

**IN VITRO ANTHELMINTHIC ACTIVITY OF *CALOTROPIS PROCERA* (AIT.) R. BR. LEAVES**

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**ABSTRACT**

**Objective:** Helminthiasis affects over two billion people worldwide, particularly in the tropical region. A number of medicinal plants have been used to treat parasitic infections in man and animals. In the present study, the anthelmintic potential of *Calotropis procera* (Ait.) R. Br. leaves extract was evaluated.

**Methods:** The ethanolic extract of *Calotropis procera* (Ait.) R. Br. leaves was separated into *n*-butanol and water fractions. The *n*-butanol fraction was subjected to column chromatography. Ethanolic extract, *n*-butanol, and water fractions as well as *n*-hexane, chloroform, chloroform: methanol (9:1); chromatographic elutes of *n*-butanol fraction were evaluated for *in-vitro* anthelmintic activity using Indian earthworm *Pheretima posthuma* as a experimental models.

**Results:** The results indicated that ethanolic extract, water fraction, *n*-hexane, and chloroform elutes showed better activity as compared to *n*-butanol fraction and chloroform: methanol (9:1) elute of *Calotropis procera* (Ait.) R. Br. leaves.

**Conclusion:** The present investigation is the scientific validation of the anthelmintic activity of *C. procera* (Ait.) R. Br. leaves in view of its usage in ethnoveterinary practice. It is suggested that further research is required for identification of active principles.

**Keywords:** *Calotropis procera*, Chromatographic elutes, *Pheretima posthuma*, Anthelmintic activity.

**INTRODUCTION**

Helminth infections are among the most widespread infections in humans. As per WHO, only a few drugs are frequently used in the treatment of helminth infestations in human beings. Anthelmintic resistance is a major problem for the control of many parasitic nematode species and has become a major constraint to livestock production in many parts of the world. Due to the prevalence of parasitic infections and the developed resistance of some anthelmintic drugs is now an enclosing area in the field of research [1].

Herbal drugs have been used since ancient times as medicines for the treatment of a range of diseases. *Calotropis procera* (Ait.) R. Br. is well known for its traditional medicinal uses belonging to the family Asclepiadaceae. It has numerous colorful names such as Sodom Apple, Akund Crown flower and Dead Sea Fruit, but the scientific name is *C. procera*. This plant has been advocated for a variety of disease conditions including leprosy, ulcers tumors, and piles [2]. Different extracts of *C. procera* (Ait.) R. Br. leaves have been found to possess the following diverse biological activities such as antidiarrheal [3], antioxidant [4-6], antipyretic [7], analgesic [8], antimicrobial [6,9,10], spasmolytic [11], schizonticidal [12], cytotoxic [13,14], hepatoprotective [15], hypoglycemic [16], and abortifacient [17].

Previously, our group reported the pharmacognostical studies including macroscopic and microscopic evaluation of *C. procera* leaves which is useful to supplement existing information with regard to the identification and standardization of *C. procera* leaves to distinguish it from substitutes [18]. The leaves were reported to contain calotropagenin, calotropin, calotoxin, calactin,  $\alpha$ -amyrin,  $\beta$ -amyrin, stigmaterol, calotropenyl acetate, calotropenol, procesterol, uscharin, voruscharin, syriogenin, multiflorenol, 12 $\beta$ -hydroxyfrugoside, 4'-O- $\beta$ -glucopyranosyl-12 $\beta$ -hydroxyfrugoside, 4'-O- $\beta$ -cellobiosyl-12 $\beta$ -hydroxyfrugoside, 16 $\alpha$ -hydroxy calotropagenin, ursolic acid, and *o*-pyrocatechuic acid on the basis of the liquid chromatography-mass

spectrometry (MS)/MS analysis of *C. procera* leaves [19]. Literature survey revealed that different parts of this plant have been used as anthelmintic [20-23]. In an attempt to confirm the claimed folk use, the present study was carried out to scientifically evaluate the anthelmintic activity of *C. procera* (Ait.) R. Br. leaves.

**METHODS****Collection and preparation of plant material**

The leaves of *C. procera* (Ait.) R. Br. were collected in the month of February from the local field of Mathura (Uttar Pradesh), India. The leaves were cleaned by washing with running water and shade dried, then powdered to pass through 100 mesh size. Powdered leaves were extracted with ethanol by maceration for 7 days at room temperature. The solvent was recovered under reduced pressure, and the ethanolic extract was obtained as a brownish green viscous residue. The dried residue was suspended in water and further extracted with *n*-butanol to obtained water and *n*-butanol fraction. The *n*-butanol fraction and water fraction was concentrated under reduced pressure and vacuum dried. The *n*-butanol fraction was subjected to column chromatography. The column was run with different solvents and ethanolic extract, *n*-butanol fraction, water fraction and chromatographic elutes of *n*-butanol fraction (*n*-hexane, chloroform, chloroform: methanol; 9:1) were investigated for their anthelmintic activity.

**Phytochemical screening**

Qualitative assay, for the presence of plant phytoconstituents, such as carbohydrates, alkaloids, glycosides, flavonoids, tannins, and saponins, were carried out on the ethanolic extract, *n*-butanol fraction and water fraction of *C. procera* (Ait.) R. Br leaves following standard procedure [24,25].

**Animal**

Healthy adult Indian earthworms, *Pheretima posthuma*, due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings [26-28] were used in this study.

All earthworms were of approximately equal size of 5-6 cm length and 0.2-0.3 cm widths used for all the experimental protocol. They were collected from the local moist place, washed and kept in water.

### Drugs

Piperazine citrate was purchased from GSK Pvt. Ltd. The solvents and other chemicals of analytical grade were used during experimental protocol.

### In-vitro anthelmintic activity

Ethanol extract, *n*-butanol fraction, water fractions and *n*-hexane, chloroform, chloroform: methanol (9:1) elutes of *C. procera* (Ait) R. Br leaves were investigated for their anthelmintic activity against *Pheretima posthuma* as a method of Ajaiyeoba et al. [29]. Six groups of equal size Indian earthworm consisting of six earthworms in each group were taken. Each group was treated with one of the following: vehicle (dimethyl sulfoxide [DMSO]), piperazine citrate (20 mg/mL), and ethanolic extract, different fractions and different elutes (10 mg/mL, 20 mg/mL) in DMSO (5.0 mL) and volume was make up to 40 mL by distilled water. Observations were made for the paralysis time and subsequently for death time of the worms. The mean paralysis and death time for each group were recorded (each reading was taken 6 times). The time taken by the worms to become motionless, was considered as paralysis time, was recorded, and the lethal time was also recorded by observing the time taken to become motionless on the application of external stimuli by pricking with pin. Piperazine citrate (20 mg/mL) was taken as reference drug.

### RESULTS AND DISCUSSION

Preliminary phytochemical screening of ethanolic extract revealed the presence of carbohydrates, glycosides, alkaloids saponins, tannins, flavanoids, and steroids. Water fraction showed the presence of carbohydrates, glycosides, alkaloids, and tannins while *n*-butanol fraction showed the presence of glycosides, alkaloids, saponins, and flavanoids.

The perusal of the data of Table 1 revealed that the ethanolic extract at the concentration of 20 mg/mL showed paralysis in 7.25 minutes and death in 14.10 minutes. Similarly *n*-butanol and water fraction, *n*-hexane, chloroform, and chloroform: methanol (9:1) elutes at the concentration of 20 mg/mL showed paralysis in 5.56, 7.3, 7.26, 7.26, and 5.4 min and death in 8.3, 13.5, 13.2, 13.26, and 10.5 min, respectively. This indicates that ethanolic extract, water fraction, *n*-hexane and chloroform elutes have paralysis time comparable with piperazine citrate but death time greater than piperazine citrate. The predominant effect of piperazine citrate on the worm is to cause a flaccid paralysis that result in the expulsion of the worm by peristalsis. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyperpolarization and reduced excitability that leads to muscle relaxation and flaccid paralysis [30].

Ethanolic extract, different fractions and chromatographic elutes caused paralysis followed by the death of the worms at all tested dose levels. The potency of the *C. procera* (Ait) R. Br. leaves was found inversely proportional to the time taken for paralysis or death of worms. The activity confirms the dose-dependent nature of extract (Fig. 1). It was observed that *n*-butanol fraction and chloroform: methanol (9:1) elutes showed better activity as compared to ethanolic extract, water fraction, *n*-hexane and chloroform elutes of *C. procera* (Ait) R. Br. leaves and reference control piperazine citrate. These results indicate that activity of *n*-butanol fraction was due to the combined effect of *n*-hexane and chloroform elutes, and activity of the ethanolic extract was due to the combined effect of *n*-butanol and water fractions.

Phytochemical analysis of the ethanolic extract, water, and *n*-butanol fractions revealed the presence of flavonoids as one of the chemical constituent. Polyphenolic compounds show anthelmintic activity [31]. Some synthetic phenolic anthelmintics, e.g., niclosamide, oxiclozanide, and bithionol are shown to interfere with energy generation in helminth

Table 1: In-vitro anthelmintic activity of *C. procera* (Ait)

R. Br. leaves				
S. No.	Name of drug/extract	Concentration (mg/mL)	Paralysis time (min)	Death time (min)
1	Piperazine citrate	10	7.33±0.012	33.95±0.022
2	Control	-	-	-
3	Ethanol	20	7.25±0.029**	14.10±0.152**
4	<i>n</i> -Butanol	10	17.81±0.200	30.5±0.120
		20	5.56±0.088*	8.3±0.152**
5	Water	10	20.03±0.48	29.9±1.750
		20	7.3±0.088**	13.5±0.115**
6	<i>n</i> -Hexane	10	18.13±0.088	21.63±0.145
		20	7.26±0.088**	13.26±0.088**
7	Chloroform	10	16.63±0.290	21.3±0.115
		20	7.2±0.057**	13.26±0.176*
8	Chloroform: Methanol (9:1)	20	16.5±0.088	21.36±0.088
		10	5.4±0.152**	10.5±0.176**
		10	12.46±0.088	23.36±0.305

Results expressed as ±SEM of six worms in each group. \*p<0.01 and \*\*p<0.001 as compared to control by Dunnett test, *C. procera*: *Calotropis procera*

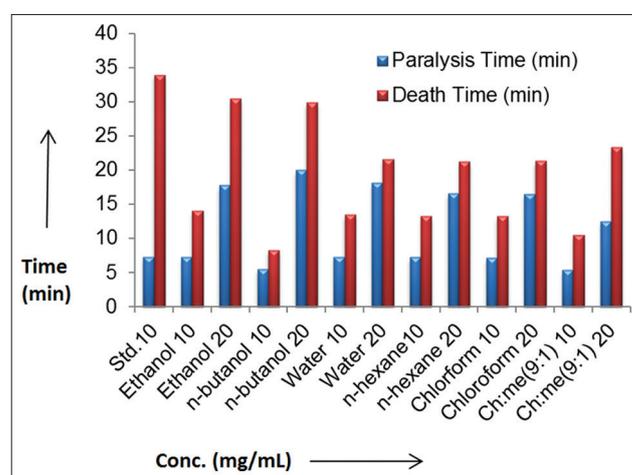


Fig. 1: In-vitro anthelmintic activity of ethanolic extract, different fractions and chromatographic elutes of *n*-butanol fraction of ethanolic extract

parasites by uncoupling oxidative phosphorylation [32]. It is possible that phenolic content in the *n*-butanol fraction of *C. procera* (Ait) R. Br. leaves produced similar effects. It is also possible that tannins contained in the ethanolic extract of *C. procera* (Ait) R. Br. leaves produced similar effects because tannins can bind to free protein in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and cause death [33].

### CONCLUSION

This study indicated the potential usefulness of *C. procera* (Ait) R. Br. leaves against earthworm infections and provide a rationale for the traditional use of this plant as anthelmintic. Further studies need to establish the mechanisms of action are required.

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