African Journal of Biotechnology Vol. 9 (5), pp. 770-774, 1 February, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB09.232
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Cytotoxicity and phytotoxicity of some selected medicinal plants of the family Polygonaceae

Farrukh Hussain[2], Ishfaq Hameed[2], Ghulam Dastagir[2], Shams-un-Nisa[3], Ibrar Khan[1] and Bashir Ahmad[1]*

[1]Pharma Biotech Research Laboratory, Centre of Biotechnology and Microbiology, University of Peshawar, Pakistan.
[2]Pharmacognosy Laboratory, Department of Botany, University of Peshawar, Pakistan.
[3]Jinnah College for Women, University of Peshawar, Pakistan.

Accepted 9 November, 2009

The cytotoxicity of the crude methanolic extracts of *Rumex hastatus, Rumex dentatus, Rumex nepalensis, Rheum australe, Polygonum persicaria and Polygonum plebejum* (Family *Polygonaceae*) was determined against *Artemia salina* at 1000, 100 and 10 µg/ml. *R. hastatus, R. dentatus* and *R. nepalensis* showed significant activity at a concentration of 1000 µg/ml against *Artemia salina. R. australe* showed low activity at 1000 µg/ml and no activity at 100 and 10 µg/ml. At concentration of 10 µg/ml, *R. australe* showed no activity. Similarly the phytotoxicity of the crude extracts of these six plants was determined against *Lemna minor.* All the plants except *R. hastatus* showed significant activity at a concentration of 1000 µg/ml. Moderate activity was shown by *R. australe*, *R. nepalensis* and *P. persicaria* at the concentration of 100 µg/ml. All the plants showed low phytotoxic activity at concentration of 10 µg/ml.

Key words: *Rumex hastatus, Rumex dentatus, Rumex nepalensis, Rheum australe, Polygonum persicaria, Polygonum plebejum, Artemia salina, Lemna minor,* cytotoxicity, phytotoxicity.

INTRODUCTION

Bioactive compounds are often toxic to *Artemia salina* (leach) shrimp larvae. The eggs of the brine shrimp *A. salina* are readily available as fish food in pet shops (Kivack et al., 2001; Carballo et al., 2002). When placed in artificial seawater, the eggs hatch within 48 h, providing large numbers of larvae. This is a rapid, inexpensive, general bioassay, which has been developed for screening, fractionation and monitoring of physiologically active natural products (Carballo et al., 2002). Members of the family Lemnaceae are suitable organisms to investigate physiological processes and effects of different chemical substances. *Lemna* plants are miniature aquatic monocot, consisting of a central oval frond or mother frond with two attached daughter fronds and a filamentous root. Under normal conditions, the plants reproduce exponentially with budding of daughter fronds from pouches on the sides of the mother frond. Using the *Lemna* assay, it is observed that natural anti-tumor compounds can inhibit *Lemna* growth. In addition, it was also discovered that some substances stimulate frond proliferation and the assay may be useful in detecting new plant growth stimulants. The commercial need for such natural, biodegradable, herbicides and plant growth stimulants may someday be filled with natural products detected by the simple and convenient *Leman* bioassay (Atta-ur-Rehman, 1991).

Rumex hastatus juice is astringent and is used in bloody dysentery. The fresh tube is chewed to relieve throat aches. The root is a laxative, alternative, tonic and anti-rheumatic and is used in skin disease (Manandhar, 2002). The leaves and shoots of *R. hastatus* and *Rumex dentatus* are diuretic, refrigerant and used as cooling agent (Islam et al., 2006; Hussain et al., 2006). The roots of *R. dentatus* and *Rumex nepalensis* are used as an astringent and in cutaneous disorders and purgative (Chopra et al., 1986; Manandhar, 2002). A strong decoction is applied to dislocated bones and to alleviate body pain. Root paste is applied externally to relieve headaches (Manandhar, 2002). The leaves of *Polygonum*

*Corresponding author. E-mail: bashirdr2001@yahoo.com. Tel: +92-91-9216485.

persicaria are astringent, rubefacient and vermifuge (Foster and Duke, 1990). Plant decoction mixed with flour, has been used as a poultice to relieve pain, for rheumatism and dried leaves are rubbed on poison ivy rash (Moermann, 1998). The roots of *Polygonum plebejum* are used in bowel complaints and the dried plant powder is taken internally in pneumonia (Baquar, 1989; Arshad and Rao, 1998). The objective of the study was to find out the cytotoxic and phytotoxic effects of the selected medicinal plants of family Polygonaceae against *Artemia salina* and *Lemna minor*.

MATERIALS AND METHODS

Sources of plant materials

Polygonum persicaria, Rumex hastatus, Rumex dentatus, Rumex nepalensis, and *Polygonum plebejum* were collected from Peshawar University Campus and *Rheum australe* was collected from Gharam Chasma (Chitral) in March - November 2005. These plants were identified with the help of Flora of Pakistan (Ali and Qaiser, 2001).

Preparation of extracts

Leaves of *Polygonum persicaria* Linn, *R. hastatus* D.Don, *R. dentatus* Linn, roots of *R. nepalensis* Spreng, rhizomes of *R. australe* D. Don and whole plant of *P. plebejum* were shade dried and then grounded to 60 mesh diameter powders using an electric grinder. Fifty grams of each sample was soaked in 250 ml 70% methanol for 72 h. Thereafter each plant extract was passed through Whatman filter paper No.1823. This process was repeated for 3 times. Each extract was concentrated by rotary evaporator at 40°C, under vacuum. The concentrates of each extracts were stored at 4°C prior to use. The methanolic extracts and the standard drug (Etopoisde for cytotoxicity and Paraquate for phytotoxicity) were dissolved in dimethylsulphoxide (DMSO) at the concentration of 10 and 1 mg/ml, respectively.

Cytotoxicity

The materials and reagents used for cytotoxicity includes, test sample, *A. salina* (shrimp eggs), sea salt (38 g/L of D/W, pH 7.4), hatching tray with perforated partition, lamp to attract brine-shrimp larvae, micro pipette (5, 50, 500 µl), vials tray, 9 vial samples, methanol, distilled water. The cytotoxic activity of the crude extract of the plants was carried out by following the method of Meyer et al. (1982).

Hatching techniques

The hatching tray [a rectangular dish (22 X 32 cm)] was half-filled with filtered brine solution and (50 mg) eggs of brine shrimps was sprinkled in it and incubate at 37°C for 24 h. After the incubation period, brine shrimps were hatched and the plants extracts were applied to find out the cytotoxic effect of these extracts.

Sample preparation

Test sample was dissolved (20 mg) in 2 ml of DMSO and from this solution 5, 50 and 500 µl was transferred to vials (3 vials/concentration). The concentration was 10, 100, and 1000 µg/ml respectively. After 2 days of hatching and maturation, 10 larvae/ vials were placed, using a Pastuer pipette. The volume was made 5 ml with seawater. It was incubated at 25 - 27°C for 24 h under illumination. Other vials were supplemented with DMSO and reference cytotoxic drug which served as negative and positive controls, respectively. The data was analyzed with Finney computer program to determine LD50 values with 95% confidence intervals.

Phytotoxicity

Phytotoxic activity of the extracts was carried out against the *L. minor* following (Ahmad et al., 2009). The medium was prepared in distilled water (1000 ml) and autoclaved at 121°C for 15 min, then pH was adjusted (5.5 - 5.6) by adding KOH pellets. The extracts (15.0 gm) dissolved in methanol (40 ml) served as stock solution. Eighteen Petri dishes, three for each extract, were inoculated with 1000, 100 and 10 µl of the stock solution to give the final concentration of 500, 50 and 5 ppm, respectively. The solvent was allowed to evaporate overnight under sterile conditions. To each plate, medium (20 ml) and plants (10), each containing a rosette of three fronds, of *L. minor* was added. Other plates supplement with methanol and reference growth inhibitor (Paraquate), which served as negative and positive control, respectively. All plates were kept in the growth cabinet at 28 (± 1) for seven days. The number of fronds per plates were counted and recorded on day seven.

RESULTS AND DISCUSSION

Cytotoxicity

The results of the cytotoxicity of the crude methanolic extracts of selected medicinal plants of family Polygonaceae are given in Table 1. *R. australe* had no cytotoxic activity. The upper and lower limit, the G value and LD50 was 0. *R. hastatus* has low cytotoxic activity. The upper and lower limits were 2926.9 and 3.4166, respectively, and the G value was 36208. LD50 was 1.701. The upper and lower limits of the *R. dentatus* were 17790 and 51.0173, respectively, and the G value was 0.1190. LD50 was 95.99. It has a significant activity. The upper and lower limit of the *R. nepalensis* was 70.846 and 13.699, respectively, and the G value was 0.1710. LD50 of the plant was 35.0. It has moderate activity. The upper and lower limit of *P. plebejum* was 30.0715 and 1.0725, respectively, and the G value was 0.2897; LD50 of the plant was 11.06. It has moderate activity. The upper and lower limit of the *P. persicaria* was 469.872 and 21.2824, respectively, and the G value was 382948; LD50 was 1.701. It has low activity.

Phytotoxicity

The results of the phytotoxicity of the crude methanolic extracts of selected medicinal plants of family Polygonaceae are given in Figures 1 - 6. The results showed that the crude extract of the *R. australe* (rhizome) showed high phytotoxic activity against *L. minor* at a concentration of the 1000 µg/ml (70.4%), moderate

Table 1. Cytotoxic activity of some selected medicinal plants of Polygonaceae against *A. salina.*

Plant name	Plant part	Dose (µg/ml)	No of survivors	LD50 (µg/ml)	Upper limit	Lower limit	G. value
R. australe	Rhizome	1000 100 10	26 30 30	0	0	0	0
R. hastatus	Leaf	1000 100 10	5 16 20	1.701	2926.9	3.4166	36208
R. dentatus	Leaf	1000 100 10	2 18 25	95.99	17790	51.0173	0.1190
R. nepalensis	Root	1000 100 10	2 8 22	35.0	70.846	13.699	0.1710
P. plebejum	Whole plant	1000 100 10	2 8 15	11.06	30.0715	1.0725	0.2897
P. persicaria	Leaf	1000	28	1.701	469.872	21.2824	382948
Standard drug	Etopoisde			7.4625			

No of shrimps used in each experiment = 30.

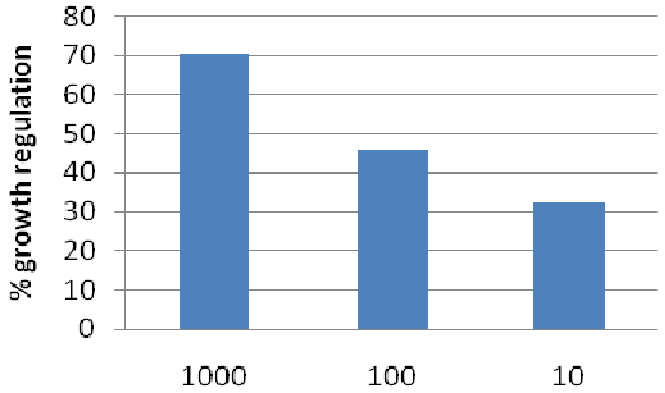

Figure 1. Phytotoxic activity of different concentrations of *R. australe* (rhizome) against *L. minor*

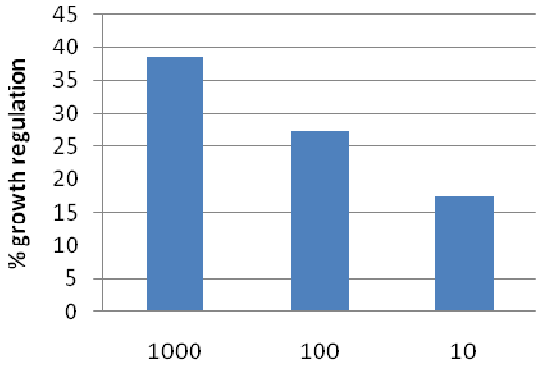

Figure 2. Phytotoxic activity of different concentrations of *R. hastatus* (leaf) against *L. minor.*

inhibition at a concentration of 100 µg/ml (45.5%) and low at a concentration of 10 µg/ml (32.2%). *R. hastatus* (leaf) showed low activity at 1000 µg/ml (38.5%), 100 µg/ml (27.3%) and 10 µg/ml (17.4%). *R. dentatus* (leaf) exhibit significant activity at a concentration of 1000 µg/ml (72.8%) and at concentration of 100 µg/ml (36.8%) and 10 µg/ml (20.2%) the phytotoxic activity was low. *R. nepalensis* (root) had high phytotoxic activity at a concentration of the 1000 µg/ml (68.8%), showed moderate phytotoxic activity at 100 µg/ml (55%) and low activity at 10 µg/ml (11.9%). *P. plebejum* (whole plant) showed high phytotoxic activity at a concentration of 1000 µg/ml (66.8%) while at a concentration of 100 µg/ml (31%) and 10 µg/ml (9.1%), the activity was low. *P. persicaria* (leaf) showed high phytotoxic activity against *L. minor* at a concentration of the 1000 µg/ml (72.8%), showed moderate inhibition at a concentration of 100 µg/ml (45.5%) and at a concentration of 10 µg/ml (21.9%), it has low inhibitory activity.

Conclusion

The results showed that *R. hastatus, R. dentatus, R. nepalensis, P. persicaria* and *P. plebejum* can be used as a source of cytotoxic agents. Similarly *R. hastatus, R. dentatus, R. nepalensis, R. australe, P. persicaria* and *P.*

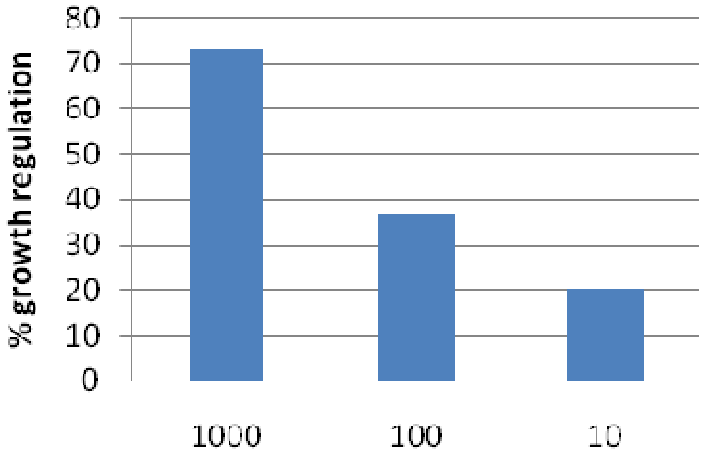

Figure 3. Phytotoxic activity of different concentrations of *R. dentatus* (leaf) against *L. minor.*

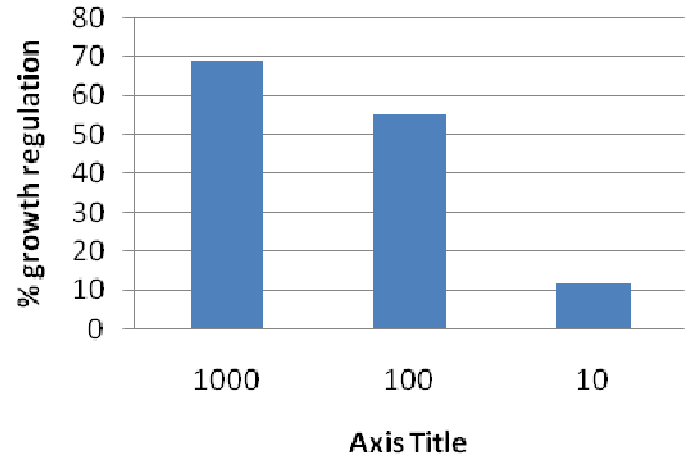

Figure 4. Phytotoxic activity of different concentrations of *R. nepalensis* (root) against *L. minor.*

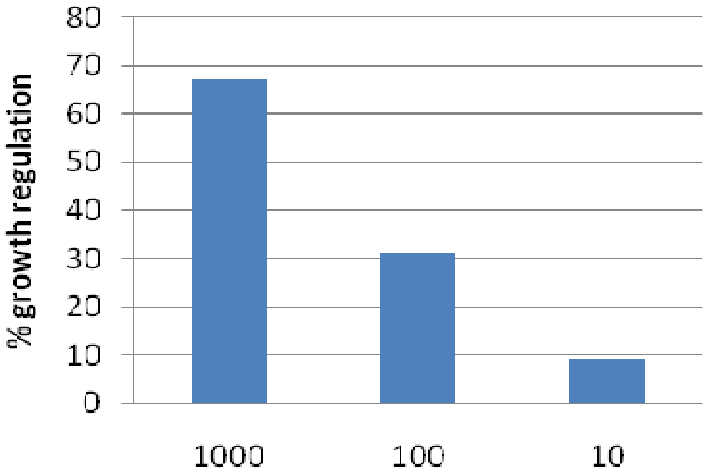

Figure 5. Phytotoxic activity of different concentrations of *P. plebejum* (whole plant) against *L. minor.*

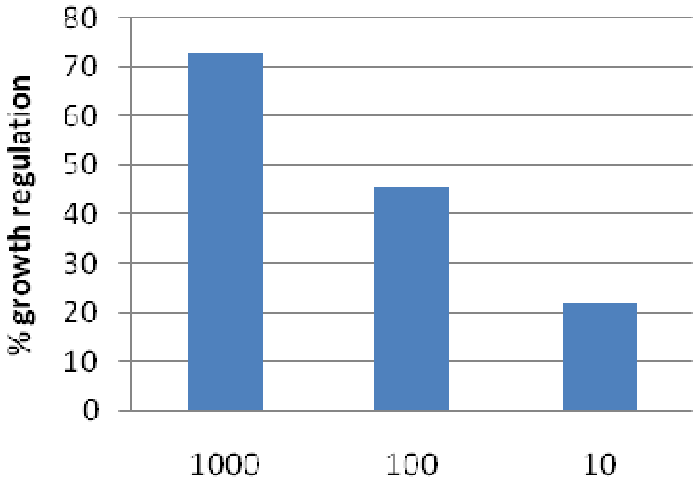

Figure 6. Phytotoxic activity of different concentrations of *P. persicaria* (leaf) against *L. minor.*

plebejum can be used as a source of phytotoxic agents at the concentration stated in Table 1 and Figures 1 – 6, respectively.

REFERENCES

Ahmad B, Ali N, Bashir S, Choudhary MI (2009). Biological activities of aerial parts of *Tylophora hirsuta* wall, Afri. J. Biotechnol. 8(18):4627-4631.

Ali SI, Qaiser M (2001). Flora of Pakistan 205: 1-190.

Arshad M, Rao AR (1998). Medicinal plants of Pakistan of Cholistan Desert. Medicinal plants of Pakistan. Proceeding of the meeting held at the Plant Genetic Resource Institute, National Agriculture Research Council, Islamabad. pp. 1-11.

Atta-ur-Rehman (1991). Studies in Natural Product Chemistry, Netherlands, Elsevier Science Publishers, 9: 383-409.

Baquar SR (1989). Medicinal and poisonous plants of Pakistan. Printed and published Printas Karachi (Pakistan).1st ed. pp. 352, 380, 393.

Carballo LJ, Hernandez-inda LZ, Perzer P, Gravalos MD (2002). A comparison between two brine shrimp assays to detect *in vitro* cytotoxicity in marine natural products, Bio. Med. Central, 2: 1-10.

Chopra RN, Nayar SL, Chopra IC (1986). Glossary of Indian Medicinal Plants (the Supplement). Council of Scientific and Industrial Research, New Delhi. p. 23.

Foster S, Duke JA (1990). A Field Guide to Medicinal Plants. Eastern and Central N. America. Houghton Mifflin Co. ISBN. 0395467225.

Hussain F, Islam M, Zaman A (2006). Ethnobotanical profile of plants of Shawar Valley, District Swat, Pakistan. Int. J. Biol. Biotechnol. 3(2): 301-307.

Islam M, Ahmad H, Rashid A, Razzaq A, Akhtar N Khan I (2006). Weeds and medicinal plants of Shawar Valley, District Swat. Pak. J. Weed Sci. Res. 12(1-2): 83-86.

Kivack B, Mert T, Tansel H (2001). Antimicrobial and cytotoxic activities of Ceratonia siliqua L. extracts. Turk. J. Biol. 26: 197-200.

Manandhar NP (2002). Plants and People of Nepal. Timber Press. Oregon. ISBN 0-8192- 527-6

Meyer BN, Ferrigni NR, Putnam JT, Jacobsen LB, Nichols DE, McLaughlin JL (1982). Brine Shrimp: A convenient general bioassay for active plant constituents. Planta Medica. 45: 31-34.

Moermann D (1998). Native American Ethnobotany, Timber Press Oregon.ISBN 0-88192- 453-9.