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Full Length Research Paper

Phytotoxic activity of crude methanolic extract of *Euphorbia prostrata* collected from Bannu District (Pakistan)

Rahmat Ali Khan[1], Abdus Saboor shah[1], Mushtaq Ahmad[1]*, Farid Ullaha Khan[1], Noor Aslam[1], Muhammad Rashid Khan[2] and Mir Sadiq Shah[1]

[1]Department of Biotechnology, Faculty of Biological Sciences, University of Science and Technology, Bannu, Khyber Pakhtunkhwa, Pakistan.
[2]Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University Islamabad, Pakistan.

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Phytotoxic activity of crude methanolic extract of *Euphorbia prostrata* from Euphorbiacae was tested on the germination of wheat seeds and on the growth of the germinated seedlings. In both the field and plate studies, the extract showed inhibitory effect on the germination of the growth of root and shoot of the seedlings. The inhibition was found to be dose dependent. The higher concentration of 1000 µg/ml showed maximum inhibitory effect on the growth of root and shoots in the studies of plate as well as on fresh and dry weight of wheat plant. Similarly, the herbicidal activity is also dependent on the concentration of extract. In this study, it was found that inhibitory potential of methanolic extract of *E. prostrata* increases as1000 >100 >10 µg/ml.

Key words: Phytotoxic activity, herbicidal activity, germination, plates and field studies, *Euphorbia prostrate*.

INTRODUCTION

Phytotherapy means the utilization of bioactive components which are extracted from natural product and which are used as medicine to cure a range of diseases. Phytotherapy, usually known as alternative medicine and considered as an essential part of modern pharmaceutical drugs according to the WHO herbal drugs, contains active ingredients plant materials in the crude state and certain excipient solvents, diluents or preservative. Almost all the active principles responsible for the pharmacological action are known (Bulletin of the WHO, 1998, 1993; Khan et al., 2010a, b). Medicinal plants have played a key role in maintaining and promoting human health and improving the quality of human life for thousand of years (Tyler, 1994; Bruneton, 1995). According to rough estimate, about 25% of all modern medicines are directly or indirectly extracted from medicinal plants for health care (Ahmed et al., 2011a,b).

*Corresponding author. E-mail: mushtaq213@yahoo.com. Tel: 92 928633425.

The secondary metabolites of plant provide protection against infections and are a source of plant survival (Ciccia et al., 2000; Luthria et al., 1993). These secondary metabolites behave as allelochemicals (Khan et al., 2010c, 2011a). The plants' extracts can act as herbicides and have great agricultural applications (Khan et al., 2007, 2011b). The natural phenomenon in which living beings affect the growth and reproduction of neighboring organism by the releasing of allelochemicals is known as allelopathy. Allelochemicals are the secondary metabolites which may have positive or harmful effects on the neighboring organism (Stamp, 2003; Khan et al., 2011c). Alleochemicals are essential for defense against herbivory (Stamp, 2003; Fraenkel and Gottfried, 1959). Allelochemicals are present in all parts of plants like leaves, stem, root and flower and have broad spectrum inhibitory effects (Atam et al., 2001; Khan et al., 2009; Sahreen et al., 2010). Synthetic herbicides cause problem to human beings and to the environment. Therefore, it is necessary to investigate non-toxic and environmental friendly compounds (Vyvyan et al., 2002;

Ahmed et al., 2010). Actually, the continuous use of artificial herbicides not only damages the agriculture products, but also causes serous biological and environmental problems (Angelini et al., 2003). It is thus necessary to study and describe the phytotoxic activity of plants and their compounds. Bioactive terpenoids play a key role in the defense system of many organisms and have effective use in agriculture and pharmaceutical fields (Tellez et al., 2002); and a large number of extremely phytotoxic compound (allelochemicals) are the derivative of terpenoid (Duke et al., 2000). That is why the plants that contain terpenoids are reported to have phytotoxic activity (Angelini et al., 2003; Arminant et al., 2006; Azirak et al., 2008; Duke et al., 2004). *Euphorbia prostrata* is a small prostrate, annual herb found all over the world. This plant has traditionally been used to treat several ailments from ancient time. The active constituents in *E. prostrata* are chiefly flavonoids, phenolic acid and tannins.

MATERIALS AND METHODS

Plant collection

Plant of *E. prostrata* was collected from Bannu District and Lakki Marwat in December 2010, identified by Mr. AbdurRehman, Chairman, Department of Botany, Government Post Graduate College, Bannu. It was dried under shadow at room temperature for 20 days and than grinded into very fine powder.

Plant extraction

600 g of plant powder of *E. prostrata* plant was socked in 2.5 L 80% methanol, with random shaking for a period of 7 days. The plant was extracted and filtered by using Whatman filter paper No 1. The filtrate of the sample was concentrated by using rotary evaporator at 37°C to obtain crude extract, which was 30 g.

Phytotoxicity bioassay/herbicidal bioassay

Stock solution preparation

5 mg/5 ml methanol stock solution is prepared, which is further subjected to the preparation of three other solutions of 10, 100 and 1000 µg/ml.

Experimental procedure

In this bioassay, eight Petri plates were taken and set with what man filter paper, for three different concentrations of 10, 100 and 1000 µg/ml. Now, 5 ml of each concentration is taken and put on Petri plate of each concentration. After that the Petri plates were kept for drying and the whole amount of methanol was evaporated. 5 ml of distilled water was put on each filter paper and set in Petri plates of all the three concentrations as well as in the control. Then, the seeds of wheat were wet in distilled water for one hour and eight seeds were put in the Petri plate of each concentration (10, 100 and 1000 µg/ml) as well as in the control. All the concentration and control were in duplicate; after that they were kept in incubator at 28°C for 72 h.

Allopathic activity in field study

For field study, 8 plastic bottles are set and 100 g soil was put in each one. 25 ml of distilled water was added to wet the soil, and then eight seeds of wheat were sown in it before proceeding for field study. A total of 8 bottles were set for three different concentrations of 10, 100 and 1000 µg/ml, with two for control.

RESULTS

Plate study for herbicidal activity of *E. prostrata*

The herbicidal potential of the extract derived from the *Euphorbia prostrata* plants was determined in Petri plates containing filter paper and extract of three different concentrations of 10, 100 and 1000 µg/ml. The results indicate that methanolic extract of *E. prostrata* possesses good herbicidal activity and has tremendous phytotoxicity at the highest concentration of 1000 µg/ml after 3rd day at which the shoots growth is only 2.5 cm and roots growth is 2.97 cm. This revealed that the growth of wheat is highly inhibited by methanolic extract of *E. prostrata*. The same concentration of extract also showed a good activity and inhibited the growth of shoots and roots after 5th day at which the shoot length was only 6.8 cm and root length was 4.65 cm. At the concentration of 100 µg/ml, the growth of roots and shoots of wheat was moderately inhibited after 3rd and 5th days. While at 10 µg/ml, only a weak inhibitory activity was observed in the roots and shoots of wheat plant after 3rd and 5th days, which is nearly equal to the control as shown in Table 1.

Phytotoxic activity

The herbicidal activity of the methanolic extract of *E. prostrata* was evaluated. The results are shown in Figure 1. The result obtained showed that the extract possesses excellent herbicidal activity. Figure 1 shows that *E. prostrata* has tremendous phytotoxic activity at the highest concentration of 1000 µg/ml and inhibits the growth of wheat. At this concentration, the shoot growth of wheat is inhibited; the plant grows to only 3.4 cm after fifteen days and 4 cm after 25 days. At 100 µg/ml, a reasonable phytotoxicity was shown which is 5.6 cm by 15 days and 7.1 cm after 25 days. While at 10 µg/ml, only a weak inhibitory activity was observed which is 7.5 cm after 15 days and 9.5 cm after 25 days. The shoot growth of control is 8.85 cm and 11 cm after 15 and 25 days, respectively.

The methanolic extract of *E. prostrata* also showed a tremendous herbicidal activity in fresh and dry weight of wheat during its plate study. Figure 2 reveals that at 1000 µg/ml, the phytotoxicity was maximum. At this concentration, the fresh weight was 0.36 g and dry weight was 0.03 g. At 100 µg/ml, the extract showed moderate phytotoxic activity and at this concentration the fresh weight was 0.57 g and dry weight was 0.05 g. The

Table 1. The herbicidal screening of *E. prostrata* during plate study.

Day	Parts studded	Control (µg/ml)	10 (µg/ml)	100 (µg/ml)	1000 (µg/ml)
3	Shoots	3.4±0.08	3.0±0.06	2.8±0.03	2.5±0.01
	Roots	3.4±0.08	3.4±0.08	3.0±0.06	2.9±0.01
5	shoots	8.7±0.3	8±0.1	7.6±0.09	6.8±0.09
	Roots	5.19±0.07	5.1±0.06	4.95±0.05	4.65±0.04

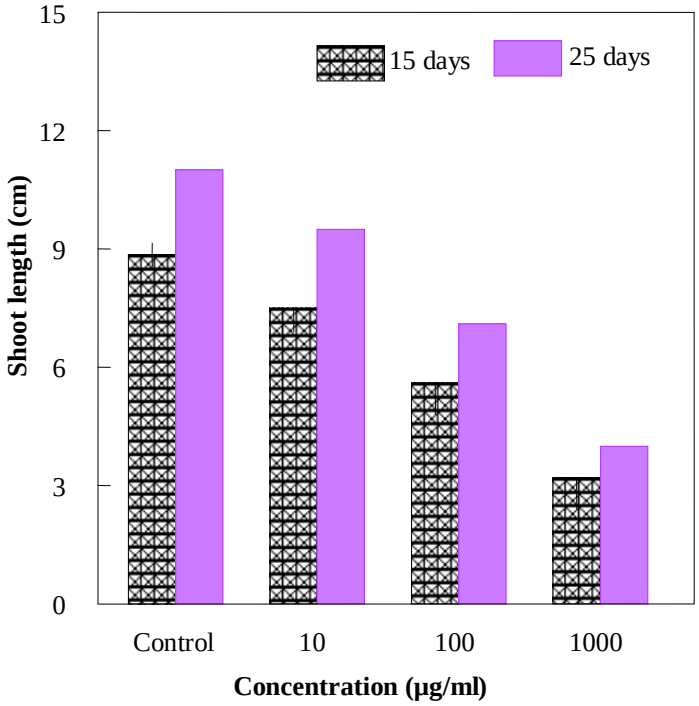

Figure 1. Effect of *E. prostrata* on growth of wheat.

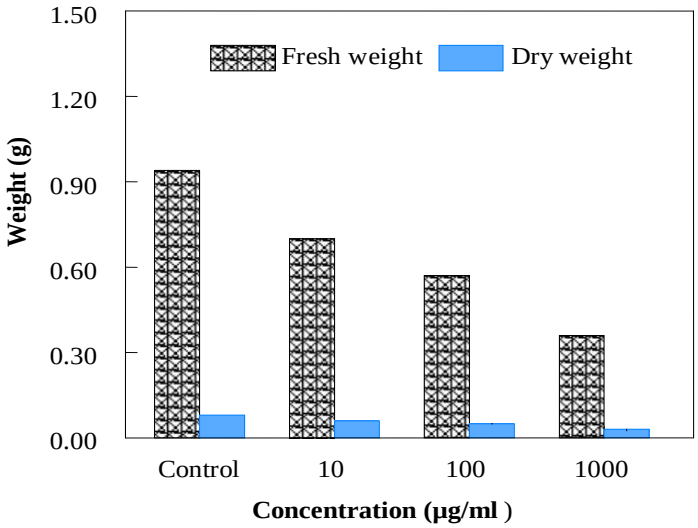

Figure 2. Effect of *E. prostrata* on the dry and fresh weight of wheat.

minimum activity was shown at 10 µg/ml at which fresh weight was 0.70 g and dry weight was only 0.06 g. The control in this activity was distilled water, having fresh weight of 0.94 g and dry weight of 0.08 g.

DISCUSSION

Native medicinal plants of Pakistan play important role in various human ailmants such as nephrotoxcity (Khan et al., 2009; Khan et al., 2010; Sahreen et al., 2011a), pulmonary oxidative damages (Khan et al., 2011a), cardio toxicity (Khan et al., 2011), antioxidant (Sahreen et al., 2011b,c;), adrenal toxicity (Khan et al., 2011b), phytoxicity (Khan et al., 2010c; Khan et al., 2011d; Khan et al., 2011e; Ahmed et al., 2011). Medicinal plants as appreciable source of antioxidant phytochemicals have received growing attention as potential chemo preventive agents. Epidemiological investigations have reported that antioxidants have a protective role in most of health diseases. Today, 30% of drugs are obtained from medicinal plants (Grabley and Thiericke, 1999). Researchers have focused on introducing new antimicrobial drugs from natural resources to overcome the emerged antibiotic resistant strains as well as adverse side effects of synthetic drugs. The data of our current study revealed that methanolic extract of plant has tremendous antibacterial property against some clinically important pathogens. The present study is well supported by Narod et al. (2004) who reported that methanolic extract of leaf and stem of *Toddalia asiatica* contained activity against gram positive and gram negative bacteria. Fungi make the food materials unfit for human by lowering their nutritive value. The collected data suggested that plant extract was tremendous in preventing bacterial and fungal growth, probably due to bioactive components like flavonoids, terpenoids and saponins. Several investigations attributed the antimicrobial activity of plant extract to the presence of phenolic compounds like flavoonids and saponins with antifungal activities.

Conclusion

The results of the present study revealed that crude methanol extract of *E. prostrate* play a crucial role in phytotoxicity and further work on isolation and characterization of the bioactive constituent responsible for this activity is in progress.

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