academicJournals

Vol. 12(45), pp. 573-576, 8 December, 2017
DOI: 10.5897/AJPP2013.2970
Article Number: 74485E367102
ISSN 1996-0816

http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

*Short Communication*

# Diuretic, insecticidal and leishmanicidal profile of the whole plant of *Viola betonicifolia*

**Naveed Muhammad[1]*, Muhammad Saeed[2]**

[1]Departmentof Pharmacy, Abdul Wali Khan University Mardan (AWKUM). Pakistan.
2Department of Pharmacy, University of Peshawar, Peshawar, Pakistan.

Received 2 January, 2013; Accepted 28 August, 2014

**In the present study, the crude methanolic and its succeeding solvent fractions of the whole plant of *Viola betonicifolia* were investigated for its diuretic, insecticidal and leishmanicidal profile. The methanolic and *n*-hexane fractions were screened (at the dose of 200, 300 and 400 mg/kg intraperitoneally [i.p.]) for diuretic effects on BALB/c mice. The methanolic extract demonstrated a weak, but not statistically significant diuretic activity compared to the standard drug, while the diuretic effect of *n*-hexane was similar to negative control. The insecticidal effect shown was in low to moderate effect range. The crude methanolic extract and aqueous fraction exhibited 40% mortality against *Callosobruchus analis*, while chloroform and *n*-hexane exhibited 40% activity against *Tribolium castaneum* and *Rhyzopertha dominica.* No leishmanicidal effect was observed against any of the tested samples.**

**Key words:** *Viola betonicifolia,* diuretic, insecticidal and leishmanicidal.

## INTRODUCTION

*Viola betonicifolia* belongs to family Violaceace locally which is known as banafsha. *V. betonicifolia* is grown in various countries of the world like Pakistan, India, Nepal, Srilanka, China, Malaysia and Australia. In Pakistan, it is available in Swat, Hazara and Dir districts. The folk use of this plant is as antipyretic, astringent, diaphoretic, anticancer, purgative and diuretic (Husain et al., 2008). It has been used in the treatment of various neurological disorders including epilepsy and insomnia (Hamayun, 2005). Additionally, it has been used in the treatment of sinusitis, skin and blood disorders and pharyngitis (Bhatt and Negi, 2006). The roots are used for kidney diseases, pneumonia and bronchitis. The flowers are recommended for the treatment of asthma, cough and colds while the leaves are useful for the treatment of boils (Husain et al., 2008). Recently, we have tested the crude methanolic as well as the subsequent solvent fraction of *V. betonicifolia* for various pharmacological activities (Muhammad and Saeed, 2011; Muhammad et al., 2012). In the current study, the diuretic, insecticidal and leishmanicidal of *V. betonicifolia* whole plant were carried out.

## MATERIALS AND METHODS

### Animals

BALB/c mice of either sex were used in all experiments. Animals were purchased from the Pharmacology Section of the Department of Pharmacy, University of Peshawar, Peshawar, Pakistan. The

*Corresponding author. E-mail: drnaveedrph@gmail.com.

animals were maintained in standard laboratory conditions (25°C and light/dark cycles 12/12 h) and were fed with standard food and water *ad libitum*. The experimental protocols were approved by the ethical committee of the Department Pharmacy, University of Peshawar, Peshawar, Pakistan.

### Plant

Whole plant of *V. betonicifolia* was collected from Swat, Khyber Pakhtunkhawa, Pakistan, in April 2010. Plant specimen was identified by Taxonomist, Department of Botany, University of Peshawar and a specimen was deposited there in the herbarium with voucher number 6410/Bot. The collected whole plant (12 kg) was air dried and powdered. The powder was extracted by maceration with methanol at room temperature for 14 days with occasional shaking. The methanolic extract was filtered and concentrated under vacuum using rotary evaporator at low temperature (45°C). The methanolic extract was dissolved in distilled water and further fractionated with chloroform, *n*-hexane, ethyl acetate, *n*-butanol and water.

### Pharmacological study

#### *Diuretic activity*

The diuretic activity of *V. betonicifolia* crude methanolic and its n-hexane fraction was determined using male BALB/c mice. The animals were divided into eight groups (n = 6). The animals were fasted for 24 h and were fed laboratory diet and water *ad libitum*. On the day of experiment, the animals of group I was treated with distilled water (15 ml/kg intraperitoneally [i.p]) and this group served as control. Similarly, the animals of group II were treated with frusemide (10 mg/kg i.p.). Groups III, IV and V were treated with 200, 300 and 400 mg/kg (i.p) methanolic extract, respectively. The remaining groups VI, VII and VIII were injected 200, 300 and 400 mg/kg (i.p) *n*-hexane fraction, respectively. All the treated animals were placed in metabolic cages (1 animal in each metabolic cage). Urine was collected in graduated cylinders and its volume was recorded at 2, 3 and 6 h. Cumulative urine excretion was calculated in relation to body weight and expressed as ml/100 g body weight.

#### *Insecticidal activity*

*In vitro* insecticidal assay was carried out against *Tribolium castaneum, Rhyzopertha dominica* and *Callosobruchus analis* following method available in literature (Saeed et al., 2010). The test sample was prepared (200 mg of crude extract was dissolved in 3 ml of methanol and served as stock solution). The sample (1019.10 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, 239.50 µg/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.

$$\text{Inhibition (\%)} = 100 - \frac{\text{Number of insects alive in test}}{\text{Number of insects alive in control}} \times 100$$

### Leishmanicidal assay

Leishmanicidal assay was carried out using previously described method (Saeed et al., 2010). *Leishmania major* (MHOM/SU/73/5-ASKH) promastigotes were cultured at 22 to 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 106 cells/ml. In a 96-well microtiter plate, 180 ml of medium was added in first row and 100 ml of medium was added in others wells. Test extracts (20 ml) was added in medium and serially diluted. 100 ml 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 to 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

### Diuretic effect

The crude methanolic of the whole plant of *V. betonicifolia* was 200, 300 and 400 mg/kg and its *n*-hexane solvent fraction was 200, 300 and 400 mg/kg. It is clear from our results that both tested extracts were devoid of diuretic effect as shown in Figure 1. The diuretic effect of methanolic extract was slightly greater than the negative control group, but was statistically non significant. In the traditional medicines, *V. betonicifolia* is used as diuretic (Husain et al., 2008), but our results were able to provide a scientific background to the folklore of this plant. The reason of the failure of our test extract as diuretic might be due to the use of *V. betonicifolia* as polypharmacy (in combination with other plants) for diuretic effect. It means that the methanolic extracts have diuretic molecules, but their action is mild. So our tested compound is mild diuretic.

### Insecticidal effect

The crude methanolic extract and its succeeding solvent fractions were screened for insecticidal effect against three insects, that is, *T. castaneum, R. dominica* and *C. analis.* The tested samples showed low to moderate insecticidal activity as shown in Table 1. The crude methanolic extract showed 20 and 40% activity against *T. castaneum* and *C. analis,* while chloroform exhibit 40% mortality against *T. castaneum* as presented in Table 1. The percent mortality of *n*-hexane was 40 and 20% against *R. dominica* and *C. analis* ethyl acetate was inactive against any of the tested insect. The butanolic extract showed low mortality (10%) against *T. castaneum,* while a moderate mortality (40%) was shown by aqueous against *C. analis.*

### Leishmanicidal effect

The leishmanicidal effect of the crude methanolic and its

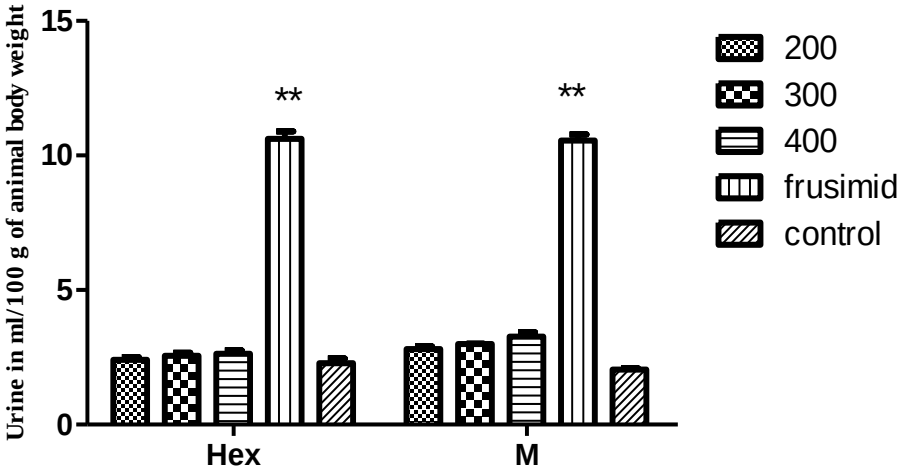


**Figure 1.** Diuretic effect of M (methanolic) and Hex (*n*-hexane) fraction of *Viola betonicifolia* in mice. Bar presents the volume of urine after 5 h of the treatment with frusemide (10 mg/kg) and extracts (200, 300 and 400 mg/kg).

**Table 1.** Insecticidal activity of *Viola betonicifolia*.

| Name of insect | Standard | Control | Mortality (%) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | M | Ch | Hex | Et | Bu | Aq |
| *Tribolium castaneum* | 100 | - | 20 | 40 | - | - | 10 | - |
| *Rhyzopertha dominica* | 100 | - | - | - | 40 | - | - | - |
| *Callosobruchus analis* | 100 | - | 40 | - | 20 | - | - | 40 |

M: Methanolic, Ch: chloroform, Hex: n-hexane, Bu: butanol, Aq: aqueous.

**Table 2.** Leishmanicidal effect of *Viola betonicifolia*.

| Test organism | Sub-fraction of oil | $IC_{50}$ (µg/ml) ± SD |
|---|---|---|
| *Leishmania major* (MHOM/SU/73/5-ASKH) | M | > 100 |
| | Ch | > 100 |
| | Hex | > 100 |
| | Et | > 100 |
| | Bu | > 100 |
| | Aq | > 100 |
| | Amphotercin-B | 0.29 ± 0.05 |
| | Pentamidine | 5.09 ± 0.04 |

M: Methanolic, Ch: chloroform, Hex: n-hexane, Bu: butanol, Aq: aqueous. $IC_{50}$: values indicate the effective concentration of a compound in µg/ml necessary to achieve 50% inhibition. Incubation period 72 h and incubation temperature was 22°C.

succeeding solvent fractions is depicted as shown in Table 2. None of the tested sample showed any leishmanicidal activity with $IC_{50}$ more than 100. The diuretic effect of methanolic extract and its subsequent solvent fractions at the test dose of 200 and 400 mg/kg of *Viola odorata* (related species of *V. betonicifolia*) have been reported (Vishal et al., 2009).

## REFERENCES

Bhatt V, Negi G (2006). Ethnomedicinal plant resources of Jaunsari tribe of Garhwal Himalaya, Uttaranchal. Indian J. Tradit. Knowl. 5:331-335.

Hamayun M (2005). Ethnobotanical profile of Utror and Gabral valleys, district Swat, Pakistan. Ethnobot. Leafl. (1):9.

Husain SZ, Malik RN, Javaid M, Bibi S (2008). Ethonobotanical properties and uses of medicinal plants of Morgah biodiversity park,

Rawalpindi. Pak. J. Botany 40:1897-1911.
Muhammad N, Saeed M, barkatullah, Ibrar M, Khan H (2012a). Pharmacognostic studies of *Viola betonicifolia* Afr. J. Pharm. Pharmacol. 6:43-47.
Muhammad N, Saeed M (2011). Biological screening of Viola betonicifolia Smith whole plant. Afr. J. Pharm. Pharmacol. 5:2323-2329.
Saeed M, Khan H, Khan MA, Simjee SU, Muhammad N Saeed AK (2010). Phytotoxic, insecticidal and leishmanicidal activities of aerial parts of *Polygonatum verticillatum*. Afr. J. Biotechnol. 1241-1244.
Vishal A, Parveen K, Pooja S, Kannappan N, Kumar S (2009) Diuretic, laxative and toxicity Studies of *Viola odorata* aerial parts. Pharmacology online 1:739-748.