can be attributed mainly due to secondary compounds (Juan and Chou, 2010). This was confirmed by Falleh *et al.,* (2013) who reported that wild *Diplotaxis* could be used as a potent source of natural antioxidants and antibiotics due to highest polyphenol content.

Allelopathic bioassay

The crude extract of *D. harra* aerial parts showed significant phytotoxic effect against on the germination and seedling growth of *C. murale* (Figures 3 and 4) at different concentrations (2.5 to 20 g.l^{-1}) over control. The data obtained indicated that the root system of *C. murale* was more affected than the shoot system to the inhibitory allelopathic activity induced by *D. harra*. At higher concentration (20 g l^{-1}) the germination of the tested seed was highly inhibited by 96.25 g l^{-1}, while the shoot and root were reduced by 82.14% and 93.50%, respectively (Figures 3 and 4). However, the opposite response was observed at the lower concentration (2.5 g l^{-1}).

The root of *C. murale* was more sensitive to the phytotoxic effect of the crude extract than shoot. This could be ascribed to the radicle was the first to emerge and consequently direct contact with the extracts or due to the organ-based sensitivity of the species to phytotoxic compounds. Previous studies (Abd El-Gawad *et al.*, 2018a; Ximenez *et al.*, 2019) also documented this aspect of greater root than shoot inhibition. The results of the present research against the weed C. *murale* agreed with the most of the previous results obtained by other researchers, which emphasized that extracts of many plant species inhibited germination of C. *murale* seeds (Arora *et al.*, 2017; Abd El-Gawad *et al.*, 2018a).

The previous phytochemical and biological investigation of *D. harra* reported its anti-inflammatory and anticancer agents (Nasri *et al.*, 2017), antidiabetic, antibacterial, antifungal, anticancer, antirheumatic and insecticidal effect (Rizk, 1986), as well as cytotoxic (Mohammed *et al.*, 2013) and antioxidant activities (Bahloul *et al.*, 2016), ascribed to the high phenolic contents which exhibited efficient free radical scavenging potential (Nabavi *et al.*, 2012). The present results showed the potent allelopathic effect of *D. harra* on the

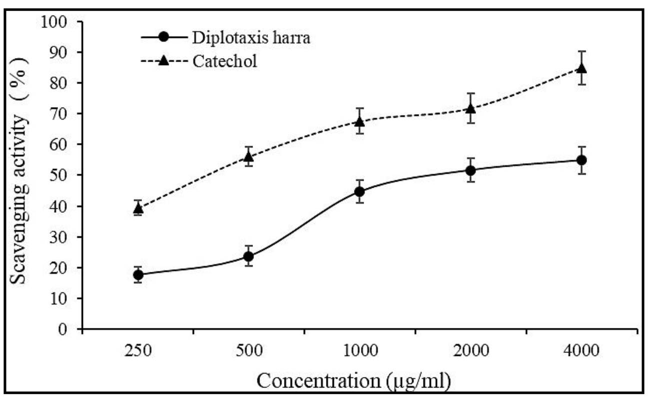

Fig. 2: % of scavenging activity of *Diplotaxis harra* extract and natural antioxidant catechol.

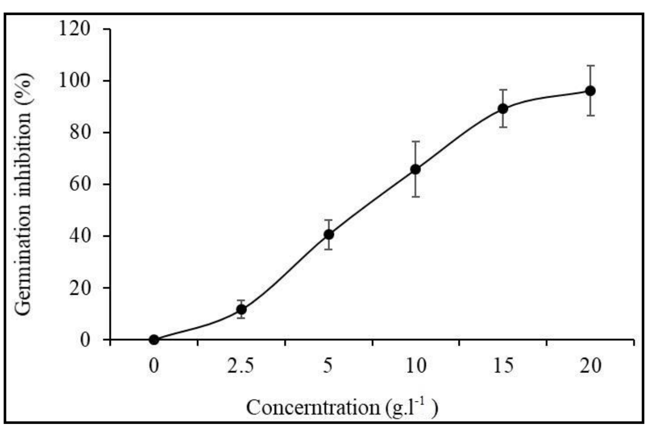

Fig. 3: Allelopathic effect of different methanol extracts from *Diplotaxis harra* aerial parts on the seed germination inhibition percentage of *C. murale* after four days of treatment.

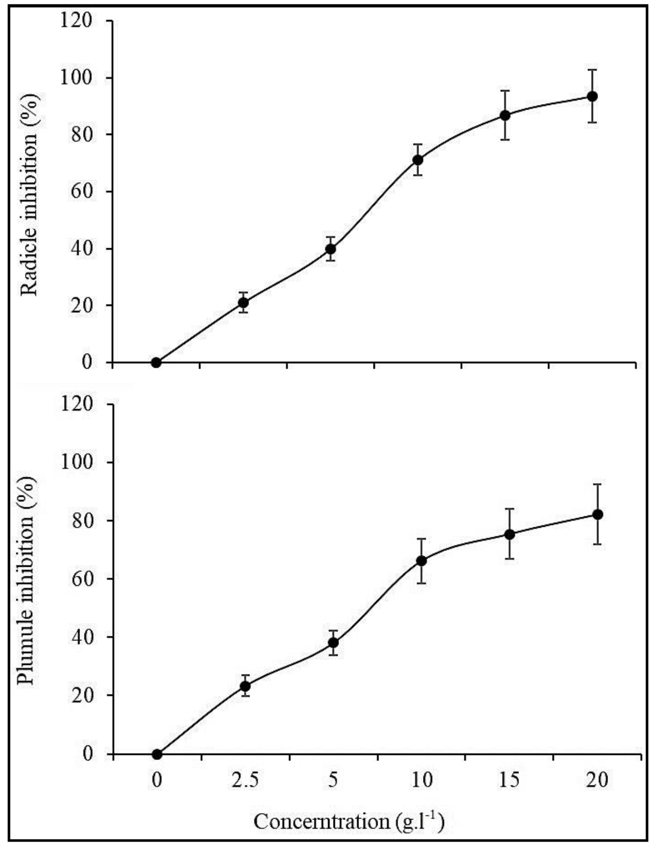

Fig. 4: Allelopathic effect of different methanol extracts from *Diplotaxis harra* aerial parts on the seedling growth inhibition percentage of *C. murale* after fourteen days of treatment.

nuisance weed C. *murale*, which could be ascribed to the high content of phenolics, tannins, and alkaloids. Moreover, the plant extract of *D. harra* contained high levels of arachidonic and palmitic acids, nonadecane, stigmasterol and sitosterol (Hashem and Saleh, 1999), phenolics (Falleh *et al.*, 2013), flavonoids (Mohammed *et al.*, 2013).

Many beneficial effects had been shown for the β-sitosterol (Yang *et al.*, 2001). Phytosterols, in general, are of interest due to their antioxidant activity and impact on health. Recently, phytosterols have been added to vegetable oils as an example of a successful functional food (Ntanios, 2001). In general, data obtained revealed that plant toxicity against weeds reduced the development of pesticides and environmental degradation. Therefore, the phytochemical compounds from *D. harra* can be used as a green, biodegradable, and environmentally friendly resource (Abd El-Gawad *et al.*, 2018 b and 2019).

Conclusion

The results of the present study have revealed the presence of flavonoids, alkaloids, and phenolics in *D.*

harra. The presence of these secondary metabolites in this plant may be the source of some pharmaceutical and biological activities. *D. harra* could also serve as a good source for antioxidant and green materials. Further studies will be undertaken to isolate, identify, and evaluate the biological activities of these secondary metabolites in these species. In conclusion, the incorporation of allelopathic substances into agricultural management might reduce the development of pesticides and environmental deterioration.

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