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PHYTOCHEMICAL SCREENING AND BIOLOGICAL STUDIES ON THE LEAVES OF *TINOSPORA CORDIFOLIA*

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ABSTRACT: Helminthiasis is also known as worm infection and a endoparasitic disease of humans and other animals in which a part of a body is infected with parasitic worms. *Tinospora cordifolia* is a well known medicinal plant and widely used in folk medicine ayurvedic system. The objective of this work is to carry out phytochemical screening and in vitro anthelmintic activity of methanolic, petroleum ether and ethylene glycol extracts of leaves of *Tinospora cordifolia* (Menispermaceae) against Indian earthworm, Pheretima posthuma. Various concentrations (2.5, 5, 10, 20 mg/ml) of the extracts were tested, which involved determination of time of paralysis and time of death of the worms using Albendazole (10 mg/ml) as standard drug and normal saline as control. The methanolic extract exhibited a maximum anthelmintic activity than the other two extracts. The extract showed anthelmintic activity in dose dependant manner giving shortest time of paralysis (P) and death (D) with 20mg/ml concentration and caused paralysis in 4 min and death in 10 min respectively. The Preliminary phytochemical analysis indicated the presence of various phytoconstituents in the extract of which alkaloids, glycosides, triterpenes and steroids might have contributed for the potent anthelmintic activity. From the above study, it significantly shown the anthelmintic activity and provides scientific rationale for the traditional use of *Tinospora cordifolia* for Helminthiasis. The future scope of *Tinospora cordifolia* provides economic, safe and efficacy of anthelmintic drug contributing to the society.

INTRODUCTION: Helminthiasis is a highly prevalent disease worldwide that is caused by species platy helminthes (flat worms) and nematoda (round worms) ¹. 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 ².

The disease may transmitted by fecal-oral route. According to the WHO, approximately 2 billion people are affected by helminthic infections worldwide ³. Children are vulnerable to complications like malnutrition ⁴. Chemical control of helminthes coupled with improved management has been an important worm control strategy throughout the world ⁵.

However, the high cost of modern anthelmintics has limited the effective control of these parasites ⁶. In some cases widespread intensive use of sometimes low quality anthelmintics has led to development of resistance and hence a reduction in the usefulness of available anthelmintics ^{7, 8}.

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However, increasing problems of development of resistance in helminthes against anthelmintics have led to the proposal of screening medicinal plants for their anthelmintic activity⁹. A number of medicinal plants have been used to treat parasitic infections in man and animals¹⁰. Anthelmintics are those agents that expel parasitic worms (helminthes) from the body, by either stunning or killing them^{11,12}.

Tinospora cordifolia (Menispermaceae) is an herbaceous vine, indigenous to the tropical areas of India, Myanmar and Sri Lanka¹³. This plant is a glabrous climbing shrub found throughout India, typically growing in deciduous and dry forests. Many investigators have evaluated various pharmacological properties of *T. cordifolia*, it has been shown to possess antiallergic¹⁴, anti-inflammatory¹⁵, antioxidant activities¹⁶, immunomodulatory¹⁶, hepatoprotective¹⁷, antipyretic¹⁷, antidiabetic¹⁷, antihyperlipidemic properties¹⁸, urinary diseases, syphilis, skin diseases, and bronchitis. *T. cordifolia* has been known to promote longevity and increase the body's resistance against various diseases¹⁶. 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 effect. *T.*

cordifolia has been traditionally used as an anthelmintic agent. However, anthelmintic activity of *T. cordifolia* stem extract has not so far been scientifically proved and reported¹⁷.

To justify the traditional claims of *T. cordifolia*, we made an efficient attempt to assess the anthelmintic activity of methanol, petroleum ether and ethylene glycol extracts of *T. cordifolia* on *Pheritima posthum*¹¹.

Collection of Plant Material: The leaves of plant *Tinospora cordifolia* was collected from Bobbili forest, Vizianagaram district, Andhra Pradesh, India and was authenticated (Voucher no. 7066_379).

EXPERIMENTAL METHODS:

Extract Preparation: The leaves were separated and washed thoroughly with sufficient water. It was dried under shade for two weeks and powdered in a whely mill the powdered material was subjected to maceration (cold extraction)¹⁹. The air dried powdered material (180 mg) of leaves of plant was extracted with petroleum ether, methanol, ethylene glycol by cold extraction kept for several hrs for about 7 days of occasional shaking as represented in **Table 1**. The extract was concentrated under vacuum in rotary evaporator, dried completely and weighed.

TABLE 1: EVALUATION OF ANTIHELMINTHIC ACTIVITY

| S. no. | Plant Material | Solvent used | Volume of solvent | Weight of the extract |
|--------|----------------|-----------------|-------------------|-----------------------|
| 1 | 180 gm | Petroleum ether | 500 ml | 0.78 gm |
| 2 | 180 gm | Methanol | 500 ml | 3.2 gm |
| 3 | 180 gm | Ethylene Glycol | 500 ml | 1.0 gm |

Phytochemical screening for major constituents was undertaken using standard qualitative methods^{20,21}. The plant extracts were screened for

the presence of alkaloids, glycosides, steroids, triterpenes and carbohydrates. Phytochemical screening represented in **Table 2**.

TABLE 2: PHYTOCHEMICAL SCREENING OF TINSPOSA CARDIFOLIA

| Name of the test | Petroleum ether extract | Methanolic extract | Ethylene glycol extract |
|----------------------------|-------------------------|--------------------|-------------------------|
| Steroids: | | | |
| a) salkowski test | + | + | - |
| b) Libermann Buchards test | + | + | + |
| Triterpenes: | | | |
| a) Salkowski test: | + | + | - |
| b) Libermann Buchards test | - | - | - |
| Alkaloids | | | |
| a) mayers test | - | - | - |
| b) Dragendroffs test | - | - | - |
| c) Wagners test | + | + | - |
| d) Hagers test | + | + | + |

| | | | |
|-------------------------------|---|---|---|
| Carbohydrates: | | | |
| a) Fehlings test | + | - | + |
| b) Molischs test | - | - | + |
| Glycosides: | | | |
| 1. Cardiac glycosides | | | |
| a) Baljet test | + | + | + |
| b) Legal test | + | + | + |
| c) Keller kiliani test | + | + | + |
| 2. Anthraquinone glycosides | | | |
| a) Modified born tragger test | + | + | - |

Antihelminthic Activity:

Worm Collection: Adult Indian earth worm, *Pheretima posthuma* (Annelida) was used to evaluate anthelmintic activity *in vitro*^{22, 23}. *Pheretima posthuma* was collected from the local regions of Bobbili. The average size of earthworm was 8 - 10 cm.

Drug and Chemicals: Albendazole was obtained as purchased from market formulation of Glaxo Smithkline for antihelminthic activity²⁴.

Sample and Standard Preparation: Test samples for *in-vitro* antihelminthic were prepared by dissolving and suspending 0.5 gms of each petroleum ether, ethylene glycol and methanolic extract of *Tinospora cordifolia* in 10 ml of distilled water to obtain a stock solution of 50mg/ml. From this stock solution, different working dilutions were prepared to get concentration range of 2.5, 5, 10 and 20 mg/ml¹⁹. Albendazole strength of 10 mg/ml

was prepared used as standard. Normal saline is used as control.

Evaluation of Antihelminthic Activity: The antihelminthic activity petroleum ether, ethylene glycol and methanolic extract of *Tinospora cordifolia* were evaluated as per the method^{19, 22, 25}. Five groups of approximately equal size earthworms consisting of three earthworms in each group were used for the present study. Group I served as control and treated with normal saline. Group II served as standard and treated with albendazole. Group III, IV and V were treated with 2.5, 5, 10, 20 mg/ml of extracts of *Tinospora cordifolia* respectively. All the observations were made from the time taken to paralysis and death of individual worms. Paralysis was stated when the worms did not revive in normal saline. Death was concluded when the worms lost their mortality followed with fading away of their body colour.



FIG. 1: THE EFFECT OF METHANOLIC EXTRACT ON P. POSTHUMA



FIG. 2: THE EFFECT OF PETROLEUM EXTRACT ON P. POSTHUMA



FIG. 3: THE EFFECT OF ETHYLENE GLYCOL EXTRACT ON PHERETIMA POSTHUMA

RESULTS: Preliminary phytochemical screening revealed the presence of steroids, triterpenes, carbohydrates, glycosides and alkaloids **Table 2.** *Tinospora cordifolia* displayed significant anthelmintic activity at higher concentrations on *Pheretima posthuma*. The extract showed potent anthelmintic activity in dose dependant manner giving shortest time of paralysis (P) and death (D) with 20mg/ml concentration. The extract solutions at 20 mg/ml concentration caused paralysis of the earth worm in 10 min (petroleum ether), 07 min

(ethylene glycol) and 04 min (methanolic) whereas the reference drug albendazole 10mg/ml showed 25 sec respectively shown in **Table 2, 3 and 4.** The extracts solutions at 20 mg/ml concentration caused death of the earth worm in 13 min (petroleum ether), 10 min (ethylene glycol and methanolic) whereas the reference drug albendazole 10mg/ml showed 50 sec respectively The extract produced dose dependent paralysis ranging from loss of motility to loss of response to external stimuli, which eventually progressed to death.

TABLE 3: IN VITRO ANTHELMINTIC ACTIVITY OF PETROLEUM ETHER EXTRACT OF TINOSPORA CORDIFOLIA AGAINST PHERETIMA POSTHUMA

| Test Sample | Concentration (mg/ml) | Time Taken for Paralysis (min) | Time Taken for Death (min) |
|-------------------------|-----------------------|--------------------------------|----------------------------|
| Control (Normal Saline) | | 64.32 ± 0.88 | 200.33 ± 2.60 |
| Petroleum ether extract | 2.5mg/ml | 24 ± 0.88 | 33 ± 0.20 |
| | 5mg/ml | 23 ± 0.53 | 31 ± 0.72 |
| | 10mg/ml | 12 ± 0.76 | 15 ± 0.31 |
| | 20mg/ml | 10 ± 0.33 | 13 ± 0.44 |
| Albendazole | 10mg/ml | 25 sec | 50 sec |

TABLE 4: IN VITRO ANTHELMINTIC ACTIVITY OF METHANOLIC EXTRACT OF TINOSPORA CORDIFOLIA AGAINST PHERETIMA POSTHUMA

| Test Sample | Concentration (mg/ml) | Time Taken for Paralysis (min) | Time Taken for Death (min) |
|-------------------------|-----------------------|--------------------------------|----------------------------|
| Control (Normal Saline) | | 64.33 ± 0.88 | 200.33 ± 2.60 |
| Methanolic extract | 2.5 mg/ml | 12 ± 0.42 | 19 ± 0.64 |
| | 5 mg/ml | 10 ± 0.26 | 16 ± 0.64 |
| | 10 mg/ml | 08 ± 0.22 | 10 ± 0.72 |
| | 20 mg/ml | 04 ± 0.70 | 16 ± 0.88 |
| Albendazole | 10 mg/ml | 25 sec | 50 sec |

TABLE 5: IN VITRO ANTHELMINTIC ACTIVITY OF ETHALENE GLYCOL EXTRACT OF TINOSPORA CORDIFOLIA AGAINST PHERETIMA POSTHUMA

| Test Sample | Concentration (mg/ml) | Time Taken for Paralysis (min) | Time Taken for Death (min) |
|-------------------------|-----------------------|--------------------------------|----------------------------|
| Control (Normal Saline) | | 64.33 ± 0.88 | 200.33 ± 2.60 |
| Ethalene Glycol extract | 2.5mg/ml | 35 ± 0.88 | 36 ± 1.02 |
| | 5mg/ml | 23 ± 1.76 | 25 ± 0.33 |
| | 10mg/ml | 15 ± 1.0 | 17 ± 0.45 |
| | 20mg/ml | 07 ± 0.88 | 10 ± 0.58 |
| Albendazole | 10mg/ml | 25 sec | 50 sec |

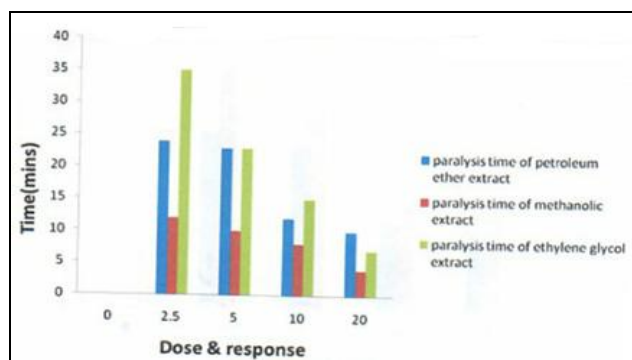


FIG. 4: COMPARATIVE STUDIES OF EXTRACTS OF TINOSPORA CORDIFOLIA ON PARALYSIS TIME OF PHERETIMA POSTHUMA

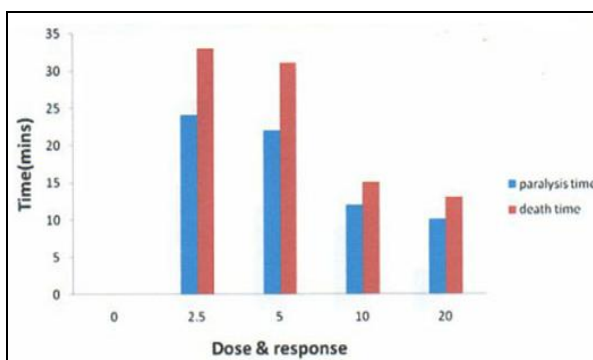


FIG. 5: COMPARATIVE STUDIES OF EXTRACTS OF TINOSPORA CORDIFOLIA ON DEATH TIME OF PHERETIMA POSTHUMA

DISCUSSION: Phytochemical analysis of the crude extracts revealed presence of alkaloids, glycosides, steroids are the important chemical constituents were shown to produce anthelmintic activities. The predominant effect of anthelmintic drugs on the worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. Albendazole act by blocking glucose uptake in larval and adult stages of susceptible parasites, and also depleting their glycogen reserves, thus decreasing ATP formation^{26, 27, 28, 29, 30}.

In the present study, all the extracts *i.e.* petroleum ether, methanol, ethylene glycol extracts of *Tinospora cordifolia* showed dose dependant and significant activity compared with the standard drug albendazole. Amongst all the extracts, methanolic extract showed better activity at higher concentration. From the above observation, the data revealed that methanolic extracts *Tinospora cordifolia* showed significant anthelmintic activity and found to be more effective in promoting paralysis as well as in causing death of the worms when compared with standard drug Albendazole at the same concentrations.

CONCLUSION: Leaf extracts of *Tinospora cordifolia* showed the anthelmintic activity against the earth worm, *Pheritima prosthuma* used in the study. The active constituents responsible for anthelmintic activity are present in the methanolic extracts of the plant. The methanolic extract has more efficient activity when compared with petroleum ether and ethylene glycol extracts at the concentration 2.5, 5, 10 and 25 mg/ml. the antihelminthic activity was found to be increased with dose (shortest time of paralysis and death was observed at 10 mg/ml and activity was comparable to the well known antihelminthic agent Albendazole.

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CONFLICT OF INTEREST: We declare that we have no conflict of interest.

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