



PHYTOCHEMICAL CONSTITUENTS, ANTIOXIDANT AND ALLELOPATHIC ACTIVITIES OF *AIZOON CANARIENSE* L. ON *ZEA MAYS* (L.) AND ASSOCIATED WEEDS

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Abstract

This study aimed to screen the chemical constituents of *Aizoon canariense* collected from North Eastern Desert (Wadi Hagul), Egypt, and evaluate its the antioxidant and allelopathic potential. Proximate composition and mineral content were calculated. Total phenolics, flavonoids, alkaloids, and tannins were determined. Antioxidant activity was evaluated based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicle scavenging. *A. canariense* has a considerable amount of the proximate composition as well as macro- and micro-minerals. However, *A. canariense* attained high content of various secondary metabolites (phenolics, tannins, flavonoids and alkaloids). In the DPPH test system, the IC₅₀ value of the antioxidant inhibition for *A. canariense* was 0.74 mg ml⁻¹. The germination percentage, shoot and root length of *Zea mays* and two associated weeds (*P. oleracea* and *A. lividus*) were reduced in a concentration-dependent manner. At higher concentration 40 g l⁻¹ inhibited the germination of *Z. mays*, *P. oleracea* and *A. lividus* by about 24.56%, 88.70% and 100%, plumule length was reduced by 39.54%, 76.64% and 95.68%, while root length was inhibited by 43.90 %, 100% and 100 %, for *Z. mays*, *P. oleracea* and *A. lividus*, respectively. From the data obtained, we concluded that the integration of allelopathic substances in agricultural management may reduce pesticide development and environmental degradation, as well as *A. canariense* can be used as a natural resource of antioxidants.

Key words: *Aizoon canariense*, Aizoaceae, phytochemical, phytotoxicity, antioxidant, Desert, Egypt.

Introduction

Arid and semi-arid habitats cover over 90% of Egypt's territory amalgamating disparate environmental ecosystems. Egypt's deserts comprise different ecological units (ecosystems), namely: Wadis, mountains, plains, rocky ridges, sand dunes, salt marshes, reed swamps, and mangrove swamps. The Eastern Desert of Egypt occupies the area extending from the Nile Valley eastward to the Gulf of Suez and the Red Sea which is about 223,000 km², *i.e.* 21% of the total area of Egypt, and is mainly formed of xerophytic shrubs and sub-shrubs (Zahran, 2010). The Eastern Desert of Egypt extends between the Nile Valley and the Red Sea. The wadi habitat in the Eastern Desert has distinctive features including a characteristic plant cover. It has the great merit of being a drainage system collecting water from

an extensive catchment area. Wadi Hagul is an extensive wadi occupying the valley depression between Gebel Ataqa to the north and the Kahaliya ridge to the south. Its main channel extends for about 35 km and collects drainage water on both sides. The vegetation of Wadi Hagul has distinctive features including a characteristic plant cover (Zahran and Willis, 2009; El-Amier and Abdul-Kader, 2015).

The xerophytic vegetation is by far the most important and characteristic type of the natural plant life in Egypt's deserts. Its communities cover vast areas of the wadis, desert plains and mountains (Youssef *et al.*, 2009; Batanouny, 2013). In Egypt, the efforts have directed towards the utilization of renewable resources of the cultivated and non-cultivated areas to produce more food and forage. Such efforts would be more successful and fruitful if they are based on previous knowledge of the

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environmental characteristics comprising soil, climate, vegetation, animal and human interference (Zahran and El-Amier, 2013).

The family Aizoaceae (Ice plant family) comprises 127 genera and 1860 species distributed in tropics and sub-tropics of South Africa and rarely in Australia (Leistner, 2000). They are commonly known as stone plants or carpet weeds (Bittrich and Hartmann, 1988). In Egypt, it is represented by 9 species included in 8 genera viz., *Aizoon*, *Mesembryanthemum*, *Sesuvium*, *Trianthema* and *Zaleya* (Boulos, 1999). All species are considered succulent but cover a wide range of life styles from pebble-like leaf succulents to small succulent shrubs. Distinctive seed capsules are valuable for identifying species. *Aizoon* was described by Linnaeus in 1753 to accommodate three species originating from the African region. Since this date, the number of described species in this genus has increased to about 20 (Dyer, 1975). *Aizoon canariense* L. is prostrate, thick-stemmed annual or perennial herb, with stems up to 40 cm long, often papillose. Leaves subcircular to obovate, entire, decurrent at base, pilose. Flowers are solitary and sessile, perianth segments yellowish inside, greenish or reddish and pilose outside. Fruit are red or pink, star-shaped, depressed in the middle (Boulos, 1999).

Many plant species that supply 90% of the world's food, fodder, fiber, drugs etc. were domesticated from wild plants found in the tropics (Myers, 2019). However, the existing wild plant in the deserts, remain interesting to plant ecologists, agronomists and genetic engineers to develop new crop strains; some of them may become important sources of various biological activities including antioxidant, allelopathic (Abd El-Gawad *et al.*, 2018a&b; Elshamy *et al.*, 2019), antifungal, antibacterial, antiviral (El-Amier *et al.*, 2014; Alghanem and El-Amier, 2017; El-Amier and Abo Aisha, 2019), anti-inflammatory (Tohidi *et al.*, 2017) and insecticidal (Castillo *et al.*, 2017) activities. In addition, the wild plant is a good source of food preservation industries, livestock fodder (Zahran and El-Amier, 2013), fibers (Zahran and El-Amier, 2014), drugs (Zaki *et al.*, 2016a & b, 2017, 2018), fragrance industries, and agro-industrial (Zuin and Ramin, 2018; Alzuaibr *et al.*, 2020). This research aims to determine the phytochemical constituents and antioxidant activity of aerial parts of *Aizoon canariense* collected from the North Eastern Desert (Wadi Hagul) in Egypt, as well as to assess the allelopathic potential against some dispraised weeds (*Portulaca oleracea* L. and *Amaranthus lividus* L.) associated with *Zea mays* L. as an important food and forage crop in Egypt.

Materials and Methods

Preparation of plant material

Aizoon canariense L. aerial parts were collected at a vegetative stage from different sites from Wadi Hagul, North Eastern Desert, Egypt. The identification of species was made according to Boulos (1999). The aerial plant parts washed with distilled water several times and were dried at room temperature. The dried sample was ground into a powder using a blender and preserved in a polyethylene bag in a refrigerator until use.

Phytochemical analysis

The moisture content, dry matter, crude fibre, ether extract, ash and crude protein (CP) of *A. canariense* sample was analyzed according to AOAC (1995). The total nitrogen was determined by the Kjeldahl method (Pirie, 1955). The method of extraction of different carbohydrate fractions adopted in this investigation was essentially that of Yemm and Willis (1954) and Handel (1968). 0.1 g of air-dried sample was submerged overnight in 10 ml of 80% (v/v) ethanol at 25 °C with periodic shaking. The ethanolic mixture was filtered and the ethanolic filtrate was made up to volume and kept in refrigerator for analysis of different sugar fractions. Glucose was determined based on the method of Feteris (1965). Sucrose was determined according to Handel (1968). Total soluble sugars were estimated by the method of Southgat (1991). The total carbohydrate content of plant sample was calculated by "difference", in this, the sum of the percentages of all the other proximate components was subtracted from 100 (AOAC, 1995).

The method of extraction of different elements in the present study was described by Allen *et al.*, (1974). Sodium (Na⁺) and potassium (K⁺) were determined in the sample by Flame Photometer (Model PHF 80 B Biologie Spectrophotometer), while calcium (Ca²⁺), and magnesium (Mg²⁺) were estimated using atomic absorption spectrometer (Perkin-Elmer, Model 2380.USA). These elements were expressed as mg/g dry weight.

Quantitative estimation of some secondary compounds

The total phenolics content was quantitatively estimated spectrophotometrically according to Chlopicka *et al.*, (2012) methods. The alkaloid was 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 activities

Antioxidant activity was determined in methanolic extract of the dried plant as described by Kosem *et al.*,

(2007) with slight modifications as follows: about 20 g of powdered samples were extracted with 200 ml of methanol 50% for a week at room temperature. The extract was then collected and filtered through Whatman No.1 filter paper in a Buchner funnel under vacuum. Antioxidant activity was determined by using a stable free radical (1,1-diphenyl-2-picrylhydrazyl) DPPH (Miguel, 2010). Briefly, 2 ml of 0.15 mM DPPH was added to 2 ml of plant extracts in different concentrations (100-1000 ppm). A control was prepared by adding 2 ml of DPPH to 2 ml solvent. The mixture was kept in the dark at 37 °C for 30 min. The absorbance was recorded at 517 nm, and the IC₅₀ was calculated graphically. The antioxidant activity was expressed as:

$$\% \text{ Radical scavenging activity} = \frac{1 - (\text{A sample})}{\text{A control}} \times 100$$

Allelopathic activity

The seeds of *Zea mays* and two associated weeds (*Portulaca oleracea* and *Amaranthus lividus*) were collected from cultivated fields in Mansoura city, Al-Dakahlia Governorate, Egypt. Uniform and ripened seeds were sterilized by soaking for 3 min in NaOCl (0.3%), then washed by sterile-distilled water three times and dried on sterilized Whatman cellulose filter paper and kept in sterilized bottles until further use.

In order to test the phytotoxic activity, two layers of Whatman No. 1 filter paper were placed in 7 cm diameter glass Petri dishes. In each petri-dish 20 seeds were placed and 4 ml of each plant extract added in a concentration of 5, 10, 20 and 40 mg ml⁻¹ and incubated in the growth chamber at 27°C (Abd El-Gawad and El-Amier, 2015). After 4 days, rate of germination and the percentage of inhibition were calculated. Meanwhile, the length of radicle and plumule was measured for each replicate after 14 days of treatment. The experiment was designed with three replications for each treatment and was repeated two times.

$$\% \text{ Inhibition percentage} = [(CG - TG / CG) \times 100]$$

$$\% \text{ Growth inhibition} = [(LC - LT)/LC] \times 100]$$

where, CG: germination rate in check treatment, TG: germination rate in extract treatment, LT: shoot or root length of powder treated weed, LC, shoot or root length of untreated check weed and crop test plants.

Results and Discussion

Nutritional value determinations

Plants have great importance due to their nutritive value and continue to be a major source of medicines as they have been found throughout human history (Balick

and Paul, 1996; Shulz *et al.*, 2001). Dietary fiber is associated with impaired nutrient utilization and reduced net energy values. However, fiber has to be included in the diet to maintain normal physiological functions in the digestive tract of animals (Noblet and Le Goff, 2001; Lindberg, 2014). Determining the dry matter content of feed provides a measure of the amount of a particular feed that is required to supply a set amount of nutrients to the animal. The dry matter of plant consists of all its constituents excluding water (Phillips, 2018). In the present study, *A. canariense* attained total ash (8.37%), moisture content (11.76%), dry matter (88.24%) and crude fiber (7.66%) (Table 1). By comparing these results to that for other plant species of the Egyptian flora, the nutritive value of *A. canariense* agree with those mentioned by El Shaer (2010), Zahran and El-Amier (2013), but lower than those reported by Tawfik *et al.*,

Table 1: Chemical composition of *Aizoon canariense*.

| Constituents analysis | Concentration |
|--|---------------|
| Moisture content % | 11.76±0.47 |
| Dry matter % | 88.24±3.53 |
| Total ash % | 8.37±0.33 |
| Crude fiber % | 7.66±0.31 |
| Crude lipid % | 2.53±0.10 |
| Crude protein % | 11.5±0.46 |
| Total nitrogen % | 1.84±0.07 |
| Carbohydrates (mg g⁻¹ dry wt.) | |
| Glucose | 2.84±0.11 |
| Sucrose | 4.31±0.17 |
| Total soluble sugar | 26.57±1.06 |
| Total carbohydrates | 69.94±2.80 |
| Macro-elements (mg/100g dry wt.) | |
| Na ⁺ | 2.28±0.52 |
| K ⁺ | 435.17±2.31 |
| Ca ⁺⁺ | 135.36±1.67 |
| Mg ⁺⁺ | 34.82±0.99 |

(2015).

The fodder crop must have high biomass, digestibility and palatability for animals. They contain high proteins, carbohydrates, low oxalate, fibre and ash content, while minerals and vitamins from comparatively a smaller part, plant materials form major portion of the diet; their nutritive value is important (El Shaer 2004; Indrayan *et al.*, 2005). In the present study, *A. canariense* attained crude protein (11.5 %) and crude lipid (2.53%), (Table 1). Comparing with the other studies, the selected plant species showed relatively comparable percentage of crude protein with the studies of Heneidy and Bidak (2003)

and Zahran *et al.*, (1999). While, the lipid contents were quite low similar to that reported by Zahran *et al.*, (1999) and El-Halawany *et al.*, (2002), but not agree with that reported by Heneidy and Bidak (1996) and Omar (2006).

The crude protein is viewed classically as an indicator of the nutritional value of plants as food for ruminants (Bryant *et al.*, 1983). Although lipids are a concentrated source of energy, they do not constitute a major source of energy from forages (Chesworth, 1996). Nevertheless, forage with high lipid content may be an asset in satisfying the energy requirements of animals when other sources are limited.

Regarding glucose content (2.84 mg g⁻¹ dry wt.) was determined in *A. halimus* with sucrose (4.31 mg g⁻¹ dry wt.) and total soluble sugars (26.57 mg g⁻¹ dry wt.) in appropriate quantities in the current study (Table 1). These results are relatively comparable to that obtained by Fernandes and Waditake (2006) on *Trifolium alexandrinum* and Hafiza *et al.*, (2002) on *Medicago sativa*.

Jeroch *et al.*, (1999) reported that optimal content of carbohydrate is 8-10% for producing high-quality silage, accordingly, most of the selected forage weeds may be considered as a good fodder species. The total carbohydrate (69.94 mg g⁻¹ dry wt., H⁺ 7%) which provides the plant itself and animal by the energy was represented by higher value than that the study of Alzuaibr (2019) and relatively comparable to that study of El-Kady (1987) and El-Amier and Ejgholi (2014). The desert grazing sheep, camels, and goats require fodder plants with improved nutritional values particularly during the long-lasting dry seasons; this will increase the average annual animal production by more than 25% (Attia-Ismail, 2016). Attempts are made to use the marginal sources, for example, saline soils and underground water for producing unconventional fodder ingredients (Attia-Ismail, 2016).

The concentrations (mg/g dry weight) of the measured macro-elements in *A. canariense* are as shown in Table 1. The sequence of macro-minerals in *A. canariense* is: K (4.35) > Ca (1.35) > Mg (0.35) > Na (0.02). Minerals play a role in four types of functions in animals: structural, physiological, catalytic, and regulatory (Suttle, 2010). The ARC (1980) system and NRC (2001) system, reported that the requirement for mineral nutrients for gestating beef cows or lactating beef cows is 0.038, 0.016, 0.003 and 0.068 mg g⁻¹ for K, Ca, Mg and Na, respectively. Deficiency or excess of dietary mineral elements may cause animal production and health concerns; therefore, mineral elements balance is very important to keep animal health (Silva *et al.*, 2015).

Quantitative determination of some secondary compounds

Plants are a major source of complex and highly structurally diverse chemical compounds (phytochemicals), this structural diversity attributed in part to the natural selection of organisms producing potent compounds to deter herbivory (feeding deterrents). Major classes of phytochemical include phenols, polyphenols, tannins, terpenes, and alkaloids (Crozier *et al.*, 2006; Dang and Van Damme, 2015). In the present study, the phytochemical screening of *A. canariense* reveals that they are good sources of natural products (Fig. 1). *A. canariense* contained high contents of tannins (28.46 mg g⁻¹ dry weight), While contained relatively contents of phenolics (18.42 mg g⁻¹ dry weight), flavonoids (9.82 mg g⁻¹ dry weight) and of alkaloids (6.66 mg g⁻¹ dry weight). These results are comparable with those of common desert plant (Hariprasad and Ramakrishna, 2011; El-Amier and Abdullah, 2014; El-Amier and Abo Aisha, 2019).

The report published by El Shaer and Attia-Ismail (2015) revealed that the majority of annual desert plants are low palatability as they produce little phytomass. However, any evaluation of plant depends on their performance both in the biological as well as its economic

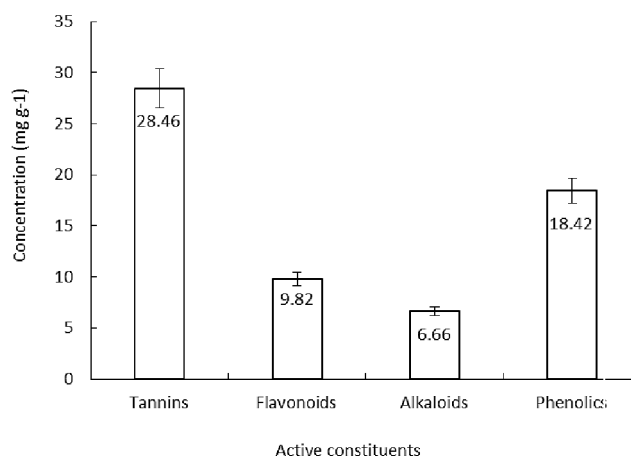


Fig. 1: The concentration of different active constituents in mg g⁻¹ dry weight in *Aizoon canariense*.

input (El Shaer and Attia-Ismail 20015).

Antioxidant activity

The evaluation of the antioxidant activity of the different plant extracts is shown in (Table 2). By increasing the plant extract concentration; there was a corresponding continuous increase in scavenging activity. At 1000 µg ml⁻¹, the extract showed scavenging activities of 54.52% while, the lowest concentration (100 µg ml⁻¹) showed the lowest antioxidant activity (12.24%). The

IC₅₀ value of *A. canariense* extract was 0.74 mg ml⁻¹ compared to standard catechol (0.15 mg ml⁻¹). These results suggest that methanol extract of *A. canariense* has an obvious effect on scavenging of DPPH radical (IC₅₀ < 1 mg ml⁻¹). Similar results were reported by Phoboo *et al.*, (2015) on same species, El-Amier and Abo Aisha, 2019; Alzuaibr *et al.*, 2020 and Salem *et al.*, (2016) on some xerophytes. The powerful antioxidant activity of shoot extract can be attributed mainly due to secondary compounds (Juan and Chou, 2010). Nowadays, wild plants are gaining popularity in developing countries as a medicinal food. Although medicinal plants may cause many biological activities in humans, very few are known. Although previous studies have shown that important

Table 2: Percentage of DPPH radical scavenging activity and IC₅₀ values of methanolic extracts of *Aizoon canariense*.

| Treatment | Concentration (µg ml ⁻¹) | Scavenging activity (%) | IC ₅₀ (mg ml ⁻¹) |
|--------------------------|--------------------------------------|-------------------------|---|
| <i>Aizoon canariense</i> | 1000 | 54.52±2.61 | 0.74 |
| | 800 | 51.68±1.87 | |
| | 600 | 49.88±1.22 | |
| | 400 | 47.67±0.98 | |
| | 200 | 20.16±0.60 | |
| | 100 | 12.24±0.18 | |
| Catechol | | | 0.15 |

Values are means ± standard error of triplicates. IC₅₀: the amount of sample necessary to decrease the absorbance of DPPH by 50%.

bioactive materials are present in methanol extract (Akowuah *et al.*, 2002).

Allelopathic activity

The germination percentage of *Zea mays* and two associated weeds (*P. oleracea* and *A. lividus*) were reduced under the effect of different concentrations (2.5-40 g l⁻¹) of *A. canariense* extract. The degree of inhibition was significantly increased in a concentration-dependent manner. *Zea mays* were more resistant to the allelopathic effect of the extract than the two other weeds at 4 DAT (Fig. 2 & 3).

The methanol extract of *A. canariense* at 40 g l⁻¹ inhibited the germination of *Z. mays*, *P. oleracea* and *A. lividus* by about 24.56%, 88.70% and 100%, while the lowest concentration (2.5 g l⁻¹) inhibited the germination by 16.95%, 40.80% and 73.19%, respectively (Fig. 2). However, the plumule length was reduced by 39.54%, 76.64% and 95.68%, while the lowest concentration reduce the plumule length by 14.65%, 28.65% and 35.89%, respectively. The root was more sensitive to the allelopathic effect compared to the shoot, where it was inhibited by 43.90 %, 100% and 100 %, for *Z. mays*, *P.*

oleracea and *A. lividus*, respectively, compared to control (Fig. 4). The allelochemicals may have phytotoxicity or stimulation depend on the nature and concentration of the compound, abiotic and biotic soil factors, the assay species, and physical/climatic factors (Abd El Gawad *et al.*, 2018a). Similar results were reported by Salem *et al.*, (2016), El-Amier and Abo Aisha (2019) and Alzuaibr *et al.*, (2020) on some desert plants.

From these results, the allelopathic effect of *A. canariense* could be attributed to several bioactive compounds that act in a synergistic manner or to compounds which regulate one another such as a flavonoid, phenolic acids, alkaloids, and tannins. Previous investigation of *A. canariense* revealed that the presence of gallic acid, chlorogenic acid, caffeic acid, protocatechuic acid and cinnamic acid (Phoboo *et al.*, 2015). Therefore, the reduction in the seedling growth of *Z. mays*, *P. oleracea* and *A. lividus* in this study may be attributed to reduction in cell division of the seedlings, altering the ultrastructure of the cells as well as led to alteration of the ion uptake, water balance, phytohormone balance, photosynthesis, respiration and inactivate several enzymes (Li *et al.*, 2010; Fahmy *et al.*, 2012). Generally, the extract of *A. canariense* showed more allelopathic activity against the tested weeds (*P. oleracea* and *A. lividus*) compared to the forage crop (*Z. mays*), where this may be useful in weed management at the field

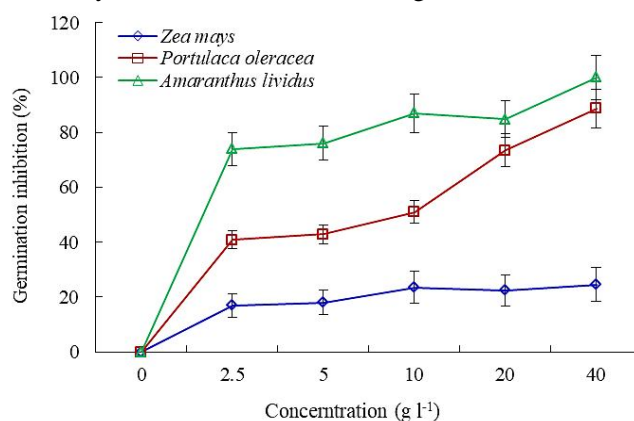


Fig. 2: Allelopathic effect of different methanol extracts from *Aizoon canariense* aerial parts on the seed germination inhibition percentage of *Zea mays* and two associated weeds after four days of treatment.

application.

Conclusion

The current study revealed that *Aizoon canariense* has appropriate amount of the proximate composition as well as macro- and micro-minerals. Based on *A. canariense* attained high content of various secondary metabolites (phenolics, tannins, flavonoids, and alkaloids),

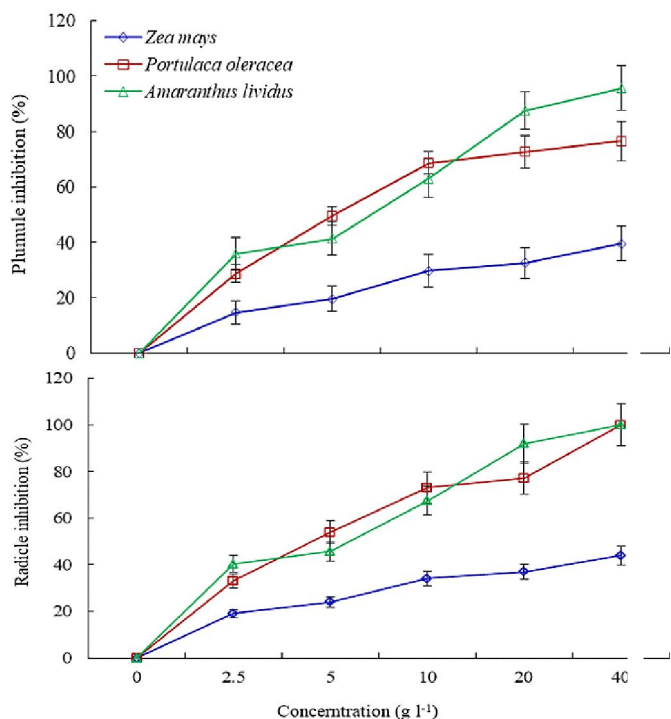


Fig. 3: Allelopathic effect of different methanol extracts from *Aizoon canariense* aerial parts on the plumule and radicle growth inhibition percentage of *Zea mays* and two associated weeds after fourteen days of treatment.

and therefore can be considered a good source of natural antioxidant. In our study, germination of *Zea mays* and two associated weeds were inhibited under treatment of *A. canariense* methanolic extracts at 40 g ml⁻¹. Moreover, both radicle and plumule were strongly inhibited under the same treatment. *Zea mays* were more resistant to the allelopathic effect of the extract than the two other weeds. Therefore, this species can be used in the method of biological control of weeds. Also, further studies are required to identify and characterize the proper allelochemicals and demonstrate their modes of action.

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