

A STUDY ON ANTHELMINTHIC ACTIVITY OF *TINOSPORA CORDIFOLIA* EXTRACTSM. RAJESHWAR REDDY¹, K. TIRUMAL REDDY¹, A. B. VEDAMURTHY¹, V. KRISHNA² AND H. JOY HOSKERI^{*1,2}

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ABSTRACT

Helminthes infections are commonly found in community and are being recognized as cause of much acute as well as chronic illness among the various human beings as well as cattle's. *Pherithema posthuma* a helminthes is commonly known as earth-worms. *Tinospora cordifolia* is an herbaceous vine, indigenous to the tropical regions. *T. cordifolia* is traditionally used as an anthelmintic agent. The anthelmintic property of *T. cordifolia* extracts was evaluated using *Pherithema posthuma* as an experimental model. Piperazine citrate was used as the standard reference. Among the various concentrations of aqueous extract tested, 250 mg/ml showed efficient anthelmintic activity and among all the concentrations ethanol extract tested, 250 mg/ml showed significant results. This investigation revealed that ethanol extract of *T. cordifolia* showed significant anthelmintic activity against *Pheretima posthuma* when compared aqueous extract. Ethanol extract also proved to be efficient than the standard drug. This investigation supported the ethnomedical claims of *T. cordifolia* as anthelmintic plant.

Keywords: Menispermaceae; *Tinospora cordifolia*; anthelmintic activity; *Pheretima posthuma*; Ethanol extract; Aqueous extract.

INTRODUCTION

Parasitic helminthes are worm-like organisms that live and feed on the living hosts by receiving nourishment and protection, while disrupting their host's nutrient absorption, causing weakness and disease in human and animals inflicting heavy production losses. Approximately 3 million people globally are infected with helminthes. Chemical control of helminthes coupled with improved management has been an important worm control strategy throughout the world. However, increasing problems of development of resistance in helminthes against anthelmintics have led to the proposal of screening medicinal plants for their anthelmintic activity^{1,2}. Plants are known to provide a rich source of botanical anthelmintics^{3,4}. A number of medicinal plants have been used to treat parasitic infections in man and animals^{5,6}. Anthelmintics are those agents that expel parasitic worms (helminthes) from the body, by either stunning or killing them.

Helminthes infections are commonly found in community and being recognized as cause of much acute as well as chronic illness among the various human beings as well as cattle's. More than half of the population of the world suffers from various types of infection and majority of cattle's suffers from worm infections⁷. However, the high cost of modern anthelmintics has limited the effective control of these parasites. In some cases widespread intensive use of sometimes low quality anthelmintics⁸ has led to development of resistance and hence a reduction in the usefulness of available anthelmintics⁹. Although the use of alternate drugs has also been advocated as a measure to avoid the development of resistant strains of helminth parasites, and as a means of reducing the cost of controlling helminthic diseases¹⁰⁻¹³.

Tinospora cordifolia (Menispermaceae) is an herbaceous vine, indigenous to the tropical areas of India, Myanmar and Sri Lanka. This plant is a glabrous climbing shrub found throughout India, typically growing in deciduous and dry forests¹⁴. Many investigators have evaluated various pharmacological properties of *T. cordifolia*, it has been shown to possess anti-allergic, anti-inflammatory, antioxidant activities¹⁵, immuno-modulatory, hepatoprotective, antipyretic¹⁶, antidiabetic¹⁷, anti-hyperlipidemic properties¹⁸, urinary diseases, syphilis, skin diseases, and bronchitis¹⁹. *T. cordifolia* has been known to promote longevity and increase the body's resistance against various diseases²⁰. In Ayurveda, *T. cordifolia* is used as a rasayana, which in Sanskrit implies circulation of nutrient. The ancient Indian physician, Charaka described rasayana as

antiaging, increasing the life span, promoting intelligence, improving memory and freedom from diseases, indicating immunostimulant effect²¹. *T. cordifolia* has been traditionally used as an anthelmintic agent²². However, anthelmintic activity of *T. cordifolia* stem extract has not so far been scientifically proved and reported. To justify the traditional claims of *T. cordifolia*, we made an efficient attempt to assess the anthelmintic activity of the ethanol and aqueous extract of *T. cordifolia*.

MATERIALS AND METHODS

Drugs and chemicals

The standard drug piperazine citrate (SD Fine Chemicals Ltd., Mumbai). Ethanol was purchased from Hong, Yang Chemical Corporation, China.

Plant Resource

Tinospora cordifolia plant material was collected from Bhadra Wildlife Sanctuary, Western Ghats, India. The plant was authenticated by Prof. V. Krishna, Kuvempu University. Fresh plant material was washed thoroughly in tap water to remove traces of soil and other contaminants. It was then shade dried. Further, the stem bark was chopped finely and shade dried, powdered mechanically and was subjected to cold extraction using ethanol as the solvent system for about 96 h, after every 24 h fresh ethanol was added and ethanol containing the crude extract was separated. After ethanol extraction, aqueous extract was followed subsequently by using double distilled water (with 5% ethanol, to avoid microorganism contamination) for 96 h. Both the extracts were filtered and concentrated in vacuum under reduced pressure and allowed for complete evaporation of the solvent on water bath and finally vacuum dried. The yield of ethanol crude and aqueous extract for 1 kg of powdered plant material was 46 g and 53 g respectively.

Test organism

Indian adult earthworms (*Pheretima posthuma*) collected from the Indo-American Hybrid Seeds, Bangalore. The earthworms were maintained under normal vermicomposting medium with adequate supply of nourishment and water, for about two weeks. Before the initiation of experiment the earthworms were washed with normal saline. Adult earthworms of approximately 4 cm in length and 0.2-0.3 cm in width were used for the experiment. This organism was selected as a model for anthelmintic activity due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings^{23,24}.

Extract preparation for experiment

The porously powdered plant material was used for extract preparation. After extraction, the crude extracts were stored in dessicator until further use. Ethanol extract and standard drug piperazine citrate were dissolved in 0.5% DMSO in normal saline (v/v). Where as, the crude aqueous extract was directly dissolved in normal saline and were used for evaluation for anthelmintic activity.

Anthelmintic activity

The anthelmintic activity of ethanol and aqueous extracts of *Tinospora cordifolia* was evaluated as per the method reported by Dash et al.²⁵. Twelve groups with three earthworms in each groups, each earthworm was separately released into 20 ml of desired formulation in normal saline, Group I earthworm were released in 20 ml normal saline in a clean petri plate. Group II, III, IV, V, VI earthworms were released in 50, 100, 150, 200 and 250 mg/ml of ethanol extract respectively. Similarly, group VII, VIII, IX, X, XI earthworms were released in 50, 100, 150, 200 and 250 mg/ml of aqueous extract respectively. Group XII earthworms were released in normal saline containing standard drug piperazine citrate (50 mg/ml). Earthworms were observed; and the time taken for paralysis and the time taken for death was monitored and

documented in minutes. Paralysis time was analyzed based on the behavior of the earthworm with no revival body state in normal saline medium. Death was concluded based on total lose of motility with faded body color²⁶. The result of anthelmintic activity is depicted in Table 1.

Statistical analysis

The data of anthelmintic evaluations were expressed as mean \pm S.E.M of three earthworms in each group. The statistical analysis was carried out using one way ANOVA followed by Tukey's *t*-test. The difference in values at $P < 0.01$ was considered as statistically significant. The analysis of variance (ANOVA) was performed using ezANOVA (version 0.98) software to determine the mean and standard error of paralysis and death time of the earthworms.

RESULTS AND DISCUSSION

Tinospora cordifolia is a well known medicinal plant and is widely used in folk medicine/ ayurvedic system of medicine. In the present study, solvents namely ethanol and water were used sequentially for crude extraction of *T. cordifolia* stem bark. To justify the ethnomedical claims of *T. cordifolia*, we made an efficient attempt in evaluating the anthelmintic property of *T. cordifolia*.

Table 1: *In vitro* anthelmintic activity of ethanol and aqueous extracts of *T. cordifolia* against *Pheretima posthuma*

| Test samples | Concentration (mg/ ml) | Time taken for paralysis (min) | Time taken for death (min) |
|-----------------------------------------|------------------------|--------------------------------|----------------------------|
| Control (Normal Saline) | | 64.33 \pm 0.88 | 200.33 \pm 2.60 |
| Ethanol extract of <i>T. cordifolia</i> | 50 | 33.33 \pm 1.76** | 51 \pm 1.53** |
| | 100 | 14.67 \pm 1.02** | 22.67 \pm 0.33** |
| | 150 | 11.67 \pm 0.88** | 20.67 \pm 1.76** |
| | 200 | 8.33 \pm 0.33** | 14.33 \pm 0.88** |
| | 250 | 6 \pm 0.58** | 12 \pm 0.58** |
| Aqueous extract of <i>T. cordifolia</i> | 50 | 105.67 \pm 1.76** | 180.44 \pm 0.88* |
| | 100 | 85.33 \pm 0.88** | 139.33 \pm 0.45** |
| | 150 | 61.67 \pm 1.76 ns | 103.67 \pm 0.76** |
| | 200 | 47.67 \pm 0.88** | 85 \pm 1.13** |
| | 250 | 33.67 \pm 1.02** | 67.67 \pm 1.20** |
| Piperazine citrate | 50 | 31.33 \pm 1.86** | 40.67 \pm 0.88** |

Values are the mean \pm S.E.M. of three earthworms. Symbols represent statistical significance. * $P < 0.05$, ** $P < 0.01$, ns: not significant as compared to compared to control group.

Aqueous extract at the concentration of 50 mg/ml showed the time of paralysis and death at 105 and 180 min respectively. For concentration of 100 mg/ml, the paralysis and the death time was found to be 85 and 139 min respectively. At the concentration of 150, 200 and 250 mg/ml, time taken to paralysis was 61, 47 and 33 min respectively and death time 103, 85 and 67 min respectively. Among the various concentrations tested, aqueous extract at 250 mg/ml showed efficient anthelmintic activity (Table 1). On the other hand ethanol extract at the concentration of 50 mg/ml showed the time of paralysis and death at 33 and 51 min respectively. For concentrations at 100, 150, 200 and 250 mg/ml paralysis was shown at 14, 11, 8 and 6 min respectively and death occurred at 22, 20, 14 and 12 min respectively. Among all the concentrations ethanol extract tested, 250 mg/ml gave significant results. Standard drug at 50 mg/ml showed paralysis at 31 min and death time was 40 min (Table 1). Various medicinal plant have proved to be efficient anthelmintic drug including *Curcuma longa* and *Zingiber officinale*^{27,28}. This investigation revealed that ethanol extract of *T. cordifolia* showed significant anthelmintic activity against *Pheretima posthuma* when compared aqueous extract. Ethanol extract also proved to be efficient than the standard drug. This investigation supported the ethnomedical claims of *T. cordifolia* as anthelmintic plant.

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