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Euphorbia retusa (Forssk.) A PROMISING SOURCE FOR BIOACTIVE COMPOUNDS IN BIOMEDICAL AND AGRICULTURE APPLICATIONS

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ABSTRACT

Euphorbia retusa (family *Euphorbiaceae*) is an annual plant in the desert of Egypt with a 20 to 60 cm high. The aerial part of the plant containing latex is used in many biological activities. This study aimed to determine the phytochemical constituents, antioxidant, antimicrobial properties and allelopathic activities of aerial parts of *E. retusa* collected from Wadi Arabah, North Eastern Desert, Egypt. Standard methods were used for the qualitative and quantitative of tannins, saponins, flavonoids, alkaloid, phenolics, steroids, terpenoids, and glycosides. The antioxidant activity was measured based on the reduction of DPPH.Antimicrobial activity was evaluated against pathogenic bacteria, as well as the allelopathic potential against *Chenopodium murale* was assayed. *E. retusa attained* the highest values of saponins, tannins, and phenolics compared. In the DPPH test system, the IC_{50} value of the antioxidant inhibition for *E. retusa* was 802.74µg mL^{-1}. The data obtained indicated that, the sensitivity of the seedling growth to the extract was higher than the germination of the test species. In addition, the root growth of C. murale was more affected than the shoot system to the inhibitory allelopathic activity induced by *E. retusa*. At higher concentration (40 g L^{-1}) the germination of the tested seed was highly inhibited by 51.77%, while the shoot and root were reduced by 60.98% and 74.55%, respectively. However, the opposite response was observed at the lower concentration (2.5 g L^{-1}).In conclusion, theextract of *E. retusa* plant has phytotoxic properties and thus contains phytotoxic substances.

Keywords: *Euphorbia retusa, Chenopodium murale*, phytochemical, phytotoxicity, antioxidant, antimicrobial

INTRODUCTION

The production of primary and secondary metabolites is a characteristic property of living organisms that could be utilized for pharmacological and technological purposes. These chemical compounds are called “natural products.” Bioactive compounds (natural products) are secondary metabolites, which possess biological activity, besides this, these secondary metabolites also contain the property of antioxidants and pharmaceutical actions on humans (Pereira *et al.*, 2012; Jain *et al.*, 2019). The great diversity of bioactive compounds reported in plant, which include a heterogeneous class of compounds mainly phenolics, carotenoids, tocopherols, phytosterols, and organosulfur compounds (Carbonell-Capella *et al.*, 2014; Shirahigue and Ceccato-Antonini, 2020).

Euphorbiaceae is one of the largest families among the Anthophyta, with 300 genera and 5000 species. The genus *Euphorbia* is sub cosmopolitan but with strong representationin the humid tropics and subtropics of both hemispheres (Uzair *et al.*, 2009).All contain latex and have unique flower structures (Chaudhry *et al.*, 2001; Barla *et al.*, 2006). The natural flora of Egypt includes 2145 species belonging to 775 genera and related to 129 families and it has been found that *Euphorbia* is the largest genus in the Egyptian flora, where it comprises 41 species (Boulos, 2009). *Euphorbia retusa* is an annual or short-perennial plant rising 20 to 60 cm high with erected stem, sessile leaves and small yellow green flowers (Zohary, 1972). It is distributed in North Africa, Palestine, Arabia to Pakistan; in Egypt it is found in Desert wadis, Sinai (Boulos, 2000). The aerial part of the plant containing latex is used for the treatment of neuralgia, cough and asthma. The plant is found to contain alkaloids, flavonoids, tannins, triterpenes, sterols and thirteen deoxyphorbol esters having skin irritant properties (Ageel *et al.*, 1985, Haba *et al.*, 2009; Refahy, 2011). *E. retusa* is used in coughs, asthmas, antiasthmatic, expectorant, nervine (Al-Shanwani, 1996).

Wild plants are one of the important components in the ecosystems. Literature indicates that plant secondary metabolism has been reported to possess various biological activities including antioxidant, allelopathic (Abd El-Gawad *et al.*, 2018 a & b; Elshamy *et al.*, 2019), antifungal, antibacterial, antiviral (El-Amier *et al.*, 2014;El-Amier and Abo Aisha, 2019), anti-inflammatory(Tohidi *et al.*, 2017), drugs (Zaki *et al.*, 2016a & b, 2017, 2018) 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), fragrance industries, and agro-industrial

(Zuin and Ramin, 2018; Alzuaibr *et al.*, 2020) and phytoremediation (El-Amier *et al.*, 2018a & b; El-Alfy *et al.*, 2020).

Management, control and phytosociology of natural vegetation have a great interest all over the world. 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 environmental characteristics comprising soil, climate, vegetation, animal and human interference (Clark, 2000; Zahran and El-Amier, 2013; El-Amier andEl Hayyany 2020).

Plant allelopathy is the 'chemical warfare' among the plants imposed by one plant on another to suppress the latter and take advantage from that suppression (Bachheti *et al.*, 2020). In this phenomenon, one organism produces certain types of specific biochemicals which affect the plant growth and development, plant diversity, dominance, succession, and climax of natural vegetation of neighboring plants and other species (Macías *et al.*, 2014; Abd El Gawad *et al.*, 2018a). Allelopathy can be used for beneficial purpose through using biochemicals as natural herbicides or pesticides. 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).

Therefore, our study aimed to determine the phytochemical constituents present in *Euphorbia retusa* collected from inland desert (Wadi Arabah, north Eastern Desert), Egypt, to evaluate the antioxidant activity, the antimicrobial potential against several pathogenic bacterial strains and the allelopathic effect of the plants extract against the noxious weed *Chenopodium murale* as potential green eco-friendly bioherbicide.

MATERIALS AND METHODS

Plant material: *Euphorbia retusa* Forssk. aerial parts were collected at a vegetative stage from different sites from Wadi Arabah, North Eastern Desert, Egypt. The identification of species was made according to Boulos (2000) by Dr Yasser A. El-Amier, Lecturer of Plant Ecology, Faculty of Science, Mansoura University. 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

Qualitative analysis: Phytochemical screening for the presence of alkaloids, saponins, tannins, phenolics, anthraquinone, steroid, flavonoids, glucosides and terpenoids in the studied species was done using the standard methods described by Sofowora (1996); Williamson *et al.* (1996); Harborne (1998); Evans (2000); Banso and Ngbede (2006); Rasineni *et al.* (2008).

Quantitative estimation of some secondary compounds: The total phenolics were measured according to the method adopted by Chlopicka *et al.* (2012), the total flavonoids were estimated according to Stankovic *et al.* (2011), and the alkaloids were measured according to the assay adopted by Joshi *et al.* (2013). Tannins were measured according to Van Buren and Robinson (1969), while saponin content was estimated by the method adopted by Obdoni and Ochuko (2001).

Antioxidant activities: The antioxidant activity was determined in methanolic extract of dried plant by using a stable free radical (1,1-diphenyl-2-picrylhydrazyl) DPPH as described by Miguel (2010).

Antibacterial Assay: Plant extract was prepared by standard method of Su and Horvat(1981), and the dried residue was dissolved in dimethyl sulfoxide (DMSO) and reserved at -20^{o}C for future use (Kant *et al.*, 2015). The plant extracts were examined for the presence of antimicrobial bioactivity by the filter paper discs method as described by Cappuccino and Sherman (2008) using different bacterial species (*Klebsiella pneumonia* (ATCC10031), *Listeria monocytoyenes* (ATCC19116), *Escherichia coli* (ATCC10536), *Salmonella typhi* (ATCC25566), *Pseudomonas aeruginosa* (ATCC9027), *Streptococcus epidermis* (EMCC1353^{t}), *Staphylococcus aureus* (ATCC6538)and *Bacillus subtilis* (DMS1088). The tested samples were taken from the Laboratory of Bacteriology, Department of Botany, Faculty of Science, Mansoura University, Egypt.

The Activity Index (AI) was used as a parameter for measuring the antibacterial potential of the studied extracts in comparison with standard antibiotics (Shekhawat and Vijayvergia 2010).

Allelopathic activity: *Chenopodium murale* seeds were gathered from cultivated fields in Mansoura city, Egypt. Uniform and ripened seeds were sterilized by immersing in NaOCl (0.3%) for three minutes then washed by bi-distilled sterilized water several times. The sterilized seeds were dried and kept for future 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.

% Inhibition percentage = [(CG – TG /CG) ×100]

% Growth inhibition = [(LC – LT)/LC) ×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.

2.6. 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 probabilitylevel.

RESULTS AND DISCUSSION

Phytochemical constituents: All results of phytochemical analysis of *Euphorbia retusa*aerial parts are showed in the Table1. This indicates that *E. retusa* contains a wide range of phytochemicals. In this study, the use of different solvents showed different response for the presence of phytoconstituents and according to the intensity of colour or precipitates produced the terms of scores are using as -, +, ++, +++. In the present study the results showed that maximum three phytocompounds i.e. phenols, saponins and tannins were present in the ethanolic extract of *E.retusa*while three minimum phytocompounds, i.e., alkaloids, flavonoids, terpenoids were present. On the other hand, there are three phytocompounds are absence from methanolic extract (Table 1). The quantitative analysis of aerial parts of wild *E. retusa*was shown in Figure 1. The results showed that the aerial parts mainly contained saponins (29.14 mg g^{-1}) and tannins (24.28 mg g^{-1}). While, the values of flavonoids, alkaloids and phenolicswere 19.44, 12.33 and 10.29mg g^{-1} dry weight, respectively. These results were consistent (compatible) with the results of Gapuz and Besagas (2018) on same genus *Euphorbia* (*Euphorbia milii, E. trigona*and *E. antiquorum*) and El-Amier *et al.* (2014) on *Senecio glaucus* (Asteraceae) present in the arid regions, but they were lower than some wild plants such as *Aizooncanariense, Diplotaxisharra* (Alzuaibr *et al.*, 2020; El-Amier and Al-hadithy, 2020).

The variation in the levels of the phytochemicals could be a result of a number of factors. They could be dependent on location,season, climatic factors,properties of phytochemicals, extraction method, the solvent used, time and temperature of extraction (Mašković *et al.*, 2016; Mostafa *et al.*, 2018). Numerous studies have confirmed that saponins and tannins possess the unique property of precipitating and coagulating red blood cells, hasten the healing of wounds and inflamed mucous membranes (Okwu, 2004; Yadav *et al.*, 2014).

Antioxidant activity: The plants and their products are found throughout human history as herbal supplements as botanicals, nutraceuticals, and drugs (Ekor, 2014). Natural antioxidants are commonly derived from plant sources, and the efficacy is determined by plant species, variety, extraction and/or processing methods, and the growing environment(Said *et al.*, 2002). 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 53.76% while, the lowest concentration (100 µg ml^{-1}) showed the lowest antioxidant activity (16.04%).

The IC_{50} values of *E. retusa*extract was 802.74µgmL^{-1} compared to standard catechol (416.38µgmL^{-1}) (Table 2). These results suggest that methanol extract of *E. retusa*has an obvious effect on scavenging of DPPH radical (IC_{50}<1 mg ml^{-1}). Similar results were reported by Basma *et al.* (2011) on *E. hirta*, El-Amier and Abo Aisha 2019; Alzuaibr *et al.* 2020 and Salem *et al.* (2016) on some xerophytes. Strong radical scavenging activity is associated with a high content of polyphenols (Dinda *et al.*, 2016; Moldovan *et al.*, 2016). Polyphenolic compounds, at least in part, are responsible for the high antioxidant activity of plants.

Antibacterial activity: Antimicrobial agents are primarily important in reducing the global burden of infectious disease. Microorganisms can cause various diseases in human beings from mild to acute and chronic (Bhatia and Narain, 2010; Rook *et al.*, 2017). The antibacterial activity of crude extract of *E.retusa* was estimated in vitro using 8 different pathogenic bacterial strains. In this study, the extract of *E. retusa* aerial parts showed a significant antimicrobial inhibitory effect ($P < 0.05$) on growth of the tested Gram positive and negative bacteria, as well as exhibited broad antimicrobial spectrum against most of the tested bacterial strains (Table 3). The ethanolic extract of *E. retusa* expressed the highest zones of inhibition against the tested gram positive bacteria *S. epidermis* (19.5±0.35 mm) and gram negative bacteria *E. coli* and *S. typhi*(22±0.0 and 26.5±0.35 mm, respectively). On the other hand, *S. typhi* was the most sensitive bacteria, while *K. pneumonia* and *P. aeruginosa* were the most resistant bacteria among the tested strains.

Table 1. Qualitative phytochemical screening of *Euphorbia retusa*

Phytochemical screened	*Euphorbia retusa*
Alkaloid	+
Saponins	+++
Tannins	++
Flavonoids	+
Phenolics	++
Glycosides	-
Anthraquinone	-
Steroids	-
Terpenoids	+

(+): presence; (++): considerable presence; (+++): abundance; (-): absence

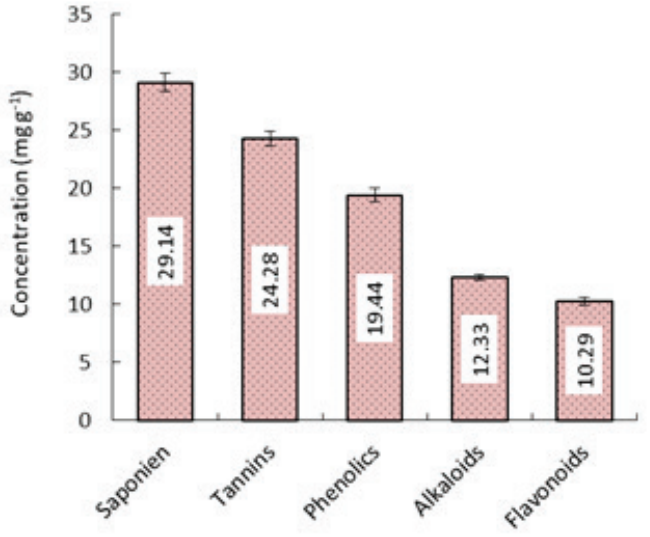

Figure 1. The active secondary constituents in *Euphorbia retusa*

This result was similar to those of other studies that reported the antibacterial activity of methanolic extract of *Senecio glaucus* and *Fagonia* species (El-Amier *et al.*, 2014; El-Amier and Abo Aisha, 2019). However, contrary to our result, they also reported antibacterial activity of methanolic extract of *Pergularia tomentosa* against the same bacteria (Alghanem and El-Amier, 2017). It is obvious from the results that that gram-negative bacteria are more sensitive to three plant extracts than gram-positive bacteria and these results agrees with the previously reported by El-Amier *et al.* (2014), Kaneria *et al.* (2009) and Kumar *et al.* (2016). Manandhar *et al.* (2019) reported that the nature of the cell wall of gram-negative bacteria make them more susceptible to different compounds than gram positive bacteria. The antimicrobial activity of plants seems to depend on the efficacy of the extraction, the solvent used and the metabolic activity of the tested microbes (Cowan 1999; Kaneria *et al.* 2009).

Moreover, the significance of using the prepared extract of *E. retusa*in comparison with the standard antibiotics (penicillin, gentamicin and chloramphenicol) against the tested pathogenic bacterial strains was estimated using the activity index (Table 4). The activity index varied in gram positive from 0.00 to 1.61 and in gram-negative bacteria from 0.00 – 3.79. The maximum activity index values were observed against *P. aeruginosa* and *L. monocytoyenes* (3.79 and 2.14, respectively) while the lowest activity index value was for *E. coli* (0.05). Penicillin expressed low effects against the tested pathogenic strains. Activity index values above one expressed higher potential of herbal extract, while those below one expressed higher antibiotics potential against tested pathogenic strain (Shekhawat & Vijayvergia 2010). The results obtained confirmed the potency of using the studied extract compared to the antibiotic penicillin.

Allelopathic activity on the germination and seedling growth of *Chenopodium murale*: The methanolic extract of *E. retusa* aerial parts showed

Table 2. The antioxidant scavenging activity of *Euphorbia retusa*

Treatment	Concentrations (µg mL^{-1})	% of DPPH scavenging	IC_{50} (μgmL^{-1})
Euphorbia retusa	100	16.04±0.76	802.74
	200	22.01±1.05	
	300	32.95±1.57	
	400	35.50±1.69	
	600	46.17±2.72	
	800	50.54±2.97	
	1000	53.76±3.16	
Catechol	50	16.17±0.70	416.38
	100	20.27±0.88	
	200	29.40±1.28	
	300	37.25±1.62	
	400	50.00±2.17	
	500	57.72±2.51	

Values are means ± standard error (n=3). IC50: the antioxidant concentration capable of diminishing 50% of the used DPPH radical.

significant phytotoxic effect against on the germination and seedling growth of *C. murale* (Figures 2) at different concentrations (2.5 to 40 mg ml^{-1}) over control. The sensitivity of the seedling growth to the extract was higher than the germination of the test species. In addition, the root growth of *C. murale* was more affected than the shoot system to the inhibitory allelopathic activity induced by *E. retusa* (Figure 2). 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.

At higher concentration (40 mg ml^{-1}) the germination of the tested seed was highly inhibited by 51.77%, while the shoot and root were reduced by 60.98% and 74.55%, respectively (Figures 2). However, the opposite response was observed at the lower concentration (2.5 g L^{-1}). The results of thepresent research against the weed *C. murale* agreedwith the most of the previous results obtained by otherresearchers, which emphasized that extracts of manyplant species inhibited germination of *C. murale* seeds (Arora *et al.*, 2017; Abd El-Gawad *et al.*, 2018a; Abd-El-Gawad *et al.*, 2019; Abd-ElGawad *et al.*, 2020a).In addition, the aerial part of the plant containing latex, alkaloids, flavonoids, tannins, triterpenes, sterols and thirteen deoxyphorbol esters (Ageel *et al.*, 1985, Haba *et al.*, 2009; Refahy, 2011).

These results indicated that *E. retusa* plant extracts have phytotoxic properties and thus contain phytotoxic substances. The concentration dependent inhibitory activities of allelopathic plant extracts on germination and seedling growth were also reported by Soltys *et al.* (2012) and Abd-ElGawad *et al.* (2020b). Therefore, the plant

Table 3: The antibacterial potential of *Euphorbia retusa* extracts against the tested pathogens

Tested microorganisms	Plant extract	DMSO*	Inhibition zone of the standard antibiotic			LSD0.05
			Penicillin	Gentamicin	Chloramphenicol	
Gram negative bacteria						
Escherichia coli (ATCC10536)	22±0.0	n.a	15±0.17	20±0.13	22±0.15	1.99***
Listeria monocytoyenes (ATCC19116)	15±0.0	n.a	7±0.06	22±0.09	n.a	1.63***
Pseudomonas aeruginosa (ATCC9027)	10.5±0.35	n.a	7±0.04	18±0.07	24±0.08	1.20***
Klebsiella pneumonia (ATCC10031)	10.5±1.06	n.a	n.a	21±0.10	32±0.14	3.15***
Salmonella typhi (ATCC25566)	26.5±0.35	n.a	11±0.08	24±0.07	21±0.21	1.29***
Gram positive bacteria						
Bacillus subtilis (DMS1088)	15±0.71	n.a	n.a	9±0.04	18±0.14	1.83***
Streptococcus epidermis (EMCC1353t)	19.5±0.35	n.a	n.a	30±0.11	29±0.11	1.41***
Staphylococcus aureus (ATCC6538)	14.5±1.06	n.a	17±0.05	26±0.13	27±0.12	3.07***

Disc diameter 6mm was subtracted from each obtained value. Values are calculated as means ± standard error of triplicates. n.a: Not active, values of significant variation at $p < 0.05$.

Table 4: Activity index analysis of *Euphorbia retusa* extracts against human pathogenic bacteria

Pathogens	Methanol extract	Antibiotics (IZ)
Klebsiella pneumoniae	-	Penicillin G (0)
	1.05	Gentamicin (21)
	0.69	Chloramphenicol (32)
Listeria monocytoyenes	2.14	Penicillin G (7)
	0.65	Gentamicin (22)
	-	Chloramphenicol (0)
Escherichia coli	0.70	Penicillin G (15)
	0.48	Gentamicin (20)
	0.05	Chloramphenicol (22)
Salmonella typhi	0.95	Penicillin G (11)
	0.44	Gentamicin (24)
	0.50	Chloramphenicol (21)
Pseudomonas aeruginosa	3.79	Penicillin G (7)
	1.47	Gentamicin (18)
	1.10	Chloramphenicol (24)
Streptococcus epidermis	-	Penicillin G (0)
	0.50	Gentamicin (30)
	0.52	Chloramphenicol (29)
Staphylococcus aureus	1.15	Penicillin G (17)
	0.75	Gentamicin (26)
	0.72	Chloramphenicol (27)
Bacillus subtilis	-	Penicillin G (0)
	1.61	Gentamicin (9)
	0.81	Chloramphenicol (18)

E: extract; A: antibiotics; E > A and > 1 values indicate extracts have higher effect against bacterial pathogens in comparison with antibiotics; E < A and < 1 values indicate antibiotics have higher effect against bacterial pathogens in comparison with extracts, IZ= inhibition zone.

could be served as an important candidate for isolation and identification of allelopathic substances, which may promote the development of new natural herbicides. Besides this, farmers will get benefits from the plant residues such as bioherbicide for weeds,

CONCLUSION

Wild plants have significant economic and medicinal uses and possess the high biological activity and antioxidant compounds. Besides this, weed management is one of the most challenging tasks in crop production.

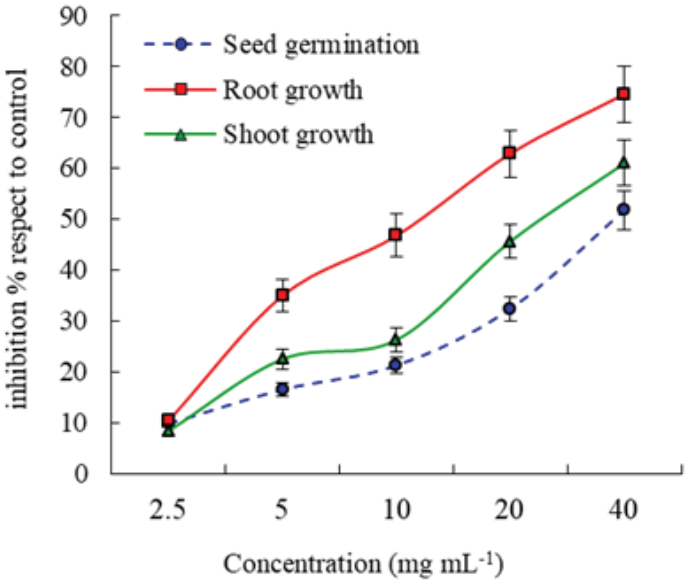

Fig. 2: Allelopathic effect of different methanol extracts from *Euphorbia retusa aerial* parts on the seed germination and seedling growth inhibition percentage of C. murale after fourteen days of treatment.

Natural products are historically successful and are still a viable alternative to synthetic antibiotics and antioxidants. Moreover, the overuse of synthetic herbicides causes severe environmental pollution. In the present study, *E. retusa* acts a promising role. Therefore, further studies will be undertaken to isolate, characterization of phytotoxic substances from *E. retusa*, and evaluate the biological activities of these secondary metabolites in these species.

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