In vitro anthelmintic studies of *Chlorophytum borivilianum* Sant. & Fernandez tubers

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The present communication deals with the laboratory studies carried out to ascertain the anthelmintic properties of *Chlorophytum borivilianum* Sant. & Fernandez saponin extracts against *Pheretima posthuma* and *Ascardia galli*. Methanolic extract (ME), crude saponin extract (CSE) and purified saponin fractions (PSF) were used as test solutions. Piperazine citrate was included as standard drug and distilled water as control. Study involved the determination of time of paralysis and time of death of the worm. The anthelmintic activity of saponin extracts of *C. borivilianum* root tubers has therefore been demonstrated for the first time.

**Keyword:** Anthelmintic activity, *Ascardia galli*, *Chlorophytum borivilianum*, *Pheretima posthuma*.

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**Introduction**

The World Health Organization (WHO) has estimated that approximately 80% of the world’s population depends on traditional medicines for meeting their primary health care needs. Parasites have been of concern to the medical field for centuries and the helminths still cause considerable problems for human beings and animals. During the past few decades, despite numerous advances made in understanding the mode of transmission and the treatment of these parasites, there are still no efficient products to control certain helminths and the indiscriminate use of some drugs has generated several cases of resistance. As an important component of complementary and alternative medicine, traditional Ayurvedic medicinal plants may be a useful model for the discovery and development of new chemical substances for helminth control which are generally considered to be very important sources of bioactive substances.

*Chlorophytum borivilianum* Sant. & Fernandez belonging to family Liliaceae is a very well known plant for its aphrodisiac as well as immunomodulatory activity in India (Plate 1). Roots holds very important position in Ayurveda and Unani system where it is mostly used to treat oligospermia, pre- and post-natal symptoms, arthritis, diabetes and dysuria, antiviral, anticancer, immunomodulatory, antidiabetic, antistress, aphrodisiac, antimicrobial and anti-inflammatory activities of root extracts have been evaluated. The root contains carbohydrates, phenolic compounds, saponins and alkaloids. As the saponins are reported to have anthelmintic activity, hence

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Plate 1—*Chlorophytum borivilianum*
present study was undertaken to prove efficacy of *C. borivilianum* root tuber extracts against selected worms.

**Materials and Methods**

**Plant Materials, Extraction and Isolation**

*C. borivilianum* roots were purchased from local cultivator and authenticated by Dr. Prabha Y. Bhogaonkar (Director, Government Vidarbha Institute of Science and Humanities, Amravati). A specimen sample (CB/SLD-02) is deposited at Department of Botany, Vidarbha Institute of Science and Humanities, (V.M.V), Amravati.

The tubers were washed, dried, powdered and defatted by petroleum ether. Marc then extracted with methanol for 3 hours at 50°C which is further concentrated to give methanol extract (ME). In order to get the crude saponins, ME extract was again dissolved in methanol and then acetone was added (1:5 v/v) to precipitate the saponins as described by Yan *et al* [17]. The precipitates were dried under vacuum. The whitish amorphous powder thus obtained as a crude saponin extract (CSE). To get the pure saponin fraction (PSF), certain amount of CSE was fractionated with silica gel-60 (230-400 mesh) by column chromatography and eluted successfully with chloroform-methanol-water (70:30:10) as described by Favel *et al* [18]. Eluted fractions were combined to give purified saponin fraction (PSF).

**Experimental**

Indian adult earthworms (*Pheretima posthuma*) collected from moist soil and washed with normal saline to remove all faecal matter were used for the anthelmintic study. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol. *Ascardia galli* worms are easily available in plenty from freshly slaughtered fowls and their use, as a suitable model for screening of anthelmintic drug was advocated earlier. Methanolic extract (ME), crude saponin extract (CSE) and purified saponin fractions (PSF) from the tubers of *C. borivilianum* were investigated for their anthelmintic activity against *Pheretima posthuma* and *Ascardia galli*. Various concentrations (10-50 mg/ml) of each extract were tested in the bioassay, which involved determination of time of paralysis and time of death of the worms.

The anthelmintic assay was carried as per the method of Ajaiyeoba *et al* [19] with minor modifications.

In the first set of experiment, six groups of six earthworms were released in to 50 ml of solutions of piperazine citrate, ME, CSE and PSF extracts of tubers of *C. borivilianum* in distilled water. Piperazine citrate was included as standard drug and distilled water as control.

Observations were made for the time taken to paralyze and death of individual worms. Time for paralysis was noted when no movement 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 colours. Same experiment was done for *Ascardia galli* worms, only the difference was of solutions which were prepared in normal saline solutions.

**Results and Discussion**

Preliminary phytochemical screening of alcoholic extract revealed the presence of saponin glycosides, sterols, phenolic compounds and alkaloids while aqueous extract showed presence of glycosides and phenolic compounds. The extracts subjected to TLC (Fig. 1) in suitable solvent system (CHCl₃: Glacial acetic acid: MeOH: Water, 16:8:3:2) derivitised with Anisaldehyde-sulphuric acid reagent was given 4 separated spots which get faded as isolation targeted towards saponins. TLC analysis of the crude extracts revealed presence of saponins as one of the chief chemical constituents and saponins are known to have anthelmintic activity.
From the results shown in Table 1, the predominant effect of piperazine citrate on the worm is to cause a flaccid paralysis that results in expulsion of the worm by peristalsis. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis. Figures 2 and 3 are showing comparative data of different fractions for paralysis and death of *Pheretima posthuma*.

All extracts showed significant anthelmintic activity on selected worms. PSF found to be more active as compared to ME and CSE. The PSF demonstrated paralysis as well as death of worms in a less time (24.2 min) as compared to piperazine citrate (26.7 min) especially at higher concentration of 25 mg/ml in case of *Pheretima posthuma*. While in case of *Ascardia galli* PSF has taken about same time as that of PC, ME and CSE caused paralysis of *Pheretima posthuma* and *Ascardia galli* in 34 min and 21.02 min, respectively and death in 82 min and 53.1 min, respectively.

**Conclusion**

From the results it can be concluded that the tubers of *C. borivilianum* can be used as anthelmintic. Phytochemical studies reveal presence of saponins is major constituents hence it is also confirmed that anthelmintic activity is due to saponins. Further studies involving isolation of responsible constituents...
and *in vivo* models of anthelmintic activity to establish the mechanisms (S) of action are required.

**References**


