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# Anticestodal property of *Strobilanthes discolor*: An experimental study in *Hymenolepis diminuta*—rat model

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**Abstract**

Use of *Strobilanthes discolor* T. Anders leaves in the treatment of intestinal worm infections is a common ethnobotanical practice in the Naga tribes of north-eastern part of India. In the present communication the anticestodal efficacy of *Strobilanthes discolor* leaf extract was investigated using *Hymenolepis diminuta*—rat experimental model. The effects of leaf extract were adjudged by monitoring the eggs per gram of faeces (EPG) counts and percentage worm recovery rates following treatment with methanol leaf extract of this plant to different groups of rats harbouring *Hymenolepis diminuta* infections. The leaf extract showed significant reductions in EPG counts as well as in recovery of surviving worms at autopsy. A notable result of the extract's efficacy was observed against the larval stages of parasite, where no single worm was recovered at its 800 mg/kg dose administered twice daily for 3 days. Effects of plant extract on adult stages were almost comparable with that of a standard drug, Praziquantel.

The study suggests that the leaf extract of *Strobilanthes discolor* possesses significant anticestodal activity and supports its use in the folk medicine.


*Keywords:* *Strobilanthes discolor*; Anticestodal activity; *Hymenolepis diminuta*; India

## 1. Introduction

*Strobilanthes* Blume (Acanthaceae) is a large tropical genus of 400–500 species distributed mainly in south and south-east Asia. Commonly named as Lavender bell, *Strobilanthes discolor* T. Anders (Acanthaceae) is a large sub-gregarious shrub which in the north-eastern part of India known as *Masupni.* Its distribution ranges from Himalayas range including Naga Hills, Bhutan, Assam and Khasia mountains. In the course of our study on ethnomedicine of Naga tribes in the north-eastern part of India, we came across about the use of its leaf decoction in the treatment of intestinal worm infections. So far no report on its biological activity or chemical constituents present is available in any literature. In view of its uses in folk medicine this study was undertaken to investigate the efficacy of leaf extract of *Strobilanthes discolor* as claimed by local people, using *Hymenolepis diminuta*—rat model.

## 2. Materials and methods

### 2.1. Plant material and preparation of the extract

Fresh leaves of *Strobilanthes discolor* were collected in January 2003 by Vareishang Tangpu from Mungreitang Paoyi, Manipur, India. A voucher specimen of the collected material has been deposited in the herbarium of Department of Zoology, NEHU (No. AKY 212). The plant material was air-dried under shade and powdered for extraction in methanol at 40 °C by Soxhlet fractional distillation method (Yadav et al., 1992). The final crude extract was recovered using a rotatory evaporator and stored at +8 °C until use. The total yield of the final extract was 51.01%.

### 2.2. Drugs

Praziquantel (Distocide®), the standard reference drug used in the study was manufactured by Shin Poong Pharm. Co. Ltd., Seoul, Korea. Plant extract and Praziquantel (PZQ) solutions were prepared fresh in 0.9% phosphate buffer saline (PBS) at pH 7.3 before administration.

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### 2.3. Experimental animals

Male and female albino rats (100–120 g) were used. They were maintained under standard environmental conditions and fed with rodent diet (Pranov Agro Industries Ltd., Delhi) and water ad libitum. Proper care was taken to protect the welfare of the experimental animals and all the experiments were performed according to the rules laid down by the Institutional Animal Care and Use Committee.

### 2.4. Maintenance of Hymenolepis diminuta infection

The infection of *Hymenolepis diminuta* has been maintaining in our laboratory for years by alternating the hosts as described by Dixon and Arai (1991). Gravid segments of *Hymenolepis diminuta* were scratched smoothly onto filter papers in Petri dishes. *Tribolium confusum* Jacquelin du Val (Tenebrionidae), the intermediate host, were allowed to feed on the eggs of *Hymenolepis diminuta* for 72 h and had free access to flour and kept for 12–14 days at room temperature or until dissected. On dissecting upon the beetles, cysticercoids were collected and suspended in normal saline and inoculated to previously uninfected rats. After 18–20 days, eggs of *Hymenolepis diminuta* could be observed in the faeces of rats, which were mixed with flour powder and fed to the beetles and the life cycle continues in the laboratory.

### 2.5. Preliminary acute toxicity test

The leaf extract was administered orally in the doses of 62.5, 125, 250, 500, 1000, and 2000 mg/kg, p.o. to six animals in each group. The general signs and symptoms of toxicity, intake of food and water, and mortality rates were observed for 72 h post-administration of extract (Tangpu and Yadav, 2004).

### 2.6. Effect of Strobilanthes discolor leaf extract on larval stages

Thirteen groups of animals ($n = 6$) were used in this experiment. All animals were inoculated with five cysticercoids each by a feeding tube and maintained in separate cages. Treatments with leaf extract at different single and double doses per day (100, 200, 400, and 800 mg/kg, p.o.) in groups 2–9, and with PZQ at single and double doses per day (5 and 25 mg/kg, p.o.) in groups 10–13 were administered to the rats on day 2–4 post-inoculation of cysticercoids.

The group 1 was used as the control and given 1.0 ml of saline per day for the same 3 days. From day 18 post-infection, 1 g of fresh faeces was collected from each cage of the treated and control rats for eggs per gram (EPG) counts using modified Mc Master method (Anonymous, 1977) for 3 days (days 18–20). Follow-up examination of EPG was done on days 28–30 following a week EPG count. Finally an autopsy was performed by chloroform anaesthesia killing of the animals on day 31 and accordingly worm recovery rate (%) was estimated as described by Rim et al. (1980).

### 2.7. Effect of Strobilanthes discolor leaf extract on immature stages

Thirteen groups of animals ($n = 6$) were used in this experiment and leaf extract was given on days 8–10 post-inoculation of cysticercoids. The EPG counts and worm recovery rate (%) were calculated out as given in the above experiment.

### 2.8. Effect of Strobilanthes discolor leaf extract on adult stages

Animals were divided into 26 groups ($n = 6$), each animal was inoculated with five cysticercoids. Groups 1 and 14 served as the control untreated. Groups 2–13 received single and double doses of leaf extract (100, 200, 400, and 800 mg/kg, p.o.) and PZQ (5 and 25 mg/kg, p.o.) for 3 days (days 21–23 post-inoculation). Groups 15–26 received same doses of leaf extract and PZQ for 5 days (days 21–25). EPG counts for each group (groups 1–26) were done for 3 days (days 18–20) before treatment, for 3 days after treatment (days 24–26 for 3-day treatment groups and days 26–28 for 5-day treatment groups) and for another 3 days after 1 week post-treatment (days 34–36 for former treatment groups and days 36–38 for the latter groups). Finally an autopsy was performed for all the groups on completion of experiments and accordingly worm recovery rates (%) were calculated.

### 2.9. Statistical analysis

All results were reported as mean ± S.E.M. These results were further analysed by using Student's *t*-test to calculate the significance of the results (Prasad, 2003). *P* values less than 5% were considered significant.

## 3. Results

### 3.1. Acute toxicity

The leaf extract when orally given to the rats at doubling doses from 62.5 up to 2000 mg/kg, p.o. showed no mortality or any adverse signs in the animals with regard to body weight, body temperature, and food and water in take up to 72 h post-treatment.

### 3.2. Effect of leaf extract on larval stages

The effects of leaf extract on larval stages of *Hymenolepis diminuta* infections in rats as monitored by EPG counts and worm recovery rate (%) are shown in Table 1. The EPG values (0-13539) of leaf extract treated groups significantly reduced in dose-dependent manner when compared to control (31,256). Treatments with double doses of the extract showed higher reductions in the EPG counts when compared to single doses. Further, there were no eggs observed in the treated group at 800 mg/kg dose given twice daily for 3 days. With regard to percentage worm recovery rate, the results were comparable with that of the standard drug PZQ and there was no worm recovered