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Full Length Research Paper

Biological activities of aerial parts of *Paeonia emodi* Wall

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The ethanolic extract derived from the aerial parts of *Paeonia emodi* was screened for various *in vitro* biological activities including antifungal, antibacterial, insecticidal, phytotoxic and haemagglutination activities. General toxicity (brine shrimp lethality assay) of this extract has also been assessed. The extract was found to possess excellent phytotoxicity against *Lemna minor* L., moderate heamagglutination activity against human erythrocytes and reasonable insecticidal activity against *Bruchus pisorum*. The crude extract did not display any antifungal or antibacterial activity against the fungi and bacteria used in this study. No significant general toxicity was observed with the extract at tested concentrations.

Key words: *Paeonia emodi*, biological activities, phytotoxicity, heamagglutination, insecticidal activity.

INTRODUCTION

Paeonia emodi Wall. (Paeoniaceae), is widely distributed in N. Pakistan, N.W. India, W. Nepal and China (De-yuan, 2004). It is an erect perennial herb, 50 cm long, glabrous, leaves biternate or ternate, lamina pale, flowers solitary, axillary (Kirtikar, 1918). The carpels are densely pubescent; flowers 3 on a stem (De-yuan, 2004). *P. emodi* finds several applications in indigenous medical system. The rhizomes are used as a tonic to cure backbone ache (Hamayun et al., 2004). The roots and rhizomes are used to cure backache, dropsy and epilepsy and are also used as a tonic, emetic, cathartic, blood purifier and colic while the seeds are purgative (Shinwari et al., 2003). The roots are used for the treatment of headache, dizziness, vomiting and to aid pregnancy (Ahmad and Sher, 2004). In our previous investigations, the extract from the aerial parts *P. emodi* and its fractions were found to possess significant enzyme inhibition (against urease and α-Chymotrypsin) and radical scavenging activities (Khan et al., 2005).The various constituents isolated from this plant include a β-glucuronidase-inhibiting triterpene, 1β,3β,5α,23,24-pentahydroxy-30-12,20(29)-dien-28-oic acid along with oleanolic acid, betulinic acid, ethyl gallate, methyl grevillate and 1,5-dihydroxy-3-methylanthraquinone (Nawaz et al., 2000). Other constituents are monoterpene glycosides, wurdin and benzoylwurdin along with paeoniflorin, lactiflorin and oxypaeoniflorin (Muhammad et al., 1999). Emodinol, an oleane type triterpene, showing significant β-glucuronidase inhibitory activity, benzoic acid and 3-hydroxybenzoic acids (Riaz et al., 2003a), paeonins A and B, monoterpene galactosides showing potent lipoxygenase inhibitory activity (Riaz et al., 2003b) are also present.

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In the current study, the crude extract derived from the aerial parts of this plant was screened for various *in vitro* biological activities including antifungal, antibacterial, insecticidal, heamagglutination activities, phytotoxicity and brine shrimp lethality. Our previous studies on the same extract (Khan et al., 2005) encouraged us to undertake the present investigations to assess the aerial parts of this plant for any further beneficial effect of medicinal and/or agricultural importance.

MATERIALS AND METHODS

Plant material

The plant *Paeonia emodi* (aerial parts) was collected from Swat, Pakistan and identified by Mehboob-ur-Rehman, plant taxonomist, Department of Botany, Government Degree College Matta, Swat, Pakistan. A voucher specimen was deposited at the Herbarium Post Graduate Jehanzeb College Swat, Pakistan.

Preparation of extract and fractionation

Air-dried and ground plant material (2.1 kg) was extracted at room temperature with ethanol (6.0 L, three weeks x 3 times). The residue was filtered and evaporated to dryness under reduced pressure at 45ºC to yield the gummy crude extract (355 g).

Antifungal activity

Antifungal activity of the extract was evaluated using agar tube dilution method (Paxton, 1991). The solution of the extract was incorporated into non-solidified sterile sabouraud dextrose agar in glass tubes (final concentration, 400 µg/ml) and inoculated with a piece of seven days inoculum of the respective fungus. For non-mycelial growth, an agar surface streak was employed. Inhibition of fungal growth was measured after 7 days of incubation at 28±1ºC. The percent growth inhibition was calculated with reference to the negative control.

Antibacterial activity

The extract was screened against various human pathogens employing agar well diffusion method (Atta-ur-Rahman et al., 1999). Nutrient agar plates were swabbed with a 2-8 h broth culture of the respective bacteria. Samples (3 mg/ml) were added in to the wells dug in the medium of these plates. Imipenum (10 µg/disc) was used as reference antibacterial drug. The plates were incubated at 37ºC for 14-19 h and the activity was determined by measuring the diameter of zones of inhibition (mm).

Phytotoxic activity

Phytotoxic activity of the crude extract was tested against the *Lemna minor* L. (McLaughlin et al., 1991). Three flasks for each 500, 50 and 5 µg/ml were inoculated with stock solution of the extract (20 mg/ml). To each flask, 20 ml medium and 10 plants each containing a rosette of three fronds, was added. Paraquat was used as reference growth inhibitor. All flasks were incubated in the growth cabinet for seven days after which the growth regulation in percentage was calculated with reference to the negative control. IC_{50} was calculated with a Finney computer program with 95% confidence interval.

Brine-shrimp cytotoxicity

Artemia salina (brine-shrimp eggs) was used to determine the cytotoxicity of the extract (Meyer et al., 1982). Ten shrimp, 5 ml seawater and different concentrations of extract (1000, 100 and 10 µg/ml) were added to separate vials. Etoposide (LD_{50} = 7.465 µg/mL) was used as reference cytotoxic drug. All the vials were incubated at 26±1ºC for 24 h and the brine shrimps that survived were counted. The data was analyzed with a Finney computer program to determine LD_{50} values with 95% confidence interval.

Insecticidal activity

The insecticidal activity of the extract was determined by direct contact application using filter paper (Ahn et al., 1995). 3 ml of the extract (1 mg/ml) was applied to filter papers (90 mm diameter). After drying, each filter paper was placed in the separate petri dish along with 10 adults of each *Tribolium castaneum*, *Bruchus pisorum* and *Rhyzopartha dominica*. Permethrin (235.71 µg/cm^2) was used as reference insecticide. All these were kept without food for 24 h after which mortality count was done.

Haemagglutination activity

The haemagglutination activity was tested against human erythrocytes blood groups ABO (Naqvi et al., 1992). 2% suspension of erythrocytes, obtained by centrifugation of the blood, was prepared in phosphate buffer (pH 7). The activity was investigated serially in different dilutions of the extract against all the blood groups. For this purpose 1 ml of each dilution was added to 1 ml of erythrocytes (2%) followed by incubation at 25ºC. Smooth button formation at the bottom showed negative activity, whereas a rough granular deposition indicated a positive reaction, the intensity of which was determined from the extent of deposition.

RESULTS AND DISCUSSION

Pakistan has a strong tradition of herbal remedies and, like most developing countries; its rural population still depends mainly on the indigenous system of medicine for their health related matters (Khattak et al., 1985). It was, therefore, seemed interesting to evaluate scientifically and determine the efficacy of the aerial parts an indigenous medicinal plant *P. emodi*, the roots of which are commonly used by traditional practitioners. We, in the current study, present the screening of the aerial parts of this plant for various in vitro biological activities including antifungal, antibacterial, insecticidal, heamagglutination activities, phytotoxicity and brine shrimp lethality.

The results of phytotoxic activity of the extract are shown in Figure 1. The extract displayed excellent phytotoxicity in the highest tested concentration (500 µg/ml) and caused complete (100%) inhibition of growth of *L. minor*. It also exhibited a moderate (50%) inhibitory activity at concentration of 50 µg/ml; but was devoid of any herbicide activity at 5 µg/ml concentration. The IC_{50} value of the extract was found to be 50 µg/ml. There is a

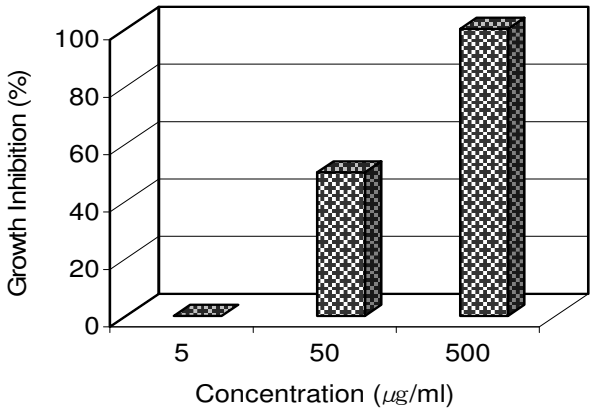

Figure 1. Phytotoxic activity of ethanolic extract of *P. emodi* against *L. minor* L.

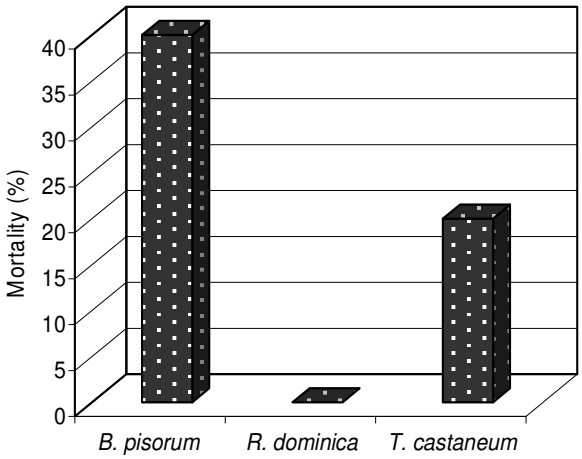

Figure 2. Insecticidal activity of ethanolic extract of *P. emodi*.

need to discover new herbicides since the number of herbicide-resistant weeds is increasing and conventional synthetic herbicides are becoming less and less effective against the resistant weed biotypes (Bhowmik and Inderjit, 2003) in addition to environmental and health related concerns. Therefore, new herbicides from natural sources are currently receiving more attention which could be appropriate and non-hazardous alternatives to the currently used synthetic agrochemicals as the natural products, generally, are effective, biodegradable and thus posing less threat to the environment. A number of plants, their extracts or their purified active constituents can act as allelochemicals to other plants and thus could be candidates for application for agricultural purposes (Khan et al., 2002). The results obtained from the current study indicated that *P. emodi* extract might be useful as natural herbicides and could be a source of bioactive agrochemicals.

Table 1. Haemagglutination activity of extract of *P. emodi* against human RBC's.

Blood Groups	Dilutions			
	1:2	1:4	1:8	1:16
A^+	++	+	-	-
A^-	++	+	-	-
B^+	++	+	-	-
B^-	++	+	-	-
AB^+	++	+	-	-
AB^-	++	+	-	-
O^+	++	+	-	-
O^-	++	+	-	-

(- = no agglutination; + = weak; ++ = moderate; +++ = strong).

The insecticidal activity of the crude extract was performed against *B. pisorum*, *T. castaneum* and *R. dominica* and the results obtained are displayed in Figure 2. The extract exhibited a moderate (40%) inhibitory activity against *B. pisorum*, a weak (20%) activity against *T. castaneum* and no inhibitory activity against *R. dominica*. The interest in the discovery of botanical insecticides as alternatives to the synthetic ones, which possess well-known adverse effects on agroecological systems, has been increased in the last few decades in integrated pest management program (Pavela, 2004). During the current study, *P. emodi* has shown a moderate insecticidal activity particularly against *B. pisorum* and thus could be a source of stored product pest control especially if used in higher concentrations than the tested one.

The haemagglutination activity of the extract was tested against human RBC's and the results are shown in Table 1. The extract showed moderate ($^{++}$) agglutination effect against all blood groups at the highest concentrations (1:2) and weak (+) activity at a dilution of 1:4; however, at further lower concentrations it did not display any agglutination properties. The heamagglutination activity is generally attributed to a group of proteins called as lectins (Benevides et al., 1999) which are valuable agents for the separation and characterization of glycoconjugates and glycopeptides, histochemistry of cells and tissues, and the study of cell differentiation (Gabius and Gabius, 1993). Because of their usefulness in investigation of cellular membranes, the phytolectins have recently attracted much attention (Rapava et al., 2001). This investigation revealed that *P. emodi*, could be a good source of important plant lectins.

Antifungal activity of the crude extract of *P. emodi* was tested against *Trichophyton longifusus, Candida albicans, Aspergilus flavus, Microsporum canis, Fusarium solani,* and *Candida glaberata* while various bacteria used in the evaluation of antibacterial activity of this extract included *Escherichia coli*, *Bacillus subtillus*, *Shigella flexenari*, *Staphyllococcus aureus*,

Pseudomonas aeruginosa and *Salmonella typhi*. The crude extract did not display any antimicrobial activity against the fungi and bacteria used in this study.

No significant general toxicity of the extract was observed at test concentrations (10, 100 and 1000 µg/ml) in the brine shrimp lethality assay as the IC_{50} value was higher than 1000 µg/ml. This study was useful in evaluating the toxicity of the extract, which declared it safe to be used.

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