

In-vitro anthelmintic activity of seeds of *Zanthoxylum armatum* DC. against *Pheretima Posthuma*

Dinesh Kumar Mehta, Rina Das, Anil Bhandari¹

Department of Pharmaceutical Chemistry, Sri Sai College of Pharmacy, Badhani, Pathankot, Punjab, ¹Department of Pharmaceutical Sciences, Jodhpur National University, Jodhpur, Rajasthan, India

Among the most common infections of digestive system in human beings are helminth infections. In developing countries, they pose a large threat to the society. Such parasitic diseases cause severe morbidity, including lymphatic filariasis, onchocerciasis and schistosomiasis. Different extracts of the plant material were tested against adult Indian earthworms *Pheretima posthuma* (Pheritimidae) as test worms. Various concentrations (10, 25 and 50 mg/ml) of all extracts were tested and results were expressed in terms of time for paralysis and time for death of worms. Piperazine citrate (10 mg/ml) was used as the standard reference drug. Results showed that the aqueous extract is more potent as compared to other extracts as it took less time to cause paralysis and death of the earthworms as compared to standard reference drug.

Key words: Anthelmintic activity, *Pheretima posthuma*, piperazine citrate, *Zanthoxylum armatum*

INTRODUCTION

Helminthiasis is the condition resulting from worm infestation, and is one of the major prevalent diseases in the world, particularly in the tropical countries. Lack of adequate sanitary facilities and supply of pure water coupled with poverty and illiteracy are some of the factors responsible for wide spread nature of this disease in the developing countries. Helminthiasis is prevalent globally (1/3 of world's population harbours them), but is more common in developing countries with poorer personal and environmental hygiene.^[1] Anthelmintics or antihelmintics are drugs that expel parasitic worms (helminthes) from the body, by either stunning or killing them.^[2] The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs; therefore, there is a foremost problem in treatment of helminthes diseases.^[3] Moreover, these drugs are unaffordable because of their high cost. These factors paved the way for herbal remedies as alternative anthelmintics.

Zanthoxylum armatum (TUMBURU) Tumburu consists of dried seeds of *Zanthoxylum armatum* DC. Syn. *Z. alatum* Roxb. *Zanthoxylum armatum* DC. belongs to family Rutaceae. Commonly known as 'timur' or 'Nepali Dhania'. Seeds of *Z. armatum* are a well-known ayurvedic medicine.^[4] The fruit, branches and thorn are used to cure toothache and other diseases of teeth. It is considered good for asthma.^[5] Bark powder mixed with honey gives relief against gum bleeding.^[6] Fruit and seeds are employed as aromatic and tonic, in fever, dyspepsia and expelling round worms.^[7,8] All the aerial parts of 'timur' are reported to contain volatile principle. Previous investigations on *Z. armatum* seeds have revealed the presence of alkaloids, terpenoids.^[9] It is a volatile oil consisting mainly of linalool and flavonoids.^[10,11] The plant has been reported to possess antioxidant,^[12] antinociceptive,^[12] anti-inflammatory,^[12,13] natural piscicide,^[14] antimicrobial,^[15] anthelmintic,^[15] hepatoprotective,^[16] antiproliferative^[17] and antifungal^[18] activities.

However, so far no study has been reported to have done the evaluation of anthelmintic activity in seed part. The present study was therefore undertaken to evaluate the *in vitro* anthelmintic activity of different extracts of seeds of *Zanthoxylum armatum* DC, against *Pheretima posthuma* (Pheritimidae).

MATERIALS AND METHODS

Seeds of *Zanthoxylum armatum* were collected from Poonch district, Jammu and Kashmir, India and plant

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Address for correspondence: Prof. Dinesh Kumar Mehta, Sri Sai College of Pharmacy, Badhani, Pathankot - 145 001, Pathankot, India.
E-mail: dkmehta17@rediffmail.com

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material was identified by Dr. S.N Sharma of Department of Botany, IIM, Jammu. A voucher specimen (19767) was deposited in the Department for future records. The seeds were dried under shade and powdered.

Preparation of Extract

The shade dried seeds were powdered with the help of an electric grinder. Coarsely powdered material (500 g) was extracted successively with petroleum ether 40-60), methanol and water by maceration process at room temperature for three days. Then all the extracts were filtered and concentrated with a rotary evaporator and kept in a refrigerator.

Phytochemical Test

The freshly prepared crude extracts of *Z. armatum* were subjected to standard phytochemical screening tests for various constituents.

Animals

Earthworms *Pheretima posthuma* (*Pheritimidae*) have been used widely for the initial evaluation of anthelmintic activity, because of their easy availability. Indian adult earthworms (*Pheretima posthuma*) were collected from the water logged areas of soils and washed with normal saline to remove all filthy matter. They were authenticated by Head, Department of Zoology, S.D College, Pathankot, Punjab. The earthworms of 3 to 5 cm in length and 0.1 to 0.2 cm in width were used for all the experimental protocol due to their anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.

Drugs and Chemicals

All the chemicals and reagents were procured from S.D. Fine Chemicals (Mumbai, India). All the chemicals were of analytical grade. The drug piperazine citrate was procured from Glaxo Smithkline Ltd, Mumbai.

Anthelmintic Activity

The assay was performed on adult Indian earthworm *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal round worm parasite of human being. Five groups of Indian earth worms each containing six earthworms approximately of equal size was used for the study. Each group of earth worms were tested with different extract conc. (10, 25 and 50 mg/ml), distilled water (control), and reference standard piperazine citrate (10 mg/ml in distilled water).^[19] Observations were made for the time taken to paralysis and death of individual worms. Time taken to paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time taken for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when

dipped in warm water at 50°C followed with fading of their body colour.

RESULTS

Preliminary phytochemical screening of different extracts of *Zanthoxylum armatum* DC seeds revealed the presence of alkaloids, phenolic compounds, flavonoids, tannins and saponins. Tannins and polyphenolic compounds were shown to produce anthelmintic activities.^[20]

Extracts exhibited more potent activity at higher concentration (50 mg/ml) against *Pheretima posthuma* (earthworm). Evaluation of anthelmintic activity was compared with reference standard piperazine citrate as shown in Table 1. The methanolic seed extract of *Zanthoxylum armatum* demonstrated paralysis as well as death of worms in a less time as compared to piperazine citrate especially at higher concentration of 50 mg/ml. While water extract showed significant activity, the petroleum ether extract was the least active among all the extracts.

DISCUSSION

In the present study, it was observed that all the extracts of *Zanthoxylum armatum* DC seeds have exhibited positive response to certain degree of anthelmintic activity. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyper polarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis.^[21] Different extracts of *Zanthoxylum armatum* DC seeds not only demonstrated paralysis, but also caused death of worms even at low concentration of 10 mg/ml as compared to standard drug piperazine citrate.

In conclusion, the traditional use of seeds of *Zanthoxylum armatum* as an anthelmintic have been confirmed as the seed extracts displayed activity against the worms used in

Table 1: Anthelmintic activity of *Zanthoxylum armatum* DC. extract

Test substance	Conc. (mg/ml)	Time taken for P and D of worms in min	
		P	D
Distilled water (control)	-	-	-
Piperazine citrate (standard)	10	19.16±0.87	61±0.88
Aqueous extract	10	23.83±0.79	74.83±1.72
	25	14.5±0.42	34.83±1.16
	50	11±0.25	20.66±1.43
Petroleum ether extract	10	28.3±0.61	86±1.84
	25	19.56±0.95	48±1.05
	50	12.10±0.87	35±1.32
Methanolic extract	10	18.06±0.65	61.83±1.32
	25	10.12±0.51	29.10±0.81
	50	5.18±0.40	15.5±0.40

P – Paralysis; D – Death

the study. The methanolic extract was more potent. Further studies need to establish the mechanism(s) of action are required.

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