



## In-vitro Anthelmintic Activity of Seed Extract of *Lagenaria siceraria* (Molina.) Standley Fruit

Thube Smita<sup>1\*</sup>, Tambe Rashmi<sup>1</sup>, Patel Mohammed Farooque<sup>1</sup>, Patel Sakil Daud<sup>1</sup>

<sup>1</sup> Department of Pharmacognosy, M.C.E.Society's Allana College of Pharmacy, Azam Campus, 2390 K.B.Hidayatullah road, Camp, Pune 411 001.India

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### ABSTRACT

The aim of present study was to evaluate anthelmintic potential of crude extracts of seeds of *Lagenaria siceraria* (Molina) Standley using *Pheretima posthuma* as test worms. Various concentrations (10 – 100 mg/ml) of the extracts were tested in the bioassay, which involved determination of time of paralysis (P) and time of death (D) of the worms. Piperazine citrate (10 mg/ml) was included as standard reference and distilled water as control. The results of present study indicated that the methanol and benzene extracts significantly demonstrated paralysis, and also caused death of worms especially at higher concentration of 100 mg/ml, as compared to standard reference Piperazine citrate. In conclusion, the traditional use of seeds of the plant *Lagenaria siceraria* as an anthelmintic have been confirmed and further studies are suggested to isolate the active principle/s responsible for the activity.

**Keywords:** Anthelmintic, *Lagenaria siceraria*, *Pheretima posthuma*, Piperazine citrate.

### INTRODUCTION

Diseases caused by helminth parasites in livestock continue to be a major productivity constraint, especially in small ruminants in the tropics and subtropics (1). In the developing world, the greatest impact of parasitic diseases is indirect and causes potential productivity losses (2). Infections by gastrointestinal helminth parasites of livestock are among the most common and economically important diseases of grazing livestock (3). Adulteration of anthelmintics has been found to be a common practice (4). Illiteracy and unfamiliarity with synthetic anthelmintics, resulting in incorrect usage, are also a problem leading to the same consequences. Moreover, these drugs are relatively expensive. As a consequence of these problems and difficulties, pastoralists and small holder farmer have continued to use indigenous plants as livestock dewormers(5). Considerable research has shown that some plants not only affect the nutrition of animals, but also have antiparasitic effects (6).

The plant, *Lagenaria siceraria* (Mol.) Standl. (Family: Cucurbitaceae), known as bottle gourd, is a common fruit vegetable used throughout the India. Since time immemorial the fruit is used as diuretic, cardio-tonic, cardio-protective and nutritive agent. The fruit is also reported to have good source of vitamin B complex and choline along with fair source of vitamin C and  $\beta$ -carotene. It is also reported to contain cucurbitacins, fibers and polyphenols (7-10). Two sterols namely campesterol and sitosterol have been identified and isolated from the petroleum ether fraction of methanol extract of *L. siceraria* fruits, which is reported to possess antihepatotoxic activity (11). *Lagenaria siceraria* fruit has been reported to possess antioxidant activity(12), hypolipidemic and antihyperlipidemic effects in

#### \*Corresponding author.

Tel.: + 91-02026442774

Telefax: +91-02026442075

E-mail: smitathube81@rediffmail.com

normocholesterolemic and triton-induced hyperlipidemic rats(13). HPLC analysis of methanolic extract from plant shows the presence of flavone-C glycosides (14). Lagenin, a novel protein has been isolated from lyophilized extract of seeds (15). Literature survey revealed that the seed extract of *Lagenaria siceraria* has yet not been screened for its traditional anthelmintic activity. Therefore the present study was carried out to provide pharmacological evidence for the folklore medicinal consideration of seeds as anthelmintic.

### MATERIALS AND METHODS

#### *Plant Collection and Authentication*

*L. siceraria* fruits were collected from the local farms of Ahmednagar District, Maharashtra, in the month of February-March.

The collected seeds were air-dried under the shade in laboratory for 7-12 days. After complete drying, seeds were powdered and extracted thoroughly with light petroleum ether (40-60°) in a Soxhlet extractor for 24-48 h. The exhausted powder after extraction with Pet Ether was successively extracted with Benzene and Methanol. The extracts were preserved properly before subjecting to anthelmintic activity.

#### *Worms collection*

Indian earthworm *Pheretima posthuma* (Annelida) were collected from the Government Agriculture college, Pune.

#### *Preparation of test sample*

Samples for in-vitro study were prepared by dissolving the extracts in distilled water to obtain different working solutions as 10, 50, and 100 mg/ml.

#### *Anthelmintic Assay*

The anthelmintic assay was carried as per the method of Ajaiyeoba E.O. et al (16) with minor modifications. The assay was performed on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings (17-20). Because of easy availability, earthworms

Test Substance	Concentration (mg/ml)	Time of Paralysis (P) And Death (D) of <i>Pheritima posthuma</i> in minutes	
		P	D
Pet Ether extract	10	90 ± 0.8	100 ± 0.45
	50	45 ± 0.04	82 ± 0.36
	100	25 ± 0.12	61 ± 0.33
Benzene extract	10	40 ± 0.04	53 ± 0.67
	50	27 ± 0.23	39 ± 0.18
	100	12 ± 0.15	22 ± 0.23
Methanol extract	10	24 ± 0.04	35 ± 0.67
	50	19 ± 0.23	26 ± 0.18
	100	9 ± 0.15	17 ± 0.23
Piperazine citrate	10	20 ± 0.07	45 ± 0.007

All values represent Mean ± SEM ; n = 6 in each group.

have been used widely for the initial evaluation of anthelmintic compounds in vitro (21-24). 50 ml formulations containing three different concentrations, each of crude extracts (10, 50 and 100 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 when 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 (50 °C) (25-26). Piperazine citrate (10 mg/ml) was used as reference standard while distilled water as the control.

### RESULT AND DISCUSSION

Preliminary phytochemical screening of the crude extracts revealed the presence of sterols, proteins, carbohydrates, glycosides and saponins. As shown in Table 1, the different extracts exhibited anthelmintic activity in dose dependent manner giving shortest time of paralysis (P) and death (D) with 100 mg/ml concentration. The methanolic extract of *L.siceraria* caused paralysis of 9 min and time of death of 17 min while benzene extract revealed paralysis of 12 min and death of 22 min against the earthworm *Pheritima posthuma*. The reference drug Piperazine citrate showed the same at 20 and 45 minutes respectively.

The predominant effect of Piperazine citrate on worm is to cause a flaccid paralysis which results in expulsion of the worm by peristalsis. 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(27). The crude extracts of *L.siceraria* not only demonstrated paralysis but also caused death of worms especially at higher concentration of 100 mg/ml in shorter time as compared to reference drug Piperazine citrate . Phytochemical analysis of crude extracts revealed the presence of saponins among the other chemical constituents

The traditional medicines prove as a source of easily available effective anthelmintic agents. The origin of many effective drugs has been found in the traditional medicines practices and in view of this it is important to undertake studies pertaining to screening of the traditional medicinal plants for their use as anthelmintics

### CONCLUSION

In conclusion, the traditional use of the seeds of *L.siceraria* as anthelmintic has been confirmed as the different extracts showed good anthelmintic activity in the study. Further it would be interesting to isolate the possible phytoconstituents which are responsible for the anthelmintic activity and the mechanism of action.

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