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In-vitro anthelmintic activity of root of *Clitoria ternatea* Linn

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ABSTRACT

The present study was undertaken to evaluate anthelmintic activity of crude alcoholic extract of *Clitoria ternatea* Linn belonging to Fabaceae family & its different fractions namely Petroleum ether, Ethyl acetate and Methanol using Pheretima posthuma as test worms. Various concentrations (10-50 mg/ml) of alcoholic extracts and its various fractions were tested in the bioassay, which involved determination of time of paralysis (P) and time of death (D) of the worms. Piperazine citrate was included as standard reference and distilled water as control. The results of present study indicated that the crude alcoholic extract and its ethyl acetate and methanol fractions significantly demonstrated paralysis and also caused death of worms especially at higher concentration of 50 mg/ml, as compared to standard reference piperazine citrate. Further studies are in process to isolate the active principle/s responsible for the activity.

KEY WORDS: Anthelmintic, *Clitoria ternatea*, *Pheretima posthuma*, Piperazine citrate.

INTRODUCTION

Clitoria ternatea Linn (Fabaceae) also known as Aparajita in India is a flowering plant that is insect pollinated .It is a vigorous, strongly persistent, herbaceous perennial legume. It is native to south-east Asia and widely distributed in S. Africa, Tanzania, Mauritius, Cameroon (1). It has been used in the indigenous system of medicine for cooling, acrid, purgative, diuretic, laxative, anthelmintic, anti-ulcer properties. In animal tests, the methanolic extract of Clitoria ternatea roots demonstrated nootropic, anxiolytic, antidepressant, antistress and learning enhancing activity (2). Phytochemical review shows the presence of tannins, resins, starch, taraxerol, and ternatins in the root of Clitoria ternatea (3-5). The present study was therefore undertaken to evaluate the in vitro anthelmintic activity of crude extract of *Clitoria ternatea* root (70% alcoholic extract) and its different fractions against *Pheretima posthuma*.

MATERIALS AND METHODS

Plant Collection and Authentication

The root of *Clitoria ternatea* was collected and its botanical identification was confirmed from Nagarjuna garden, Punjabrao Krishi Vidyapeeth, Akola, Maharashtra. The plants species were cultivated and preserved in the Institute.

Plant Extraction

The plant material (root) was dried for several days and powdered with the help of an electric grinder. It was air dried and extracted exhaustively with 70% alcohol. The liquid extract was evaporated in vacuum to yield 23.63% brownish residue. The dried residue was taken in minimum quantity of water and was successively extracted with petroleum ether, ethyl acetate and methanol to yield 11.57 % w/w, 9.69 % w/w and 12.47 % w/w residue respectively. Alcoholic extract and all the fractions were preserved in refrigerator.

Worms Collection and Authentication

Indian earthworm *Pheretima posthuma* (Annelida) were collected from the water logged areas of soil and identified at the National Institute of Veterinary and Animal husbandry Sciences, Akola, Maharashtra.

Preparation of test sample

Samples for *in-vitro* study were prepared by dissolving and suspending 2.5 gms of each crude alcoholic extract and its petroleum ether, ethyl acetate and methanol fractions in 25 ml of distilled water to obtain a stock solution of 100 mg/ml. From this stock solution, different working dilutions were prepared to get concentration range of 10, 25 and 50 mg/ml.

Anthelmintic assay

The anthelmintic assay was carried as per the method of Ajayieoba E. O. et al (6) with minor modifications. The assay was performed on adult Indian earthworm *Pheretima posthuma*, due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings (7-9). *Pheretima*

posthuma worms are easily available and used as a suitable model for screening of anthelmintic drug was advocated earlier (10-15). 50 ml formulations containing three different concentrations, each of crude alcoholic extract and its various fractions (10, 25 and 50 mg/ml in distilled water) were prepared and six worms (same type) were placed in it. Time for paralysis was noted when no movement of any sort could be observed except the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50^0 C (16,17). Piperazine citrate (10 mg/ml) was used as reference standard while distilled water as the control.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of root of *Clitoria ternatea* has shown the presence of tannins, resins, taraxerol and ternatins. As shown in table 1, alcoholic extract of *Clitoria ternatea* and its different fractions exhibited anthelmintic activity in dose-dependant manner giving shortest time of paralysis (P) and death (D) with 50 mg/ml concentration. The alcoholic extract caused paralysis of 4.27 min. and time of death of 10.58 min. while ethyl acetate and methanol fractions revealed paralysis of 6.28 and 5.63 min and time of death of 21.59 and 11.92 min. respectively against the earthworm *Pheretima posthuma*. The reference drug Piperazine citrate showed the same at 19.26 and 63.25 minutes, respectively.

Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyper polarization and reduced excitability that leads to muscle relaxation and flaccid paralysis (18). The root extract of *Clitoria ternatea* not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 50 mg/ml, in shorter time as compared to reference drug Piperazine citrate. Phytochemical analysis of the crude extract revealed the presence of tannins among other chemical constituents contained within them. Tannins were shown to produce anthelmintic activities (19). Chemically tannins are polyphenolic compounds (20). It is possible that tannins contained in the extracts of *Clitoria ternatea* produced similar effects. Reported anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal (21) or glycoprotein on the cuticle of the parasite (22) and may cause death.

In conclusion, the study has shown that, alcoholic extracts of *Clitoria ternatea* roots have significantly determined anthelmintic activity. Further studies are in process to identify the possible phytoconstituents responsible for anthelmintic activity.

Table 1: Anthelmintic activity of alcoholic extract of Clitoria ternetea and its fractions.

Test subs	Concentration (mg/ml)	Time taken for Paralysis (P) and for death of *Pheretima posthuma* worms (D) in min.	
		P	D
Alcoholic extract	10	13.28±0.49	25.51±1.12
	25	7.39±0.47	18.66±0.81
	50	4.27±0.99	10.58±0.46
Petroleum ether fraction	10	28.62±0.31	78.57±0.71
	25	18.35±0.97	61.24±0.58
	50	10.29±0.63	48.21±0.35
Ethyl acetate fraction	10	16.63±0.31	45.92±0.16
	25	10.18±0.42	35.27±0.33
	50	6.28±0.67	21.59±0.38
Methanol fraction	10	14.56±0.83	33.69±0.82
	25	9.52±0.15	21.76±0.71
	50	5.63±0.84	11.92±0.37
Piperazine citrate	10	19.26±0.62	63.25±0.58

(Results expressed as Mean ± SEM from six observations)

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