African Journal of Pharmacy and Pharmacology Vol. 6(10), pp. 698-701, 15 March, 2012
Available online at http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP11.339
ISSN 1996-0816 ©2012 Academic Journals

Full Length Research Paper

Evaluation of *Viola betonicifolia* for anthelmintic activity

Naveed Muhammad[1], Muhammad Saeed[1]*, Haroon Khan[1], Mughal Qayum[1], Barkatullah[2] and Amir Badshah[1]

[1]Department of Pharmacy, University of Peshawar, 25120, Peshawar, Pakistan.
[2]Department of Botany, University of Peshawar, 25120, Peshawar, Pakistan.

Accepted 23 January, 2012

In the present study, crude methanolic extract and its subsequent solvent fractions of *Viola betonicifolia* were tested against the adult species *Pheretima posthuma* and Juvenile species (*Cephalobus litoralis* and *Helicotylenchus indicus*) of worms. The anthelmintic activity of ethyl acetate and chloroform fractions was very significant against *P. posthuma*, in a concentration dependent manner (100 mg/ml), mortality time was 42 and 58 min, respectively. Mortality of chloroform and ethyl acetate fractions was 66 and 62% after 48 h against *C. littoralis*, respectively. Nevertheless, chloroform and ethyl acetate fractions exhibited 49 and 57% mortality against *H. indicus*. It is concluded that the extracts of *V. betonicifolia* could be a significant natural source of vermicides.

Key words: *Viola betonicifolia, Pheretima posthuma, Cephalobus litoralis, Helicotylenchus indicus.*

INTRODUCTION

Approximately two billions individuals of the world harbor parasitic worm infections of all ages, especially in the third world countries (Geary et al., 2010). Parasitic worms also infect livestock and crops, and have negative effect on food production. Despite this prevalence of parasitic infections, research on anthelmintic drugs is poor. Natural products are considered as the best healing agents for the treatment of different diseases (Saeed et al., 2010a).

Viola betonicifolia belongs to family Violaceace. Locally, it is known as banafsha. It is a perennial herb of 8 to 20 cm in height. The stem of the plant is absent and leaves are triangular or obtuse and petiole is longer than lamina. Roots are slender, unbranched and rhizome is short. *V. betonicifolia* is available in various countries of the world like Pakistan, India, Nepal, Sri Lanka, China, Malaysia and Australia (Flora of Pakistan). In Pakistan, it is available in Swat, Hazara and Dir. Traditionally, this plant has been used as antipyretic, astringent, diaphoretic, anticancer, purgative, epilepsy and nervous disorders (Ilyas and Hamayun, 2010) and cough (Tiwari et al., 2010). Some other uses are sinusitis, skin disorders, blood disorders, pharyngitis (Bhatt and Negi, 2006), kidney diseases, pneumonia and bronchitis. Flowers are used in lung troubles, cough and boil (Hussain et al., 2008). In continuation of our research, work on Pakistani medicinal plants (Saeed et al., 2010a 2010b; Khan et al., 2008, 2010; Muhammad and Saeed, 2011; Muhammad et al., 2012a, 2012b), we investigated *V. betonicifolia* for various biological activities. However, in this piece of research work, we discussed the results of anthelmintic activities of the crude extract and its various solvent fractions.

MATERIALS AND METHODS

Plant and extraction

Whole plant of *V. betonicifolia* was collected from Swat, Khyber Pakhtonkhawa in April 2010. Plant specimen was identified by Professor Dr. Muhammad Ibrar, Department of Botany, University of Peshawar and specimen was deposited there in the herbarium under voucher number 6410/Bot. The collected whole plant (12 kg) was air dried and powdered. The powdered material was extracted by maceration with methanol at room temperature for 14 days with occasional shaking (Khan et al., 2008). The methanolic extract was filtered and concentrated by rotary evaporator at low temperature (45°C). The methanolic extract was dissolved in distilled water and

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

Table 1. Anthelmintic activity of *V. betonicifolia* against *Pheretima posthuma*.

Sample	Concentration (mg/ml)	Time for paralysis (min)	Time for death (min)
Piperazine citrate	25	1.6 ± 0.7	54.5 ± 0.4
	50	1.0 ± 0.16	31.2 ± 0.6
	100	0.6 ± 0.27	19.3 ± 0.4
Butanol	25	99.03 ± 0.36	123.21 ± 0.6
	50	82.00 ± 0.3	107.98 ± 0.1
	100	74.15 ± 0.4	97.00 ± 0.34
Hexane	25	77.5 ± 33.2	101.00 ± 0.30
	50	55.0 ± 0.35	67.00 ± 0.19
	100	43.89 ± 0.31	71.0 ± 0.28
Aqueous	25	81.69 ± 0.95	101.94 ± 0.38
	50	66.69 ± 0.26	93.69 ± 0.57
	100	52.83 ± 0.34	84.23 ± 0.38
Methanolic	25	74.5 ± 0.34	133.21 ± 0.6
	50	60.2 ± 0.11	117.98 ± 0.1
	100	46.2 ± 0.21	87.00 ± 0.34
Ethyl acetate	25	25 ± 0.33	60 ± 0.18
	50	22 ± 0.02	45 ± 0.19
	100	16 ± 0.21	42 ± 0.32
Chloroform	25	34 ± 0.19	70 ± 0.25
	50	25 ± 0.35	65 ± 0.23
	100	16 ± 0.20	58 ± 0.32

was further fractioned with chloroform, hexane, ethyl acetate, butanol and aqueous fractions.

Anthelmintic activity

The assay was performed on adult earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings (Deore et al., 2009). Earthworms (3 to 5 cm in length and 0.1 to 0.2 cm in width) were collected from moist soil and were washed with normal saline, released in 50 ml of solutions of Piperazine citrate (standard drug) and extracts (25, 50 and 100 mg/ml each) in distilled water Table 1. Distilled water was used as control. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with fading away of their body colors.

Nematicidal activity

Culture preparation

Culture of *Cephalobus litoralis* was prepared by using a single egg. Green peas (*Pisum sativum*) were mashed in small Petri dishes. A single female was picked and placed beside pea meal paste. Female laid eggs within 12 h and then nematode eggs were hatched within 72 h and after 10 days, large number of nematodes in various stages of life cycle was obtained. These were used for screening crude extracts (Qamar et al., 1989). For isolation of *Helicotylenchus indicus* nematodes, 500 mg soil samples (Depth 15 to 25 cm) were collected from paddy (*Oryza sativa* L.) fields. Soil samples were processed by Cobb sieving (Cobb 1918) and modified with Baermann (1917) funnel method. Soil sample was put in a large bucket containing water, vigorously stirred into a suspension, which was allowed to settle for about 2 min. The heavy soil particles sank to the bottom but nematodes remained suspended in the water. The remaining suspension was slowly poured over a coarse sieve (60 mesh aperture), which was continuously tapped by hand to avoid blocking. The deposit on the sieve was washed with a gentle jet of water into a beaker. This water suspension, containing eel-shaped nematode, was passed through 200 and 300 mesh sieves. The nematodes thus recovered were mixed and water was decanted after allowing sufficient time for nematodes to settle down. Then, nematode suspension was poured over a piece of tissue paper attached to a perforated plastic sheet placed in a funnel fitted with a rubber tube and clamped at the lower end. The water contained in the funnel barely touched the bottom of the tissue paper. Care was taken not to allow the debris to float off the edges of the tissue paper. After 24 h, the nematodes wriggled out into the clear water in the funnel and settled at the bottom, and then 100 ml of water containing the nematodes was drawn into a beaker. The nematode suspension was allowed to

Table 2. Nematicidal activity *V. betonicifolia* against *Cephalobus littoralis.*

Plant	Mortality observed against different concentration (%)						
	24 h			48 h			Control
	2	1	0.5	2	1	0.5	
Butanol	20	12	-	50	28	14	2
Hexane	12	7	3	30	18	10	1
Aqueous	4	2	-	10	7	4	2
Methanolic	30	21	16	58	40	25	1
Ethyl acetate	33	25	12	62	39	30	2
Chloroform	37	22	17	66	45	35	2

Table 3. Nematicidal activity *V. betonicifolia* against *H. indicus.*

Plant	Mortality observed against different concentration (%)						
	24 h			48 h			Control
	2	1	0.5	2	1	0.5	
Butanol	18	12	5	29	16	8	1
Hexane	10	5	-	26	12	8	1
Aqueous	3	-	-	7	5	-	2
Methanolic	28	19	13	52	36	20	2
Ethyl acetate	30	18	12	57	34	22	1
Chloroform	25	16	11	49	32	20	1

settle for 2 h or more, the excess supernatant water was poured off, and the remaining concentrated content was transferred into a cavity block for examination under the stereomicroscope and nematodes picked (Naqvi et al., 1992).

Mortality test

Crude extracts were dissolved in water (passed through Whatman filter paper No.1) to make dilutions of 2, 1 and 0.5%. Experiments were performed under laboratory conditions at 28 ± 2°C. Glass tubes 15 cm long and 8 cm were taken for bioassay. 3 ml were taken from all dilutions in each tube. The required amount of nematode suspension (100 freshly hatched second stage juveniles/3 ml suspension) were poured in to the tubes to each of which equal amount of plant extract had already been poured). Distilled water with nematode larvae was taken as control. The dead nematodes were observed under stereoscopic binocular microscope after 24, 48 and 72 h and percentage mortality was calculated. Nematodes were considered dead if they did not move when probed with a fine needle (Muhammad and Saeed, 2011).

RESULTS AND DISCUSSION

The anthelmintics drugs that are commonly used for the treatment of nematodes have little efficacy especially as a single dose regimen. Moreover, the clinical significance has been reduced by the resistance of different nematodes to these drugs (McCarthy, 2005).

Considerable efforts have been made to find out more effective and safe nematicidal. For a period of time now, medicinal plants have been screened to find components with nematicidal activity because they are comparatively safer than synthetic agents. In this context, we have carried out anthelmintic activity of *V. betonicifolia* against various species of worms. The crude extract and subsequent solvent fractions of whole plant of *V. betonicifolia* demonstrated marked vermicidal activity. The overall anthelminintic activity was in a concentration dependent manner. The anthelmintic activity of ethyl acetate and chloroform fractions were very significant against the tested worms. As shown in Table 1, ethyl acetate and chloroform fractions time for death was 42 and 58 min against *P. posthuma*, respectively. Mortality of chloroform and ethyl acetate fractions were 66 and 62% after 48 h against *C. littoralis*, respectively as illustrated in Table 2. Nevertheless, chloroform and ethyl acetate fractions exhibited 49 and 57% mortality against *H. indicus* as shown in Table 3. The current finding can be attributed to the anthelminintic activities of isolated pure entities from *Viola odorata* (Colgrave et al., 2008). Based on the results, it can be concluded that *V. betonicifolia* could be a significant natural source of vermicidal. Further detail studies on the extract especially chloroform and ethyl acetate fractions may lead to the isolation of pure molecules with more prominent efficacy and safety. In this regard, we have already started work

on isolation of active moieties.

ACKNOWLEDGMENT

We are thankful to Higher Education Commission (HEC) Pakistan for providing scholarships for this research study.

REFERENCES

Baermann G (1917). Eine einfache Methode zur Auffindung von Ankylostomum (Nematoden)-Larven in Erdproben. Welteureden. Batavia.Geneesk. Tijschr. Ned. Ind., 57: 131-137.

Bhatt VP, Negi GCS (2006). Ethnomedicinal plant resources of *Jaunsari* tribe of Garhwal Himalaya, Uttaranchal. Ind. J. trad. Know., 5(3): 331-335.

Cobb NA (1918). Estimating the nema population of soil. Agric. Tech. Circ. U.S. Deptt. Agric., 1: 4

Colgrave ML, Kotze AC, Ireland DC, Wang CK, Craik DJ (2008). The Anthelmintic Activity of the Cyclotides: Natural Variants with Enhanced Activity. Chem. Biochem., 9: 1939-1945.

Deore SL, Khadabadi SS, kamdi KS, Ingle VP, Kawalkar NGS, Patil UA, Vyas AJ (2009). In vitro anthelmintic activity of *Cassia tora*, Int. J. Chem. Tech. Res., 2: 177-179.

Geary GT, Woo K, McCarthy JS, Mackenzie CD, Horton J, Prichard RK, Olliaro PL, Lazdins-Helds JK, Engels DA, Bundy DA (2010). Unresolved issues in anthelmintic pharmacology for helminthiases of humans. Int. J. Parasitol., 40: 1-13.

Hussain SZ, Malik RN, Javaid M, Bibi S (2008). Ethonobotanical properties and uses of medicinal plants of biodiversity morgah park, Rawalpindi. Pak. J. Bot., 40(5): 1897-1911.

Ilyas I, Hamayun M (2005). Studies on the traditional uses of plants of Malam Jabba valley, District Swat, Pakistan. Ethnobot. Leaflets.

Khan H, Ali KM, Mahmood T, Choudhary M (2008). Antimicrobial activities of *Gloriosa superba* Linn (Colchicaceae) extracts. J. Enz. 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.

Mccarthy J (2005). Is anthelmintic resistance a threat to the program to eliminate lymphatic filariasis. Am. J. Trop. Med. Hyg., 73: 232-233.

Muhammad N, Saeed M (2011). Biological screening of Viola betonicifolia Smith whole plant. Afr. J. Pharm. Pharmacol., 5(20): 2323-29.

Muhammad N, Saeed M, barkatullah, Ibrar M, Khan H (2012a). Pharmacognostic studies of Viola betonicifolia. Afr. J. Pharm. Pharmacol., 6(1):43-47.

Muhammad N, Saeed M, Khan H, Haq I (2012b). Evaluation of n-hexane extract of Viola betonicifoliafor its neuropharmacological properties. J. Nat. Med: DOI 10.1007/s11418-012-0636-0.

Naqvi B, Khan SA, Shaikh D, Shaikh MR (1992). Nematicidal properties of selected marine algae from Karachi coast. J. Islamic Academy Sci., 5(3): 171-172.

Saeed S, Tariq P (2010a). Antimicrobial activities of *Emblica officinalis* and *Coriandrum sativum* against Gram-positive bacteria and *Candida albicans*. Pak. J. Bot., 39(3): 913-917.

Saeed S, Naim A, Tariq P (2010b). A study on prevalence of multi-drug-resistant Gram negative bacteria. Int. J. Biol. Biotech., 4(1): 71-74.

Tiwari, Radha B, Prabhawati T (2010). Ethnopaediatrics in Garhwal Himalaya, Uttarakhand, India (Psychomedicine and Medicine). New York Sci. J., 3(4): 123-126.