

## EVALUATION OF ANTHELMINTIC POTENTIAL OF *PUNICA GRANATUM* FLOWER EXTRACT AND ITS FORMULATION

Shinde M. B.<sup>1\*</sup>, Nagarkar R. D.<sup>2</sup>, Waghchoure D. R.<sup>3</sup> and Bhutada S. A.<sup>4</sup>

<sup>1,2,4</sup>Sanjivani Arts, Commerec and Science College Kopargaon, Ahmednagar, Maharashtra, India.

<sup>3</sup>Ashwamedh Agritech & Farm Solution Systems, Kopargaon, Maharashtra, India.

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### \*Corresponding Author

**Dr. Shinde M. B.**

Sanjivani Arts, Commerec  
and Science College  
Kopargaon, Ahmednagar,  
Maharashtra, India.

### ABSTRACT

The *Punica granatum* belongs to family Punicaceae. *Punica granatum* (Pomegranate) plant parts are used traditionally for the treatment of various disorders such as anthelmintic, antidiabetic and other activities. *Punica granatum* flower extract was used to evaluate the anthelmintic activity. As earthworm (*Pheretima posthuma*) resembles intestinal parasites, it was selected for *in vitro* study. The experiment was performed in petri dishes with *Pheretima posthuma*. For that sterile 7 petri plates were used with different concentration of *Punica granatum* flower extract (5, 10, 15, 20, 25mg/ml and positive and negative control). The standard drug Albendazole (20mg/ml) was used

as positive control while normal saline as negative control. The anthelmintic activity of aqueous extract of *Punica granatum* flower was showed the longest and shortest time required for paralysis and death which was 5mg/ml and 25mg/ml concentration respectively. This study concludes that the aqueous extract of *Punica granatum* flower have the anthelmintic activity and can be used against parasitic worm.

**KEYWORDS:** Flower extract, Punica Granatum, Anthelmintic, Pheretima Posthuma.

### INTRODUCTION

The *Punica granatum* (Pomegranate) is an ancient plant and has been used for centuries for health benefits in number of diseases. Pomegranate plant parts are used traditionally for the treatment of various disorder, e.g. different parts of plant like fruits, leaves, bark, peel, seed has numerous therapeutic properties and can be used in treatment such as antimicrobial activity, anthelminthic activity, antidiabetic activity, antioxidant activity, anticancer activity,

cardiovascular activity etc (Leena *et al.*, 2016). In addition to this, the flowers of pomegranate also have medicinal properties, decreases the blood glucose, reduces the cholesterol and antiallergic (Nitave *et al.*, 2014). Hence, only pomegranate flower has been prescribed in Unani (Greek Medicine) is a form of traditional medicine and widely practised in south Asia. In Ayurvedic it is used as medicines for the treatment of diabetes and other disorders.

Helminthiasis is a macroparasitic disease observed in human and animals in which the part of the body is infested with parasitic worms such as round worms (Nematodes), tapeworms (Cestodes), or flukes (trematode), Hook worm, thread worm, necator etc. Some of the above worm can also infect to the organ – tissues and their larval may enter in it. They may cause intestinal injury, enterobiasis and lymphatic obstruction by secreting different toxins. Typically the worm's reside in the gastro intestinal tract. Ascariasis is the most common disease and generally children under 15 years are infected. Children infected with ascaris have proven to be lower in weight and height and have lower hemoglobin concentration and I.Q. (Sharma *et al.*, 2015). Destruction of parasitic intestinal worms from the body, anthelmintic drugs are used. Most of the marketed, commercial drugs produces side effects such as abdominal pain, loss of appetite, nausea, vomiting and diarrhoea. Hence overcome this side effects, herbal drugs are best remedies for the treatment of parasitic diseases in human without any side effect. The selection of drug for the treatment is not only based on its efficacy but also on its side effect/toxicity, low cost and availability of products. The present study focuses on the evaluation of anthelmintic activity of aqueous extract of *Punica granatum* flower and its formulation with significance to the public health.

## MATERIALS AND METHODS

**Collection of plant Material:** Fresh flowers of *Punica granatum* (Pomegranate) family Punicaceae were collected from local farm of village Badapur, Yeola, Maharashtra, India. These flowers were washed under running tap water and then rinsed with distilled water. After washing thoroughly the flowers were subjected to shade drying for a week. The dried flowers were powdered using mixture grinder.

**Preparation of Aqueous extract:** The extract of pomegranate flower was prepared in 10 % concentration i.e. 10 gm of Pomegranate flower powder added into 100 ml of sterile distilled water. Aqueous extract were prepared by boiling in waterbath at 50-55°C for 40-45 mins. The mixture was stirred continuously for 24hrs. This extract were filter through Whatman filter

paper no. 2 and filtered were concentrated in hot air oven at 55<sup>0</sup>C. The extract was used for further experimental work.

**Worm Collection:** The *invitro* anthelmintic activities were carried out on Indian adult earthworms (*Pheretima posthuma*). The earthworms were collected from moist soil, washed with water and then with normal saline to remove all the soil particles and fecal matter adhere to it. The earthworm of 6 – 18 cm in length were selected for present study. As earthworms resembles with the intestinal roundworm parasite *Ascaris lumbricoids*, and tapeworms (*Taenia solium*) used for the initial evaluation of anthelmintic activity.

**Anthelmintic activity:** The anthelmintic activity was evaluated on adult Indian earthworm which was collected from moist soil. The experiment was carried out at normal room temperature. The standard drug Albendazole (20mg/ml) was used as positive control while normal saline as a negative control. The experiments were performed in petri dishes. Three earthworm of approximately equal size were released in each petri plate and different concentrations of aqueous flower extract (250, 500, 750, 1000 and 1250/50ml) were added to it. Observations were made for the time of paralysis and death of earthworms. Paralysis time was noted down when there was no self-movement in the body of the worm. Death time was noted when the worms lose their motility followed with fading away of their body color.

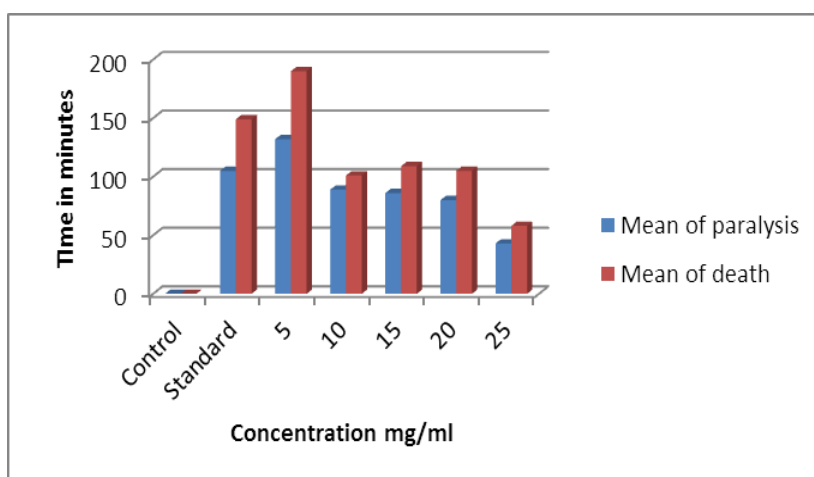
## RESULT AND DISCUSSION

### Anthelmintic test

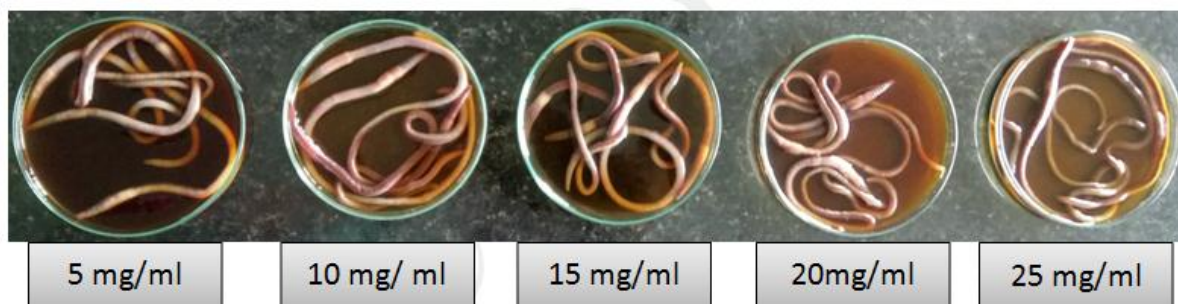
**Table No. 1: Anthelmintic activity of aqueous extract of *Punica granatum* flower.**

S.N.	Treatment	Conc.	Length (cm)	Time of paralysis in min.	Time of death in min.	Mean of Paralysis in min.	Mean of Death in min.
1	Normal Saline (Control)	-	10	-	-	0	0
			13	-	-		
			15	-	-		
2	Albendazole (Standard)	20 mg/ml	19	106	152	105	149
			12	106	150		
			08	104	147		
3	Aqueous extract Of <i>Punica granatum</i> flower	5 mg/ ml	10	121	183	132	190
			14	132	191		
			16	145	196		
		10 mg/ml	15	88	97	89	101
			19	71	101		
			27	75	105		
		15 mg/ml	8	78	101	86	109

			12	87	111		
			17	93	117		
		20 mg/ml	6	71	93	80	105
			18	81	111		
			18	88	111		
		25 mg/ml	17	36	59	43	58
			18	41	60		
			18	53	57		



**Graph No. 1: Anthelmintic activity of aqueous extract of *Punica granatum* flower.**



**Photograph No. 1: shows the death of earthworms after treatment of aqueous extract of *Punica granatum* flower.**

The experimental data shows anthelmintic activity against earthworm. The time of paralysis and death was noted against various concentration of aqueous flower extract.

It was found that (table no.1) the standard drug (20mg/ml) paralyze the earthworms in 105 minutes whereas the earthworm were killed in 149 minutes. In the normal saline the earthworm were neither paralyzed nor killed. From the observation it is found that the time required for earthworm is 132 minutes for paralysis and 190 minutes for death at 5mg/ml concentration of aqueous extract, 89 min for paralysis and 101 min for death at 10mg/ml, 86

min for paralysis and 109 min for death at 15mg/ml, 80 min for paralysis and 105 min for death at 20 mg/ml, 43 min for paralysis and 58 min for death at 25 mg/ml concentration of aqueous extract.

From the above result it was found that the longest time required for paralysis and death was observed with 5mg/ml concentration of aqueous extract of *Punica granatum* flower whereas shortest time required for paralysis and death was observed with 25mg/ml.

Leena *et al.*, (2016) reported that the time of paralysis of earthworm for aqueous at low concentration (25mg/ml) was 66 minutes and at high concentration (150 mg/ml) was 14 minutes. Death time for aqueous at low concentration (25mg/ml) was 86 minutes and at high concentration (150 mg/ml) was 36 minutes.

Aggrawal *et al.*, (2015) used three plant materials like flowers of *C. procera*, Fresh leaves of *A. indica* and fruit peel of *P. granatum* for the study of anthelmintic activity under in vitro condition.

Nitave *et al.*, (2014) reported that the time of paralysis of earthworm for aqueous extract of *Nerium oleander* flower at low concentration (25mg/ml) was 34 min and at high concentration (100 mg/ml) was 18.50 minutes. Death time for aqueous extract at low concentration (25mg/ml) was 47.16 minutes and at high conc. (100mg/ml) was 34.83 minutes.

## CONCLUSION

The present study concludes that the aqueous extract of *Punica granatum* flower have the anthelmintic activity. As the concentration of aqueous extract increases the time for paralysis and death decreases. The standard requires more time as compare to the aqueous extract for paralysis and death of earthworms. Hence, this herbal formulation is more effective as compared to standard drug. Therefore this formulation of *Punica granatum* flower powder can be taken with hot water for the treatment of ascaris, tapeworms, flukes, Hook worm, Thread worm etc. which are present in the intestine of human being.

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