African Journal of Biotechnology Vol. 9(8), pp. 1241-1244, 22 February, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Phytotoxic, insecticidal and leishmanicidal activities of aerial parts of *Polygonatum verticillatum*

Muhammad Saeed[1]*, Haroon Khan[1], Murad Ali Khan[2], Shabana Usman Simjee[3], Naveed Muhammad[1] and Saeed Ahmad Khan[1]

[1]Department of Pharmacy, University of Peshawar, Peshawar 25120, Pakistan.
[2]Department of chemistry, Kohat university of science and technology Kohat, Pakistan.
[3]International Centre for Chemical Sciences, HEJ Research Institute of Chemistry University of Karachi, Karachi 75270, Pakistan.

Accepted 7 January, 2010

The aim of the present study was to explore the aerial parts of the *Polygonatum verticillatum* for various biological activities such as phytotoxic, insecticidal and leishmanicidal properties. Outstanding phytotoxicity was observed for the crude extract and its subsequent solvent fractions against *Lemna acquinoctialis* Welv at tested doses of 5, 50 and 500 µg/ml. Complete growth inhibition (100%) was demonstrated by the crude extract and aqueous fraction at maximum tested dose (500 µg/ml). Among the tested insects, moderate insecticidal activity was recorded against *Rhyzopertha dominica*. However, neither crude extract nor its solvent fraction registered any significant (> 100 µg/ml) leishmanicidal activity against *Leishmania major*. Based on the phytotoxicity, the aerial parts of the plant could be a significant source of natural herbicidal for sustainable weed control.

Key words: *Polygonatum verticillatum*, phytotoxicity, insecticidal activity, leishmanicidal activity.

INTRODUCTION

Polygonatum verticillatum [L.]. All. (Nooreallam) is a perennial rhizomatous herb belongs to family *Convallariaceae* (Tamura, 1993; Monika et al., 2006). In the traditional system of treatment, *Polygonatum* has been used for thousands of years. The rhizomes of *P. verticillatum* are indicated in the treatment of pain, pyrexia, burning sensation and for phthisis (Amrit, 2006). As a polypharmacy, it has been practiced to promote urine discharge (diuretic) and attenuate painful urination (Ballabh et al., 2008). Some of the other documented uses of the plant are as emollient, aphrodisiac, galactagogue (increases milk release), weakness, appetizer and tonic (Alam, 2004). The antinociceptive activity of the rhizomes of the plant has been recently reported (Khan et al., 2010). Affinity chromatography has led to the purification of lectins from fresh rhizomes of the plant and was estimated 120 mg/kg (Antoniuk, 1993). While considering the diverse folk uses of the plant, the present study was designed to analyze the crude extract of the aerial parts of plant and its subsequent solvent fractions for various biological activities such as phytotoxic, insecticidal and leishmanicidal activities.

MATERIALS AND METHODS

Plant material

The whole plant, *P. verticillatum* [L.] All. was collected from District Swat N.W.F.P, Pakistan, in July - August, 2007. The botanical identity of the plant material was done by the Taxonomy Department of PCSIR Laboratories Peshawar and a specimen with catalogue No: 9970 (PES) was deposited in the herbarium of PCSIR Laboratories Peshawar.

Plant extraction and fractionation

The aerial parts of the plant (10 kg) were air dried in shade, chopped into small pieces and powdered. The extraction of plant material was carried out by soaking in methanol at ambient temperature for 14 days. The methanolic extract was filtered through filter

*Corresponding author. E-mail: saeedrph2000@yahoo.com.

Abbreviations: FBS, Fetal bovine serum; **DMSO,** dimethylsulfoxide; **IC_{50}**, 50% inhibitory concentrations; **LD_{50}**, half maximum lethal dose.

Table 1. Phytotoxic activity of the crude extract and subsequent fractions of aerial parts of *Polygonatum verticillatum* against the *Lemna acquinoctialis* Welv.

Name of extracts		No of fronds					
		5 µg/ml		50 µg/ml		500 µg/ml	
	Control	Sample	GI (%)	Sample	GI (%)	Sample	GI (%)
Crude extract	19.33	18	6.88	15	22.4	10	48.26
n-hexane	19.33	18	6.88	10	48.26	5	74.13
Chloroform	19.33	17	12.05	9	53.44	Nil	100
Ethyl acetate	19.33	18	6.88	10	48.26	4	79.3
n-butanol	19.33	15	22.40	9	53.44	Nil	100
Aqueous	19.33	18	6.88	11	43.09	6	68.96

GI = % Growth Inhibition, Standard drug = Paraquat (3.142µg/ml).

paper and the marc obtained was again macerated with methanol. The same process of extraction was repeated three times and the combined filtrates were concentrated under vacuum at low temperature (40°C) using rotary evaporator (Khan et al., 2007). Finally, the crude methanolic extract (2.410 kg, 24.10% w/w) was obtained. The crude extract (1.8 kg) was dissolved in distilled water and sequentially partitioned with various solvents to obtained n-hexane (275 g), chloroform fraction (295 g), ethyl acetate fraction (210 g), n-butanol fraction (317 g) and aqueous fraction (445 g).

In vitro phytotoxicity assay

In vitro phytotoxicity assay was carried out for the crude extract and subsequent solvent fractions against *Lemna acquinoctialis* Welv (Atta-ur-Rahman, 1991; Finney, 1991). The medium was prepared by mixing various inorganic components in 100 ml of distilled water and KOH solution was added for the adjustment of pH at 6.0 - 7.0. The medium was autoclaved at 121°C for 15 min. Test samples (15 mg) dissolved in ethanol (1.5 ml) served as stock solution. Nine flasks (three for each dilution) were inoculated with 1000, 100 and 10 µl of the stock solution for 500, 50 and 5 ppm. The solvent was then evaporated overnight under sterilized conditions. Each flask was supplemented with 20 ml of the medium. Thereafter, 10 plants each containing a rosette of three fronds, were added to each flask. One other flask, supplemented with solvent as control and reference plant growth inhibitor (Paraquat), served as a standard phytotoxic drug. The flasks were plugged with cotton and placed in growth cabinet for 7 days. On the 7th day, the number of fronds per flask was counted. Results were analyzed as growth regulation in percentage, calculated with reference to the negative control.

In vitro insecticidal activity

In vitro insecticidal assay was carried out for the crude extract and its various fractions against *Tribolium castaneum*, *Sitophilus oryzea*, *Rhyzopertha dominica and Callosobruchus analis* following method available in literature (Tabassum et al., 1997). The test sample was prepared (200 mg of crude extract was dissolved in 3 ml of methanol and served as stock solution). The sample (1572.7 µg/cm^2) was loaded over the filter paper of appropriate size (9 cm or 90 mm) on Petri plate using micropipette. The plate was left overnight (24 h) to evaporate the solvent. Next morning, 10 healthy and active insects of each species of same size and age were added to each plate including control (methanol) and standard drug (Permethrin, 393.17µg/cm^2). Thereafter the plates were incubated in growth chamber at 27°C for 24 h with 50% relative humidity. For calculation, the number of survived insects was counted and the mortality (%) was determined using following formula. Results were the mean of three different experiments.

In vitro leishmanicidal activity

Leishmania major (DESTO) promastigotes were cultured at 22 - 25°C in RPMI-1640 (Sigma). The medium was supplemented with 10% heat-inactivated (56°C for 30 min) fetal bovine serum (FBS). Promastigote culture in the logarithmic phase of growth was centrifuged at 2000 rpm for 10 min, and washed with saline three times in the same condition. Parasites were diluted with fresh culture medium to a final density of 10^6cells/ml. In a 96-well micro titer plate, 180 µl of medium was added in first row and 100 µl of medium was added in others wells. Test extracts (20 µl) was added in medium and serially diluted. 100 µl of parasite culture was added in all wells. One row was used for control (DMSO) which received medium while one for standard drugs (Amphotericin B, Pantamidine). The plate was incubated at 21-22°C for 72 h and the numbers of surviving parasites were counted microscopically in Neubauer chamber. Results are the replicates of three different experiments. The 50% inhibitory concentrations (IC_{50}) were calculated by a Windows based EZ-Fit 5.03 Perrella Scientific Software.

RESULTS AND DISCUSSION

The practice of herbal treatment is well established in Pakistan like most other developing countries of the world. Large number of Hakims and Tabbies are involved in this practice especially in the rural areas of the country. The dynamic features of local system of treatment are their safety, affordability and availability to large population. Traditional health care systems using medicinal plants can be recognized and used as a starting point for the development of novelties in drugs (Khan et al., 2008). Therefore, the current study was designed to explore some of the biological properties of the aerial parts of the *P. verticillatum* in the light of established *in vitro* protocols.

The results of the phytotoxic assay are presented in Table 1. It is evident from the results that the crude form of the aerial parts showed 6.88, 22.40 and 48.26% growth inhibition at 5, 50 and 500 µg/ml, respectively. Upon fractionation, prominent increase in the inhibitory

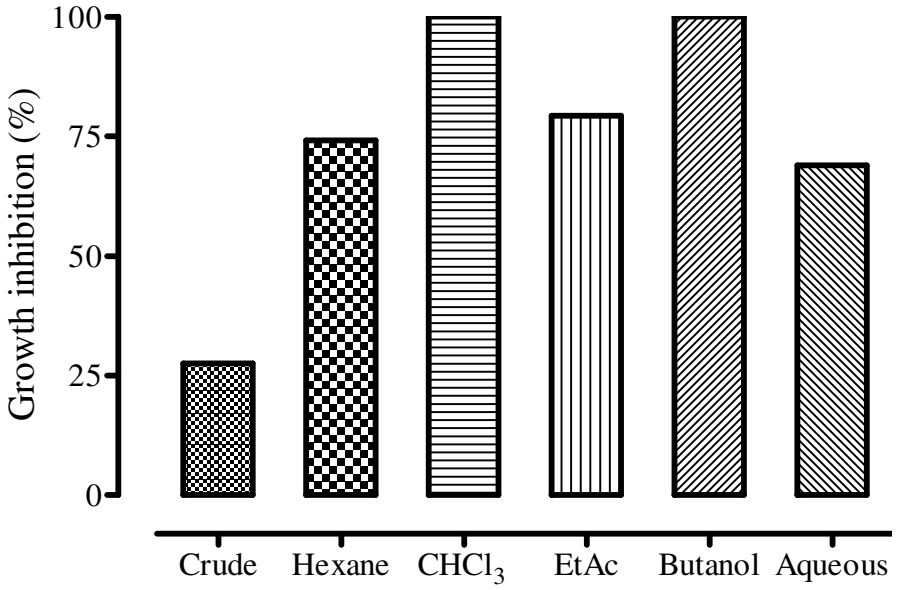

Figure 1. Phytotoxic activity of the crude extract and its subsequent solvent fraction of aerial parts of *Polygonatum verticillatum* at 500 µg/ml.

Table 2. Insecticidal activities of crude extract and various fractions of aerial parts of *Polygonatum verticillatum*.

Name of insect	Mortality (%)							
	Std	NC	Crude	Hexane	Chloroform	Ethyl acetate	Butanol	Aqueous
Rhyzopertha dominica	100	Nil	Nil	50	30	Nil	Nil	Nil
Tribolium castaneium	100	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Callosdruchus analis	100	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Sitophilus oryzae	100	Nil	Nil	Nil	Nil	Nil	Nil	Nil

NC = negative control; SP = Sample (1019.9 µg/cm^2); Std = Standard (Permethrin) = 235.9 µg/cm^2.

activity was found in some of the fractions. Complete growth inhibition (100%) of *L. acquinoctialis* Welv was demonstrated by the chloroform and butanol fractions at 500µg/ml as shown in Figure 1. In addition to that, the phytotoxic potential of hexane and ethyl acetate fractions were also worth mentioning: 74.13 and 79.30% at 500µg/ml, respectively. The aqueous fraction, however, produced only 68.96% activity at 500µg/ml.

Interference of weeds obviously reduces the quality and quantity of agricultural crops and is responsible for huge economic losses all-over the world. It is estimated in US alone that weeds cause a loss of around 12% costing to nearly US$ 33 billion and the situation is more alarming in developing countries (Piment et al., 2001). Synthetic herbicides are extensively used for the control of weeds in agricultural sectors. However, various factors that restricted the use of synthetic herbicides include water and soil pollution, herbicide-resistant weed populations, herbicide residues and detrimental effects on non-target (Li et al., 2003). In recent times, more emphasis has been laid on the natural allelochemicals from plants for weed control in crop production especially to cope with the problem of weed resistance. It has been proved that the phytotoxicity of the plant reduced the growth of weeds without any negative effect on the crops growth and overall yield under normal field conditions, rather significant increase has been recorded in crops production (Batish et al., 2007). It is therefore, assumed on the basis of results that the phytotoxic principle(s) of the aerial parts of the plant could be a significant source of natural herbicides for weeds control in a sustainable manner for better crop production.

Regarding the results of insecticidal activity of crude extract and its various solvent fractions, as depicted in Table 2, moderate activity was exhibited by hexane (50 %) and chloroform fraction (30%) against *R. dominica*. On the other hand, none of the tested samples showed any activity against various insects used in the assay. Similarly, the experimental findings of leishmanicidal assay are posted in Table 3. Neither crude extract nor its various solvent fractions were able to produced any significant (LD_{50}: > 100 µg/ml) activity against *L. major*.

Table 3. *In vitro* antileishmanial activity of the methanol extract and fractions of aerial parts of *Polygonatum verticillatum* against *Leishmania* major.

Test organism	Extracts/Fractions	IC_{50} (µg/ml)
Leishmania major DESTO)	Crude methanol extract	> 100
	n-Hexane	> 100
	Chloroform	> 100
	Ethyl acetate	> 100
	n-Butanol	> 100
	Aqueous	> 100
	Amphotercin-B	0.50 ± 0.02,
	Pentamidine	2.56 ± 0.02

Incubation period was 72 h at 22°C.

REFERENCES

Piment D, McNair S, Janecka J, Wightman J, Simmonds C, O'Connell C, Wong E, Russel L, Zern J, Aquino T, Tsomondo T, (2001). Economic and environmental threats of alien plants, animals and microbial invasions. Agric. Ecosyst. Environ. 84: 1-20.

Amrit PS (2006). *Ashtavarga*- Rare Medicinal Plants. Ethnobot. Leaflets, 10: 104-108

Antoniuk VO (1993). Purification and properties of lectins of *Polygonatum multiflorum* [L.] All. and *Polygonatum verticillatum* [L.] All. Ukr. Biokhim. Zh. 65: 41-48.

Atta-ur-Rahman (1991). Studies in Natural Products Chemistry: Bench-Top Bioassay for the Discovery of Bioactive Natural Products an update (Vol. 9). Structure and Chemistry (Part-B). Elsevier Science Publishers, B.V., Netherland.

Ballabh B, Chaurasia OP, Ahmed Z, Singh SB (2008). Traditional medicinal plants of cold desert Ladakh used against kidney and urinary disorders. J. Ethnopharmacol. 118: 331-339.

Batish DR, Kaur M, Singh HP, Kohli RK (2007). Phytotoxicity of a medicinal plant, Anisomeles indica, against Phalaris minor and its potential use as natural herbicide in wheat fields Crop Prot. 26: 948-952.

Finney DJ (1991). Prohit Analysis (3rd edn). Cambridge University Press, Cambridge

Khan H, Khan MA, Hussan I (2007). Enzymes inhibition activities of the rhizomes of *Gloriosa superba* Linn (*Colchicaceae*). J. Enzyme Inhib. Med. Chem. 22: 722-725

Khan H, Khan MA, Mahmood T, Choudhary IQ (2008). Antimicrobial activities of *Gloriosa superba* Linn (Colchicaceae) extracts. J. Enzyme Inhib. Med. Chem. 23: 855-859.

Khan H, Saeed M, Gilani AH, Khan MA, Dar A, Khan I (2010). The antinociceptive activity of *Polygonatum verticillatum* rhizomes in pain models. J. Ethnopharmacol. 127: 521-527.

Li Y, Sun Z, Zhuang X, Xu L, Chen S, Li M (2003). Research progress on microbial herbicides Crop Prot. 22: 247-252.

Monika S, Jakub S, Kornelia P, Czeslaw H., Roman Z (2006). Comparison of three *Polygonatum* species from Poland based on DNA markers. Ann. Bot. Fenn. 43: 378-388.

Tabassum R, Naqvi SNH, Azmi MA, Nurulain SM, Khan MF (1997). Residual effect of a neem fraction, nimolicine and an insect growth regulator, dimilin, against stored grain pest *Callosobruchus analis*. Proc. Pak. Congr. Zool. 17: 165-170.

Tamura MN (1993). Biosystematic studies on the genus *Polygonatum* (Liliaceae) morphology of staminal filaments and karyology of elevan *Eusasian* species. Bot. Jahrb. Syst. 115: 1-26.