

# Asian Journal of Phytomedicine and Clinical Research

Journal home page: [www.ajpcrjournal.com](http://www.ajpcrjournal.com)

<https://doi.org/10.36673/AJPCR.2021.v09.i01.A03>



## BIOSTATISTICAL STUDIES OF *IN-VITRO* ANTHELMINTIC ACTIVITY OF *CLITORIA TERNATEA*

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### ABSTRACT

Medicinal plants are gift of God, to cure infinite number of diseases in human beings and other living organism. These Plant materials have been extensively used in the indigenous system of medicine which is mention in the Ayurveda and other Indian literature. In this present experiment we had studied about to evaluate *in-vitro* anthelmintic activity of *Clitoria ternatea* by using leaves extract. Anthelmintic activity was tested against Indian earthworm *Pheretima posthuma*. Extraction of *Clitoria ternatea* demonstrated potent anthelmintic activity tested against Indian earthworm *Pheretima posthuma*. The dose-dependent anthelmintic efficacy of the fractions was quite similar to that of piperazine citrate The result obtained in the study led to the conclude that leaves of the mangrove plant, high level of polyphenolics and show significant anthelmintic activity. *Clitoria ternatea* Linn known as Tulsi in India is a sacred plant for Hindus known from centuries and being used in Ayurveda for its varied healing properties belonging to the Fabaceae (Pipilionaceae) family. To this purpose we have studied *in vitro* antihelminthic activity of *C. ternatea* in comparison with albendazole.

### KEYWORDS

Anthelmintic activity, *Pheretima posthumous*, *Clitoria ternatea* and Fabaceae.

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### INTRODUCTION

Since while immemorial nature has been a mere source of medicinal plants. These medicinal plants are gift of God, to cure infinite number of diseases in human beings and other living organism. They have been the main source of drugs altogether system of medicine and other ancient systems within the world. In the various systems of medicine, many plants and herbs are used to treat

various infirmities. In all ancient scriptures of Ayurveda, Aparajita is mentioned together of the important herb. It is a good looking twing herb. *Clitoria ternatea* is the botanical name of Aparajita's and belongs to Fabaceae (Papilionaceae) family. It is probably originated in tropical Asia<sup>1</sup>. It is cosmopolitan all-round the humid, lowland tropics of Asia, Africa and Central America. It is found in lower altitude and medium altitudes of the resolved areas. *C. ternatea* is a strongly persistent, sparsely pubescent, legume. It is perennial climber with slender downy stem, found throughout the tropical regions of the country being cultivated in gardens everywhere and often also found growing over hedges and thickets. It is seen that Aparajita is being adapted to clay soils and has been tested as a forage and cover crop, but never developed as a pasture cultivar<sup>2</sup>. In various Ayurvedic preparations different parts of this plant are used as a lively ingredient which is employed for treatment of several disorders. Ayurvedic 'medha' drugs are reported which contain *C. ternatea* along with other plants. This plant has been scientifically studied for various pharmacological activities like antihistaminic, anthelmintic, hypoglycemic, antidepressant, sedative etc<sup>3</sup>. It is commonly found in Jamaica, Puerto Rico, Turks and Caicos Islands etc. It is found in all over India, especially in southern India up to an altitude of 1,500m and in the Andaman Islands<sup>4</sup>. It has been used for the treatment of various neurological disorders as an active ingredient in 'Medhya Rasayana'. By various group of persons it is considered as medicine which is useful in skin diseases, eye and throat infections also in urinary disorders, ulcers and antidote activity. In the scientific studies it had been found that extracts of *C. ternatea* can raise the concentration of acetyl choline quantity and acetyl choline esterase activity in rat brain during a similar fashion to the quality cerebral drug pyritinol<sup>5</sup>. In other treatments of varied ailments like infections, as anthelmintics, antidote to animal stings, urinogenital disorders and body aches *C. ternatea* is also used<sup>6</sup>. In severe asthma especially the roots of *C. ternatea* are useful, remittent fever and

bronchitis. These are wont to administer with ghee and honey as a tonic to children for step up in their mental abilities, muscular strength, complexation, pertussis, goiter and epilepsy<sup>7</sup>. Roots used by tribal to cause abortion and its paste applied on cattle stomach for curing abdominal swelling<sup>8</sup>. Research suggested that the methanolic extract of *C. ternatea* roots shown anxiolytic, anti-depressant, nootropic, anticonvulsant and anti-stress activity in animals. The decoction or powder of root is given in rheumatism and ear disease. Root and leaves have emetic and antiperiodic<sup>9</sup>.

## MATERIAL AND METHODS

### Plant materials and preparation of extracts

The collected *Clitoria ternatea* were processed on the same day itself. The plants part were washed thoroughly with distilled water and freeze dried. The dried samples were ground to powder and stored in air tight until further analysis. The powdered material was soaked in the different solvents of varying polarity such as methanol, acetone and at room temperature for 24 h with mass to volume ratio of 1:40 (g/ml). The solvents were filtered through Whatmans No. 1 filter paper to remove the solid particles. The filtrates were evaporated to dryness under vacuum on a rotary evaporator at 40°C. Water extract of *Clitoria ternatea* was prepared as above by soaking dried powder in distilled water and stirred using a magnetic stirrer at a low speed for 24h.

### Anthelmintic Activity

Physiological and anatomical characteristics of Indian earth worm (*Pheretima posthuma*) resemblance with the intestinal round worm parasite of human being, therefore *Pheretima posthuma* have taken in this study to assess anthelmintic activity of *Clitoria ternatea*. Indian earth worms (*Pheretima posthuma*) are divided into three groups each containing six earthworms approximately of equal size in following manner:

#### Group I

Control (2% Tween 80 in normal saline)

#### Group II

Standard (15, 30 and 45mg/ml)

### Group III

Plant extract (15, 30 and 45mg/ml).

50ml of respective drug solutions were taken in petri dishes and the earthworms (*Pheretima posthuma*) were released in to the solution. Earth worms (*Pheretima posthuma*) were monitored carefully and observations were made for the time taken to paralyze and death of individual worms. Time taken to till paralysis (*Pheretima posthuma*) was recorded when no movement could be observed except when the *Pheretima posthuma* were shaken vigorously. Times taken for death of *Pheretima posthuma* were noted after ascertaining that the worms lost their motility completely with fading of their body colour. To confirm, the death of *Pheretima posthuma* were shaken vigorously or dipped in warm water at 50°C but no movement was observed.

### RESULTS AND DISCUSSION

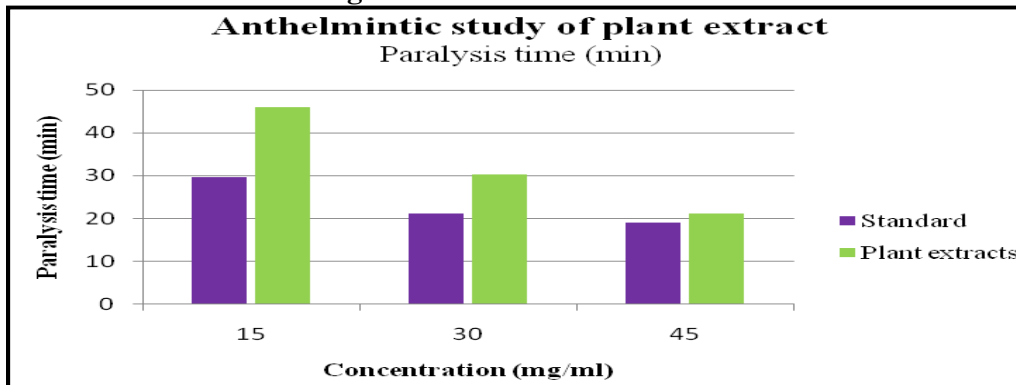
Anthelmintic activity of plant extract of *Clitoria ternatea* was performed against Indian earthworm *Pheretima posthuma*. *Clitoria ternatea* extract produced moderate activity. At 15, 30 and 45mg/ml concentration, extract produced paralysis in worms after  $45.87 \pm 0.29$ ,  $30.28 \pm 0.37$  and  $21.17 \pm 0.42$ min, while at same concentration after  $118.34 \pm 1.41$ ,  $106.21 \pm 1.88$  and  $76.47 \pm 0.47$  min produced death in earthworms respectively. Standard drug piperazine citrate at a 15 and 30mg/ml, 45mg/ml concentration, showed the potent activity which was evident by the quick paralysis time  $29.71 \pm 0.61$ ,  $21.19 \pm 0.51$  and  $19.11 \pm 0.77$  respectively and death time  $60.42 \pm 1.21$ ,  $47.15 \pm 1.71$  and  $21.13 \pm 1.43$ min respectively. The paralysis and death times of the extract, fractions and standard drug are given in Table No.1. Depicts the *Pheretima posthuma* state with control, extract and piperazine citrate.

**Table No.1: In Vitro Anthelmintic Effect of *Clitoria ternatea* Extract**

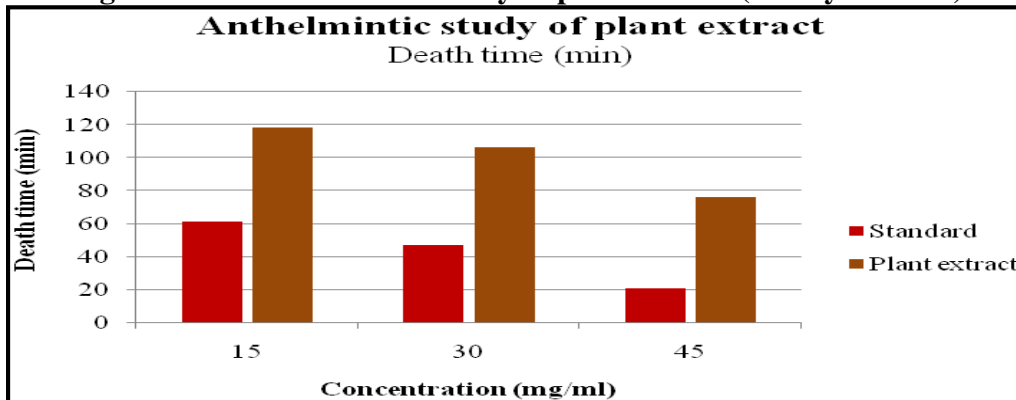
S.No	Groups	Concentration (mg/ml)	Paralysis time (min)	Death time (min)
1	Control	----	----	----
2	Standard	15	29.71±0.61	60.42±1.21
		30	21.19±0.51	47.15±1.71
		45	19.11±0.77	21.13±1.43
3	Plant extracts	15	45.87±0.29	118.34±1.41
		30	30.28±0.37	106.21±1.88
		45	21.17±0.42	76.47±0.47



**Figure No.1: *Clitoria ternatea***



**Figure No.2: Anthelmintic study of plant extract (Paralysis Time)**



**Figure No.3: Anthelmintic study of plant extractb (Death Time)**

## CONCLUSION

Aqueous extract of *Clitoria ternatea* Linn is more potent than control and lesser antihelmintic activity than albendazole. Time to paralysis and consequent death were significantly higher in aqueous extract of Ocimum than of Albendazole at same concentrations. According to the above study it was concluded that the Extraction of *Clitoria ternatea* demonstrated potent anthelmintic activity tested against Indian earthworm *Pheretima posthuma* but it did not give clear inference at that stage and hence we considered the work for further extensive research. The wormicidal activity of alcoholic extracts suggests that it is effective against parasitic infections of humans. Further, in future it is necessary to identify and isolate the possible active phytoconstituents responsible for the anthelmintic activity and study its pharmacological actions.

## ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to KIPM College of Engineering and Technology, GIDA, Gorakhpur, Uttar Pradesh, India for providing necessary facilities to carry out this research work.

## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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**Please cite this article in press as:** Satya Prakash Singh et al. Biostatistical studies of *in-vitro* anthelmintic activity of *Clitoria ternatea*, *Asian Journal of Phytomedicine and Clinical Research*, 9(1), 2021, 10-14.