

Full Length Research Paper

Phytotoxic characterization of crude methanolic extract of *Periploca aphylla*

Rahmat Ali Khan^{1*}, Noor Aslam Khan¹, Mustaq Ahmed¹, Muhammad Rashid Khan²,
Farid Ullah Khan¹, Abdus Saboor Shah¹ and Mir Sadiq Shah¹

¹Department of Biotechnology, Faculty of Biological Sciences, University of Science and Technology, Bannu, Khyber Pakhtunkhwa, Pakistan.

²Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan.

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***Periploca aphylla* is traditionally used in the treatment of various ailments. Phytotoxic activity of crude methanolic extract of *P. aphylla* 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 *P. aphylla* increased as 1000 >100 >10 µg/ml.**

Key words: Phytotoxic activity, herbicidal activity, germination, plates and field studies, *Perifloca aphylla*.

INTRODUCTION

Allelopathy is the chemical relationship between the different plants species, to express the harmful, stimulatory enhanced and beneficial effects of one plant species upon the other by formation and releasing of specific chemicals into the environment (Molisch, 1937). According to Nandakumar, (2009) allelopathy is stated as, the direct or indirect interaction among the plants or organisms through chemicals and their released breakdown metabolites. Thus, these released chemicals affect the physiological processes of the neighboring plants and or organisms. Generally, the chemical interactions among the different living organisms such as plants, microorganisms and insects are known as allelopathy, and the different organic compounds which are used in allelopathy, are known as allelochemicals (Fujii and Hiradate, 2003). It was reported that in allelopathy process, the different chemicals are released into the environment by the aerial or underground parts of

the plants in the form of root exudation, leaching by dews and rains, and volatilization or decaying plant tissue. Thus, these release chemical compounds into the environment affecting other organisms, such as weeds, plants, animals and microorganism by inhibiting or exciting their life processes. Nazir et al. (2006) shows that these released chemicals accumulate and persist or stay for a long period of time and thus provide a strong interference on the growth and development of neighboring weeds and plants.

Many allelochemicals of plant sources exert their influence by such a mechanism which is not shown by commercial herbicide. Thus for new herbicide discovery, these natural allelochemicals play the role of ideal lead compounds. Therefore recently, scientists have focused their great attention on searching for new secondary plant products to develop bio-herbicides and bio-pesticides. To enhance the synthesis and exudation of allelochemicals, the two major factors, genetic characteristics and environmental conditions have played a very important role in this field. Many plants of different species are studied and reported to exhibit allelochemicals/allelopathy.

*Corresponding author. E-mail: Rahmatgul_81@yahoo.com.
Tel: 92 928633425.

Recently, scientists have focused their attention greatly to enhance the crop production and thus by full, filling the demands of the world growing population positively. But unfortunately, crop production decreases every year due to the different types of insects and plant diseases caused by various pathogens and slow biodegradation of synthetic herbicides. To control these serious flaws, researchers have focused their attention greatly on allelochemicals and bio-herbicides, for which the different plant species are responsible for their product. In short, medicinal plants produced different types of secondary metabolites which can be used to better humanity. Medicinal plants play an important role in modulation/treatment of various diseases. In the present study some important biological activities of methanolic crude extract of plant were explored *in vitro* assays.

Periploca aphylla Dcne is applied as a poultice in tumor and swellings/obstivity, while the decoction of the bark is used as a purgative or against fever (Kazimier, 1996). The present study was therefore arranged to investigate the phytotoxic efficacy of the extract.

MATERIALS AND METHODS

Plant collection

Plant of *P. aphylla* was collected from the main Township campus of the University of Science and Technology Bannu Khyber Pakhtunkhwa Pakistan in the month of July, 2010. The plant was identified by Taxonomist Prof Abdur-Rahman, Chairman Department of Botany Government Post Graduate College Bannu. The plant materials were washed by deionised water and were shade dried at room temperature for two weeks, chopped and ground mechanically to mesh size of 1 mm.

Preparation of plant extract

2 kg powder of *Periploca aphylla* was extracted in 3 L of 70% methanol by random shaking. After a week, the extract was filtered by using Whitman filter paper No. 1. After filtration, the filtrate was further concentrated by using rotary vacuum evaporator at 38°C in order to get the methanolic crude extract of the plant. The methanolic crude extract was stored at 4°C in the refrigerator for further investigation.

In vitro phytotoxic assay

Methanolic crude extract of *P. aphylla*, wheat seed, dis:H₂O, HgCl₂, methanol, micro pipettes, tips, autoclaved Petri plates, filter paper, beakers, electronic digital balance, mud, disposable glasses, spray bottle, and magnetic stirrer, according to the modified protocol of McLaughlin and Rogers (1998) was used in this experiment. For this experiment, first of all, filter papers were set in the autoclaved Petri plates. The experiment was performed in triplicates. 5 ml from 10 µg/ml solution was sprayed/poured on the filter paper of each Petri plates very carefully by micro pipette. The same process was repeated for 100 and 1000 µg/ml Petri plates already labeled. But for the control, Petri plates were not treated by the sample solution. All the treated Petri plates were placed in the oven at 40°C for complete evaporation of methanol from the filter papers. After complete evaporation, then 5 ml of distilled water were sprayed/

poured in all the treated Petri plates as well as the three controlled (not treated) Petri plates. Then, eight wheat seeds, first washed by distilled water and then by 1% HgCl₂ solution, were placed in each plate at equal distance. Then, all the Petri plates were incubated in growth room for five days. After three days, hypocotyls/shoot and radical/root inhibition was noted by ruler with respect to control and was averaged and again after five days, the inhibition was noted by the same way and average was taken. Similarly, the fresh and dry weight of all the treated and controlled fractions was recorded by using electronic digital balance and was averaged. Then, the difference between the fresh and dried weight of the treated fractions with respect to the control was examined.

In vivo phytotoxic assay

This experiment was also performed in triplicates. 102 g mud was put in each disposable glass labeled as control and sample treated. Then, seven clean wheat seeds were sown in each glass after adding 25 ml dH₂O. After this, all the glasses were kept in the open environment. After seven days, length of hypocotyls/shoot of all the grown wheat seeds were noted by ruler and average was taken. Then, 5 ml sample solution of each concentration was sprayed separately on the concerned hypocotyls/shoot of grown wheat of labeled glass except the controlled one. The control (not treated) was sprayed by 5 ml of distilled water. After seven days, the length of hypocotyls/shoot of grown wheat of all the treated and control was measured by ruler carefully once again and average was taken. The difference was noted between the treated, and non-treated with respect to the control. Then, the fresh weight of the entire treated and control fraction was recorded very carefully by digital electronic balance separately and average was made. After this, all the treated and control fractions were packed separately and were placed in the oven at 80°C for several hours until complete dryness. After complete dryness, the total dry weight was noted by digital balance and was averaged. Then the difference was noted between the weights of treated fractions with respect to the control.

Statistical analysis

Data of *in vitro* assays recorded were analyzed with the help of slide writer and SPSS 13.0 a computer software was used for one way analysis of variance and checked significance at 0.05%, 0.01% level of probability among the various treatments.

RESULTS AND DISCUSSION

Phytotoxic activity of *Periploca aphylla* Petri plate study

Three different concentrations (10, 100, and 1000 µ/ml) were used for the phytotoxic efficacy of *P. aphylla* methanolic extract (PAME). The result reveal that the extract significantly ($P>0.05$) inhibited the shoot (hypocotyls) growth compared to the control as shown in Figure 1 after three and five days. The data also indicate that PAME significantly inhibited roots growth as compared to non treated water (control) group as shown in Figure 2. After complete treatment, fresh and dry weight of all replicates were calculated which exposed that PAME extensively ($P<0.05$) controlled the fresh as well dry weight (Table 1). Our findings show similarity

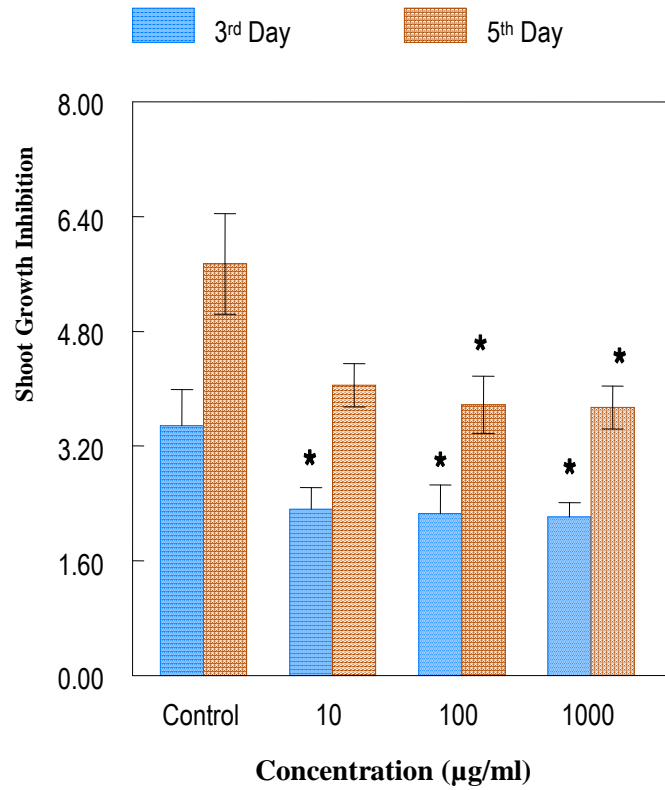


Figure 1. Shoot growth of various groups after the 3rd and 5th day treatment.

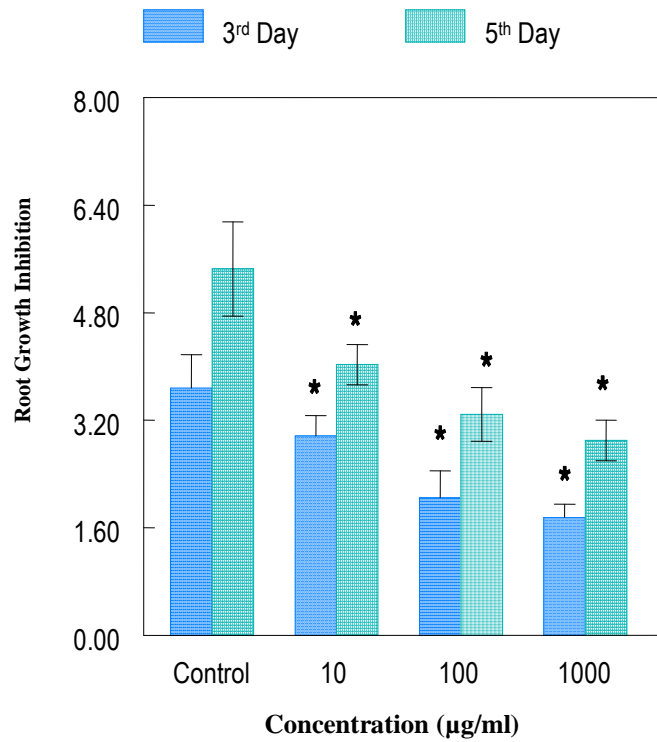
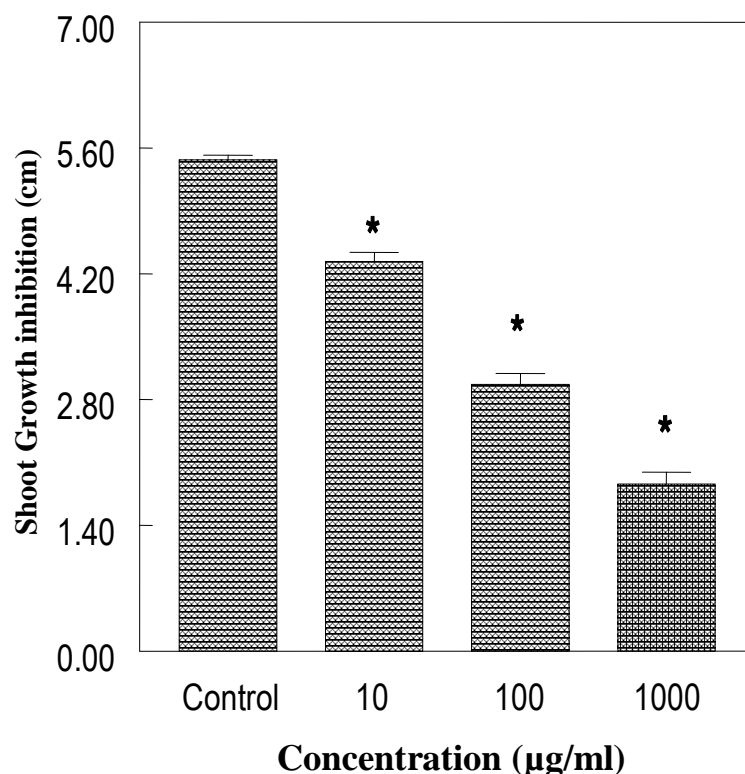


Figure 2. Root growth of various groups after the 3rd and 5th day treatment.

Table 1. Shows fresh and dry weight of various groups in Petri plates.

S/N	Treatment (μml)	Fresh weight	Dry weight
1	control	1.26 ± 0.03	0.18 ± 0.02
2	10	1.22 ± 0.05	0.16 ± 0.08
3	100	1.12 ± 0.01	0.10 ± 0.06
4	1000	1.07 ± 0.03	0.07 ± 0.05

**Figure 3.** Root growth of various groups after complete treatment.

with the results reported by Javaid (2009) that water extract of *Withania somnifera* and *Datura alba* possessed some bioactive compounds which significantly inhibited the growth of root and shoot of *Rumex dentatus* L.; highly competitive weed in wheat during allelopathic screening. A similar trend was also reported previously (Fujii et al., 2003; Gilani et al., 2008).

Field study

Various concentration of PAME was used for phytotoxic activity against wheat growth. The data shows that PAME markedly ($P < 0.05$) inhibited growth of shoot growth compared to the control (Figure 3). After complete treatment, fresh and dry weight of all replicates were measured which revealed that PAME significantly

($P < 0.05$) controlled the fresh as well dry weight (Table 2). Similar investigations were found by Kordali et al. (2008) that essential oil isolated from Turkish *Origanum acutidens* and their phenolic compounds completely inhibited the growth of seedling and roots and possessed activity when compared to standards compounds. Other studies of allelopathic behaviour (Bias et al., 2003; Pervaiz et al., 2004; Golisz et al., 2007) also revealed similar findings.

Conclusion

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

Table 2. Shows fresh and dry weight of various groups in field study.

S/N	Treatment (μ /ml)	Fresh weight	Dry weight
1	control	1.26 \pm 0.04	0.18 \pm 0.032
2	10	1.22 \pm 0.08	0.16 \pm 0.07
3	100	1.12 \pm 0.09	0.10 \pm 0.02
4	1000	1.07 \pm 0.02	0.07 \pm 0.05

in progress.

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REFERENCES

- Bias HP, Walker TS, Kennan AJ, Stermitz FR, Vivanco JM (2003). Structure-Dependent Phytotoxicity of Catechins and Other Flavonoids: Flavonoid Conversions by Cell-free Protein Extracts of *Centaurea maculosa* (Spotted Knapweed) Roots. *J. Agric. Food Chem.*, 51: 897-901.
- Fujii Y, Parvez SS, Parvez MM, Ohmae Y, Iida O (2003). Screening of 239 medicinal plant species for allelopathic activity using sandwich method. *Weed Biol. Manage.*, 3: 233-241.
- Gilani SA, Fujii Y, Adnan SM, Kikuchi A, Shinwari ZK, Watanabe KN (2008). Allelopathic study of 81 medicinal plants of Pakistan. *Weed Biol. Manage.*, (In press).
- Golisz A, Lata B, Gawronski SW, Fujii Y (2007). Specific and total activities of the allelochemicals identified in buckwheat. *Weed Biol. Manage.*, 7: 164-171.
- McLaughlin JL, Rogers LL (1998). The use of biological assays to evaluate botanicals. *Drug Info. J.*, 32: 513-524.
- Molisch H (1937). Der einfluss einer pflanze auf die andere-Allelopathie. *The role of chemical inhibition in vegetation I.* Pp. 99-106.
- Nandakumar J (2009). *Ex situ* conservation of medicinal plants in Valikamam area of the Jaffna Peninsula, Sri Lanka. *New Biot. J.*, 25: 370
- Nazir T, Uniyal AK, Todaria NP (2006). Allelopathic behavior medicinal plant species on traditional agriculture crops Himalaya, India. *Agroforestry Syst.*, 69(3): 183-187.
- Pervaiz MM, Yokotani KT, Fujii Y, Konishi T, Iwashina T (2004). Effects of quercetin and its seven derivatives on the growth of *Arabidopsis thaliana* and *Neurospora crassa*. *Biochem. Syst. Ecol.*, 32: 631-635.